FISH DISEASE

Diagnosis and Treatment

SECOND EDITION

EDWARD J. NOGA

WILEY-BLACKWELL

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Second Edition

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Contents

Preface to the First Edition

Over 80% of the Earth's surface is covered by water. Fish are ubiquitous inhabitants of this ecosystem. With over 20,000 named species and up to twice that number that may yet be discovered, they are the most successful vertebrate group and play an extremely important ecological role. In both natural environments and in culture, disease has a serious impact on fish. There is an acute awareness of and concern for the diseases affecting our fishery stocks (Noga 1988a). Fish health experts are increasingly called upon to provide answers about disease outbreaks in fishery populations. Largely due to the decline in fishery stocks and increased consumer demand, fish culture both for food and as pets is the fastest growing segment of animal agriculture in both the United States and worldwide. Disease is universally recognized as one of the most serious threats to the commercial success of aquaculture.

This book is intended to guide you, the reader, through the most commonly encountered fish diseases, and to provide you with the knowledge to manage these problems effectively. While the focus of this book is on cultured fish, most of the information also applies to wild populations.

Many readers will immediately notice that this book is not designed like traditional textbooks that cover fish disease. I have used a systems-based approach to describing fish diseases rather than the taxonomic approach, where all virus diseases are covered as a group, then all bacterial diseases, then all fungal diseases, then all parasitic diseases, etc. In my experience, diagnosticians mainly identify problems by systems rather than by taxonomic groups. For example, water quality is examined (environmental system), then the skin (dermal system), then the gills, then the internal organs, etc. I feel that design of a diagnostic guide along these lines makes for a much more understandable and user-friendly method for diagnosis because you, the reader, can literally "follow along" with the flow of the book and identify problems as they are encountered during the clinical workup.

Another feature that I felt was very important is detailed descriptions of pathogens. A knowledge of most of the common fish disease problems involves fairly simple techniques. This is in part due to our relatively unsophisticated methods used to diagnose problems, which are heavily based upon the morphologic recognition of a pathogen. For example, diagnosis of parasites, a very common problem, is made by identifying the parasite in a tissue sample. This necessitates that high-quality, representative examples be provided as illustrative material. I have tried to do this.

Finally, in order to identify and manage the problems, one needs an adequate understanding of the methods used for disease diagnosis and treatment. Thus, I have included detailed explanations and illustrations of common procedures.

The end result is what I hope will be for you a useful and practical guide that you will find valuable for everyday problems that you might encounter in working with this fascinating group of animals.

Preface to the Second Edition

Concerns about diseases affecting fish continue to increase as aquaculture remains the fastest growing segment of food animal agriculture and as the popularity of keeping fish as pets also expands. Diseases in wild populations also remain an important concern, especially as environmental degradation due to climate change and human activity impacts their health.

Since the publication of the first edition, a number of important, transformational changes have occurred in the diagnosis and treatment of fish diseases, which have necessitated many important modifications and additions to the second edition. Among these "sea changes" is the rapid rise to prominence of molecular methods for the identification of fish pathogens, which has greatly increased the speed and sensitivity in detecting agents and has provided a much better understanding of their epidemiology and pathogenesis. Also coming to prominence has been the widespread implementation of biosecurity and with it an increased emphasis on health management, which has been geared toward reducing the use of drugs in disease treatment and reducing adverse effects of fish diseases on the fish, the consumer, and the environment. Consequently, the second edition has expanded coverage of biosecurity principles and health protection strategies. A number of other issues not directly related to fish health, including food safety and environmental safety, have also become more important, and these areas are now integrated into the text. Nonetheless, drug treatment remains essential to the effective control of most fish diseases and thus the "Pharmacopoeia" section has been considerably expanded with both new drugs and dosing regimens.

With the increasing sophistication by which many cases of individual fish diseases are managed, especially in pet fish, the clinical workup section has been considerably expanded. Now included are a number of techniques that, while not used routinely on every fish disease case, are being increasingly applied, along with the standard clinical workup.

There is expanded coverage of most of the PROBLEMS, which, together with the other changes in the book, have increased its content by more than 130 pages. Virtually all illustrations are now in color. Several new diseases have been discovered or have gained prominence since the first edition; these now merit their inclusion as separate PROBLEMS. This has added 10 more problems, making a total of 103 problems in part II.

As with the first edition, I trust that this new edition will assist you, the reader, in more effectively addressing the health and welfare of the most prevalent vertebrate group on Earth.

Acknowledgments

This work would not have been possible without the invaluable assistance of many people, including those who contributed to the first edition and whose work is included in the second edition. Notable among these are the medical illustrators. I would especially like to thank Anne Runyon, who did a marvelous job in creating almost all of the line drawings in the text. Brenda Bunch also contributed significantly to the illustrations by producing the computer-generated composite photographs. Bruce Kendall, Susan Rosenvinge, Melinda Fine, and Helen Bolen also helped in this effort. I also thank the entire photography staff of the North Carolina State University Biomedical Communications Center, especially Wendy Savage, for their assistance with production of most of the photographs that I have accumulated over the years. Philip Ruckart also took some of the photographs in the new edition. Douglas Wagner and Bruce Kendall created the original cover design, which was modified in the second edition.

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Edward J. Noga, M.S., D.V.M.

Anne Runyon drew Figures II-13D, II-14A,B, II-15D, II-16C, II-17A,B, II-18A, II-21C, II-22A, II-23A, II-25A, II-26A, II-29A, II-30A, II-31C, II-32A, II-33C, II-44A, II-58A,K, II-60A, II-61F,G, II-62A, II-63A,B, II-70L, II-73B, II-74A,B, and III-4D. Brenda Bunch produced the original versions of the composite photographs in Figures II-14, II-17, II-20, II-34, II-58, II-60, II-61, II-63, II-70, II-74, and II-76. Alice Harvey created Figures II-4A,B, II-7, III-2B, and III-4E. The cover is a computer-generated composite photograph of a striped bass with skin ulcers. The original subject was laser-scanned, using a flatbed scanner, and modified using a Macintosh computer and then with Adobe Photoshop. Computer-generated montages were created by Douglas Wagner and the cover design was created by Bruce Kendall, both of the Biomedical Communications Center, North Carolina State University College of Veterinary Medicine. This cover was modified by the staff of Wiley-Blackwell for the second edition.

HOW TO USE THE BOOK

Prior to reading the text, familiarize yourself with the flow diagram of a clinical case workup (Fig. I-1). The book is organized exactly as shown in the flow diagram.

The book is divided into three major parts: (part I) "Methods for Diagnosing Fish Diseases," (part II) "Problem List," and (part III) "Methods for Treating Fish Diseases." Part I provides a detailed guide to the methods used to diagnose fish diseases. The methods are covered in the order in which they would be performed during a case workup (see Fig. I-1).

Part II is a comprehensive coverage of fish diseases. Note that the problems are listed in the order in which they are encountered in the clinical workup, as described in part I. For example, water-quality problems that are routinely identified in the clinical workup are listed first, followed by problems that are identified from examining the skin and gills, followed by internal/systemic diseases, and then problems that cannot be definitively diagnosed in a routine clinical workup but are suspected to be the cause based upon the clinical workup and rule-out of other problems (see Fig. I-1). Confirmation of a rule-out diagnosis usually requires submission of samples to a specialized reference laboratory to obtain a definitive diagnosis. The last group of rule-out diagnoses is of unknown cause ("idiopathic") and is also diagnosed by ruling out all other possible causes. This sequential arrangement of problems allows you to "follow along" through the problem list as you do the clinical workup, facilitating diagnosis.

Part III provides a detailed description of how fish are treated and the drugs that are effective for various problems.

Fish Disease

Diagnosis and Treatment

Second Edition

PART I

METHODS FOR DIAGNOSING FISH DISEASES

CHAPTER 1

Major Cultured Species

Before discussing methods used for diagnosing fish diseases, it is important to have an understanding of the numerous types of fish species that are cultured, as well as the diversity of culture systems. Understanding the different requirements for maintaining these different groups is essential to both short- and long-term health management.

AQUARIUM (PET) FISH

Aquarium fish constitute an extremely large segment of the pet animal industry (Winfree 1989). The bulk of aquarium fish are kept in the United States, Europe, and Japan (Chapman et al. 1997). In 2008, expenditures for the entire U.S. pet industry (including livestock and all products and services) were valued at over $41 billion, with 63% of U.S. households (an estimated 71 million homes) having pets and 15% of all households owning aquarium fish (APPA 2008). The great majority (nearly 95%) owned freshwater fish, but marine fish have continued to gain in popularity. Worldwide, between 1.5 and 2 million people keep marine aquaria, with 600,000 of those being in the United States (Wabnitz et al. 2003). The global market for marine aquarium products is substantial and growing rapidly, with worldwide trade estimated to exceed $7 billion (Falls et al. 2003). Worldwide trade in live marine animals (exclusive of food animal species) is estimated to be worth $200–330 million annually, with the main markets being the United States, the European Union, and, to a lesser extent, Japan (Wabnitz et al. 2003). According to the United Nations Environmental Program World Conservation Monitoring Centre's report on global trade in marine species, only 1–10% of marine fish are captive bred, with the remainder being collected from coral reefs.

Expensive and highly sophisticated aquaria are becoming increasingly more common, and it is becoming more common for an owner to have several hundred to several thousand dollars invested in fish alone. Thousands of types of pet fish (from the commonplace guppy to the more exotic and often more expensive species) are kept by hobbyists. While some aquarium fish, such as the common goldfish, cost only a few dollars, many fish command high prices, often costing several hundred dollars and some commanding over $100,000. The average freshwater aquarium fish probably costs somewhere between $3 and $10; marine fish are usually considerably more expensive, averaging $20–50. The average cost incurred by owners of fish in the United States is estimated to be about $235 per year, which compares to $200 for a bird, $645 for a guinea pig, $911 for a rabbit, and $1,200 for a dog (www.spca.bc.ca). Some pet fish owners, like owners of other animals, also become emotionally attached to their pets and are willing to spend considerable sums for proper medical care. It is also interesting to note that over 80% of pet fish owners also own other pets.

Many hobbyists specialize in a single group of fish (e.g., African cichlids, bettas, catfish, koi), and there are a number of local, national, and international breed associations for these various groups. The reader should refer to Axelrod et al. (1980), Bower (1983), Moe (1992a, 1992b), Goldstein (1997, 2008), Debelius and Baensch (1998), and other reference texts for specific details on taxonomy, biology, and husbandry. Schmidt (2002) and Axelrod et al. (2007) provide comprehensive photographic compendia of freshwater aquarium fish but nothing on husbandry.

Tropical Freshwater Aquarium Fish

The largest segment of the aquarium fish industry is the freshwater aquarium fish sector. Major groups include the poeciliids and the egg-layers.

- Poeciliids (guppies, mollies, swordtails, platies)—These are also known as livebearers because they are viviparous. (A few other nonpoeciliid fish are also viviparous.) They are prolific, with many line bred strains. These fish are often relatively inexpensive, although certain strains may be high priced.

The so-called egg-layers encompass all other freshwater aquarium fish. Major groups include the following:

- Characins (tetras)—These are active, schooling fish that usually stay in the upper water column. Some species may be a bit aggressive and nip fins or chase tankmates. Most make good members of a community aquarium. This group also includes the piranhas,

which are not good for the community aquarium (see oddball fish).
- Tropical Cyprinids (barbs, danios)—These are active, schooling fish that usually stay in the upper water column. Like the characins, they may be a bit aggressive and nip fins or chase tankmates. Most make good members of a community aquarium.
- Anabantids (bettas, gouramies, paradise fish, etc.)—These are generally peaceful fish that are good candidates for a community aquarium, except for the popular Siamese fighting fish, which, although aggressive toward conspecifics, are shy toward unrelated species. Anabantids breathe air by using an accessory organ modified from gill tissue (labyrinth organ).
- Cyprinodonts (killifishes, topminnows)—These are generally small, often brilliantly colored fish, many of which have short natural life spans (e.g., annual fish). They are often shy among other types of fish and do best in a separate aquarium. There is usually marked sexual dimorphism.
- Catfish (*Corydoras*, *Pimelodella*, *Plecostomus*, etc.) and Loaches (clown loach, kuhli loach, etc.)—These are generally peaceful, bottom-feeding fish that are useful as scavengers to keep the gravel clean. Most make good members of a community aquarium.
- Cichlids (freshwater angelfish, discus, oscar, African rift lake cichlids, etc.)—These are a popular group of fish that include a wide range of species having diverse behaviors. Some make excellent members for a community aquarium (e.g., angelfish), while others are extremely territorial and can only be kept with equally aggressive species (e.g., oscar). Some species have marked sexual dimorphism.
- Oddball fish (archerfish, piranha, freshwater butterfly fish, etc.)—These are species that are occasionally kept by aquarists as novelties. They include a diverse array of species.

Cool Freshwater Aquarium Fish

This includes the cool water cyprinids (goldfish and koi). These are hardy fish that are popular for both aquarium and pond culture. They are not tropical fish and can thrive in a wide range of temperatures. They do best in slightly cooler water. Koi culture has been one of the most rapidly growing areas of the pet fish industry and owners often spend large sums of money on both the fish and their culture environment. Details about koi husbandry and diseases peculiar to this species can be found in Stoskopf (1993), Saint-Erne (2002), and Johnson (2006).

Tropical Marine Aquarium Fish

Marine fish are becoming an increasingly larger component of the pet fish industry. At least part of this growth is because of the recent strides that have been made in successfully keeping these fish in captivity. Better tank design and its integration with more reliable and efficient pumps, filters, and other apparatus have helped to greatly improve water quality, which is essential for marine fish health. Although proper veterinary care is still sorely lacking in many situations, owners and retailers also have a better awareness of diseases and the proper means of treating them compared to years past. Another factor that may contribute to the surge in marine aquarium keeping relates to the greater amount of disposable income in many households, which has allowed more people to afford these beautiful but expensive creatures. Many marine hobbyists have reef tanks, which are elaborate and usually expensive setups that are used for the display of live invertebrates (corals, anemones, etc.) as well as fish. When a dozen or more such animals are kept in a single tank, this can become a sizable economic investment.

The majority of marine fish come from Indo-Pacific reefs (Indonesia, Philippines, Pacific Islands), with some from the Florida Keys, the Bahamas, the Caribbean, and the Red Sea (Lewbart 1992; M. Weiss, personal communication). Despite some significant advances in captive propagation, the great majority of marine aquarium fish are wild caught. A striking example of the dramatic difference in adaptation to culture between wild-caught and captive-raised marine fish is the clownfish: The relative survival rate of this group of 28 species as wild-caught individuals is markedly less than that of captive-produced stocks. Brooklynellosis (PROBLEM 24), relatively rare in captive-bred clownfish, is often called "clownfish disease" because of its common presence on wild-caught fish (Fenner 1998).

Important ecological differences between marine and freshwater fish have a direct bearing on their health in captivity (Table I-1). Compared with freshwater ecosystems, the tropical marine environment has little natural fluctuation in temperature, oxygen, or other water-quality conditions. Thus, marine reef fish are not adapted to withstand the poor water conditions to which they are often exposed in captivity; this is exacerbated by the fact that most marine aquarium fish are wild caught

Table I-1. Differences between tropical freshwater and marine aquarium fish (from Noga 1992).

	Freshwater	Marine
Many inbred strains	Yes	No
Many bred in captivity	Yes	No
Specialized feeding habits or nutritional requirements	Relatively few	Relatively many
Sensitivity to environmental changes	Relatively small	Relatively great
Territorial	Many	Almost all

and must also acclimate to the confines of culture. Reef fish are highly territorial, adding to the stress of capture. They can carry latent infections, which recrudesce under captive conditions. Parasites are especially common. Many reef fish have specialized diets, such as feeding on sponges or corals. Many cannot adapt to standard aquarium food and starve to death in captivity. Unfortunately, certain reef fish are imported and sold in stores with little regard for whether they will ever accept food. All of these factors add up to an increased susceptibility to disease and the marine fish' deserved reputation for being more difficult to keep than their freshwater counterparts. This emphasizes the need for competent health care.

Fish chosen for a marine aquarium should be species with histories of successful maintenance in captivity, and the fish should be eating well. To avoid aggression problems, a good rule of thumb is to have only one fish of any color, color pattern, or shape. Extreme range in size should also be avoided. Bower (1983) and Moe (1992a, 1992b) provide an excellent discussion on choosing fish and proper management of the marine aquarium. In general the best families for the home aquarium are, in descending order, the following: anemonefish, damselfish, angelfish, gobies, wrasses, parrotfish, and butterflyfish. Note that there are many exceptions to this rule of thumb.

Making poor choices of fish for an aquarium not only increases the likelihood of disease and other problems but also might have serious negative impacts on the natural reef environments from where the fish were collected (Helfman 2007). For example, some marine fish are captured using cyanide to temporarily stun them to ease collection. The survivors that make it to the pet store may be seriously weakened by such treatment and of even greater concern is the indiscriminate damage that this collection method does to the other reef inhabitants, including the corals (see PROBLEM 94). Organizations such as the nonprofit Marine Aquarium Council (www.aquariumcouncil.org) are promoting environmentally responsible marine aquarium keeping via certification of wholesalers and pet shops to encourage responsible collection and husbandry. The Reef Fish Guide published by Reef Protection International (www.reefprotect.org) provides a list of fish species that are either recommended or should be avoided.

It is becoming extremely popular for invertebrates to be kept with marine fish in reef tanks or other less elaborate setups. Hard and soft corals, anemones, sea urchins, starfish, shrimps, and crabs are commonly sold in aquarium stores and online. A number of excellent books on biology and husbandry of reef fish and invertebrates are available, including Goldstein (1997), Debelius and Baensch (1998), Fossa and Nilsen (1996, 2000, 2002), Tullock (2001), and Fenner (1998).

Aquarium fish include a diverse array of species from many different habitats, and while they can often withstand a wide range of environments, they do best under more defined conditions (see PROBLEMS 2 and 7 through 10).

BAIT FISH

Several species comprise an important industry that produces bait fish for sport fishermen. Included in this group are various minnow species (Cyprinidae, Cyprinodontidae), such as the fathead minnow and golden shiner. In the United States, farms are concentrated in the Southeast, especially Arkansas. Fish are typically raised in small ponds.

FOOD FISH

According to the Food and Agricultural Organization (FAO), aquaculture is the fastest growing agri-industry worldwide, with an average compounded growth rate of 8.8% per year from 1950 to 2004, compared with only 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO 2000, 2006). This is not just one industry but actually an amalgamation of many different industries that culture many different species of aquatic animals (Pillay 1993). Among the most commonly cultured fish are carp (family Cyprinidae), trout and salmon (Salmonidae), catfish (Ictaluridae, Clariidae, Pangasidae, Siluridae), eel (Anguillidae), tilapia (Cichlidae), mullet (Mugilidae), milkfish (Channidae), yellowtail (Carangidae), flounder (Pleuronectiformes), sea bass/grouper (Serranidae, Centropomidae), and sea bream (Sparidae). Pillay (1993) provides a good introduction to culture of various groups. With such a diverse enterprise, only generalizations can be made about the types of fish and culture systems. Representative species, mainly exemplified by those cultured in the United States, are covered below. Detailed coverage of diseases of salmonids and carp can be found in Bruno and Poppe (1996), Kent and Poppe (1998), and Hoole et al. (2001).

Warm Water Food Fish

This category includes fish that thrive at temperatures generally greater than about 20°C (about 68°F). In the United States, the most important member of the warm water food fish is the channel catfish (Ictaluridae). Annual U.S. production is over 225 million kg (500 million lb), having a farm value of about $400 million. This translates into over half of all aquaculture production in the United States. Major producing states are concentrated in the Southeast, especially in the southern Mississippi River floodplain, because of an ample clean water supply

and a long growing season. However, significant catfish production also exists in other areas, ranging from California to North Carolina and Missouri to Florida.

Most channel catfish are less demanding of water-quality conditions than cold water species and are usually raised in earthen ponds. Channel catfish are typically spawned in late spring or summer, with the young fish being kept in small ponds or other small facilities until they reach an adequate size (usually 13–20 cm or 5–8 inches) to fend for themselves in larger ponds, where they remain until they are harvested.

Many channel catfish farms are vertically integrated, with broodstock for spawning, hatchery and nursery facilities, and grow-out operations on the same farm. Some farms specialize in supplying fingerlings to other producers. Commercial channel catfish farms typically raise fish in 2–8 ha (5–20 ac) ponds. Annual yields average 6,500 kg/ha (5,800 lb/ac). This represents an annual harvest income of $9,750–$11,375/ha ($4,060–$4,640/ac) at farm gate price of catfish of $1.50–1.75/kg (= $0.70–0.80/lb). With such a substantial investment at stake, proper medical care is a worthwhile expenditure.

Tilapias are also raised in the U.S. on a limited basis where high tropical temperatures can be maintained (far southern states or areas having geothermal well water) or in intensive, closed culture systems. Redfish (Sciaenidae) is a marine species that is cultured extensively in states that border the Gulf of Mexico (mainly to replenish natural stocks).

Cold Water Food Fish

This category includes fish that thrive at temperatures generally below about 20°C (about 68°F). The principal members of this group in the United States and worldwide are the salmonids (salmon and trout). Rainbow trout production currently exceeds 500,000 tons worldwide, having an estimated value in excess of $1 billion, and Atlantic salmon has a similar market value. Rainbow trout is the most important cultured species in the United States, but others (e.g., Atlantic salmon, brown trout) are also valuable. Annual farm value of U.S.-produced trout and salmon is over $70 million.

Salmonids are anadromous (spawn in freshwater and then migrate to the sea to mature) and can be grown in both freshwater and seawater. Because they are demanding in their water-quality requirements, most salmonids are raised in open or semi-open systems. Most commercial salmonid production in the United States is in freshwater raceways, but increasing numbers of salmon are being raised in marine net-pens, and marine Atlantic salmon and freshwater cyprinid production are the most valuable fish aquaculture industries worldwide.

Other species of importance in the United States include sturgeon (Acipenseridae), flatfish (Pleuronectidae), and hybrid striped bass, especially striped bass × white bass hybrids (Percichthyidae). Hybrid striped bass and some flatfish species are more appropriately considered cool water groups, since they can tolerate much higher temperatures than salmonids.

LABORATORY FISH

Fish are now widely used as animal models in biomedical research (Ostrander 2000). While several larger fish species are used as animal models, including salmonids, the most important are smaller aquarium species, especially medaka and zebrafish. Zebrafish has become the most important aquatic model for comparative medicine research. Many different inbred and transgenic lines have been developed to study various biological processes and diseases. Consequently, many stocks are maintained for very long periods and thus chronic diseases (e.g., mycobacteriosis [PROBLEM 55]) can be a serious problem. An online manual of common zebrafish diseases is available at http://zfin.org/cgi-bin/webdriver?MIval=aa-ZDB_home.apg.

CHAPTER 2

Types of Culture Systems

Environment has a major influence on virtually every important disease affecting cultured fish (Snieszko 1974; Smart 1981), and thus it is only appropriate that a treatise on fish diseases includes a discussion of culture systems. The following four major types of systems are used to culture fish: aquaria, ponds, cages, and raceways. The major difference among these types of systems is simply how quickly water turns over (i.e., how quickly it is exchanged with new water). This ranges from aquaria and ponds, where no water is exchanged, to flow-through systems, where new water is being replaced continuously. This dictates the fish density that can be kept in each system, unless the culturist provides additional life-support systems.

All basic life-support processes, including providing oxygen and removing toxins, are performed by properly designed culture systems. In flow-through systems, such as raceways, these processes are accomplished by the constant addition of new, well-oxygenated water, which dilutes out toxins. Constant inflow of new water allows for high fish densities. Ponds have virtually no regular water exchange, and aside from rainfall, no new water is added naturally. Thus, ponds must rely on resident biological processes to provide oxygen and remove toxins (see "**Closed Culture Systems: Ponds**," **p. 10**). These biological processes occur in all bodies of water but have a certain finite capacity to support a fish population. This carrying capacity dictates the number of fish that a pond can sustain. Aquaria can typically hold higher fish densities than ponds because of supplemental life-support systems, including air pumps for oxygen and filters for toxin removal.

The amount of water turnover also tremendously influences the available therapeutic options. Systems with high water turnover are difficult to manipulate environmentally (e.g., to change temperature, salinity, etc.), mainly because of economic costs and environmental concerns. Also, water-borne medication, which is the most common method of treating fish disease, is more difficult in flow-through systems for the same reasons.

CLOSED CULTURE SYSTEMS: AQUARIA

Aquaria are mainly used for maintaining pet fish, although some food fish are also cultured in these intensive systems. Space does not permit a detailed discussion of the types of aquarium culture systems used for maintaining fish. The reader is referred to standard texts (Axelrod et al. 1980; Spotte 1979a, 1979b, 1992; Moe 1992a, 1992b) for details. The purpose of this discussion is to describe the basic components that are needed for aquarium culture, with emphasis on pet fish.

An aquarium is analogous to a spaceship in that all essential life-support systems must be provided; this includes removing toxins and supplying oxygen, proper temperature, and food. The basic components include the following:

1. Aquarium (tank)—It is usually made entirely of glass. Tanks are less frequently made of plastic (acrylic) or fiberglass. Sizes typically range from 1 gallon to over 100,000 gallons (4–400,000 liters) in large public aquaria. Most hobbyists have aquaria ranging from 5 to 125 gallons (20–500 liters).
2. Substrate—This consists of various types of gravel, sand, or limestone. Some substrates are inert, while others may leach minerals (e.g., crushed coral reacts with acids in the tank to release calcium and magnesium, increasing the hardness) or other substances. Some types of gravels may also leach toxins, such as heavy metals; these should not be used in aquaria. The most inert types of minerals are quartz, granite, and mica.
3. Filters—The major types of filters are corner, undergravel, outside, and canister types. Some have a water pump for increased circulation (power filter). Some may be elaborate (wet-dry filter for marine reef tanks). Filters usually perform multiple functions that can be classified into either mechanical, chemical, or biological filtration; the two most important functions are to circulate the water for oxygenation (mechanical) and to remove nitrogenous waste products via the bacteria that colonize the filter bed (biological). Filters also

remove particulates and/or pigments (chemical) that reduce the aesthetics of the tank and may also be harmful to fish. Along with the tank size, the sizes and types of filters are primary factors that dictate the amount of fish biomass that can be held in any given aquarium.

4. Aerators—These include airstones and other devices driven by pneumatic pumps that increase circulation (i.e., increase contact with the air-water interface) and thus oxygen levels.
5. Other water purification devices—These are primarily used in marine aquaria and include equipment to perform foam fractionation and protein skimming, which helps to remove excess nitrogenous wastes. Also included are reverse osmosis (R/O) units to purify water prior to using it to prepare artificial seawater.
6. Live plants—Many different types of plants are maintained in aquaria, including mainly vascular plants (i.e., higher plants) in freshwater tanks and macroalgae in marine aquaria. Plants provide oxygen, remove nutrients, and act as refuges for shy fish.
7. Decorations—These include coral, ornaments, and various types of artificial plants. All items should have been tested as safe for use in aquaria.
8. Heater—This is a thermostatically controlled electrical unit that maintains a constant temperature. Some are only partly submerged, while others are completely submersible.
9. Disinfection units—These are used to remove pathogens from the water. Most popular are units that produce ozone or ultraviolet light to kill microorganisms. While they are useful when water is being recirculated among multiple aquaria, their utility, when used for only one aquarium, is questionable.

CLOSED CULTURE SYSTEMS: PONDS

The Pond as an Ecosystem

Many of the principles that apply to aquarium ecology also apply to pond ecology. It is useful to consider the pond itself as a single, functioning entity, since the pond's health is vital to the fish's health. In many ways the pond's vital functions are similar to that of a single organism (Noga and Francis-Floyd 1991). Respiration, acid-base balance, elimination of nitrogenous wastes, and other biological functions must be maintained. Some factors, such as temperature, are beyond control; however, others can be modified considerably through active intervention of the farmer and as an indirect consequence of management practices. It is also important to realize that changing a single parameter, such as increasing pH, can have a profound effect on many other variables (Table I-2).

Thus, it is not possible to treat the pond without affecting the fish, and conversely, it is not possible to treat the fish without affecting the pond ecosystem (Tucker 1985; Tucker et al. 1979). This makes water-quality analysis as important to assessing a fish disease problem as the physical examination is to routine clinical assessment of land animals. Adjacent ponds may be identical in size, soil substrate, source of water, and number of fish stocked, but each will develop as a unique ecosystem and must be treated as such.

Several routine management practices are performed to maintain proper pond health, including the following:

1. Fertilization—May be used to stimulate growth of algae, which is the major producer of oxygen in the pond and which removes much of the ammonia.
2. Aerators—Supplemental aeration is used when oxygen is low. Paddlewheels, diffusers, and other devices may be used.

Table I-2. Interrelationships between some important water-quality factors in a fish pond (from Noga and Francis-Floyd 1991).

	Effect[a] of increase in factor on:			
Factor	Dissolved oxygen	Dissolved CO_2	Ammonia toxicity	Copper toxicity
Temperature	Decrease	Decrease	Increase	Increase[b]
pH	No direct effect	Decrease	Increase	Decrease
Alkalinity	No direct effect	Decrease	No direct effect	Decrease
Phytoplankton[c]	Increased fluctuation	Increased fluctuation	Complicated effect	Complicated effect
Hardness[d]	No direct effect	No direct effect	No direct effect	No direct effect

[a]Only direct causal relationships are presented. These relationships hold if all other factors remain constant. For example, only the direct effect of alkalinity is considered, although methods used to increase alkalinity (buffering capacity) may also increase pH.
[b]Fish become ill or succumb more quickly when the temperature is higher.
[c]Increases in the duration or intensity of light may have similar effects because of increased photosynthesis.
[d]If attributable mainly to calcium carbonate.

3. Liming—Is used to neutralize acids and to maintain a proper pH. It also provides carbonate ion needed for algae growth and calcium and magnesium needed by fish. Liming is often done after draining a pond at the end of a production cycle (see PROBLEM 7).

In addition, algicide treatment has been used to control excessive algal growth but is usually not recommended (see PROBLEM 1).

Commercial Ponds

Commercial fish ponds are typically earthen, rectangular, 0.9–1.2 m (3–4 feet) deep, and 0.4–8 ha (1–20 ac) in size. Commercial pond fish production faces problems that are similar to those in other forms of intensive animal agriculture. High stocking densities mandate high nutrient input from feed, which in turn causes the buildup of toxic wastes. High nutrient levels also stimulate algae growth, causing large fluctuations in dissolved oxygen. These suboptimal conditions place considerable stress on the fish. Water is an excellent medium for the transmission of infectious agents, and diseases can spread rapidly through susceptible populations. Diseases must be diagnosed rapidly and accurately; even a matter of several hours can be crucial to the outcome of an epidemic. Thus, herd health management with proper intervention to prevent problems is the best approach.

Some ponds are also stocked with fish that the owners then charge customers to fish (fee-fishing ponds). These ponds are frequently restocked with large fish. Owners must keep fish actively feeding to provide a quality experience for customers.

Farm Ponds

Many landowners raise fish in farm ponds that are stocked with channel catfish, as well as game fish (e.g., bass, bluegill). In the state of North Carolina alone, it is estimated that over 100,000 farm ponds exist. While such ponds usually do not constitute a primary source of income for the owner, they often represent a significant investment in time and/or money, and provide a considerable amount of enjoyment, as well as a source of food. Some individuals start out with such small production units, hoping to expand later if the business is profitable. While these systems are usually not as intensively managed as the larger commercial operations, the concepts regarding proper management are the same. Medical advice on these fish could be incorporated into routine calls that are made to care for other farm animals.

Farm ponds vary greatly in size and depth but are usually relatively small; they may be deep, leading to stratification problems (see PROBLEM 3).

Pet Fish Ponds

Ponds are also popular for keeping some pet fish (e.g., goldfish and koi). These ponds may have no filtration or aeration (Andrews et al. 1988), but supplementary life support is needed if the fish are in a high density, which may occur as the fish begin to outgrow a small pond.

FLOW-THROUGH CULTURE SYSTEMS

In the United States, flow-through (also known as open culture) systems are primarily used to raise salmonids. General characteristics include a high water turnover rate and the dependence on a flushing effect to maintain water quality. A flow-through system is any system that uses continuously flowing water that enters at one point in the system and exits at another point. The major limitation to flow-through culture is the amount of water available for use. While small systems can rely on dechlorinated tap water or low-capacity wells, larger systems usually need a source of surface water (e.g., stream, impounded lake).

The most common type of flow-through system is the raceway, a long, narrow ditch made of concrete, earth (e.g., Danish pond), or fiberglass (Stevenson 1987). Raceways are often longitudinally divided into compartments, with a 0.3 m (1 foot) deep waterfall between each compartment. This waterfall adds more oxygen to the water; oxygen is the major limiting factor to the number of fish (and thus number of compartments) possible. Some farms use liquid oxygen to increase stocking densities; in such cases, ammonia toxicity and low pH become the major concerns (see PROBLEMS 4 and 7). It is usually not feasible to control other water-quality variables, such as temperature, pH, or hardness, in flow-through systems.

The major advantage to a flow-through system is the ability to have a high stocking density and still have high-quality water (see **Table III-1**). However, disadvantages include the need for a large amount of high-quality water, which severely restricts the sites suitable for this type of culture. Increasingly stringent local and national regulations restrict the type and amount of effluents that can be released by such farms.

Many flow-through systems must use surface (e.g., stream) water, whose quality and quantity are highly dependent on rainfall (runoff). This can cause overcrowding and stressfully high temperatures in summer or during droughts.

Many diseases are transmitted via water, and important pathogens are often endemic in feral fish populations that inhabit the water source. This also makes the system susceptible to pathogens or toxins that may originate upstream of the system. Exceptions to this environmental variability are flow-through systems that use a ground

water (i.e., well or spring) source. Ground water is usually free of pathogens and not chemically influenced by rainfall. Ground water sources are chemically stable and vary little over time.

SEMI-OPEN CULTURE SYSTEMS

Cages (also called net-pens) are intermediate in water exchange between open and closed systems. There are four basic types of cages: floating, fixed, submerged, and submersible. The most common is the floating cage, in which a buoyant collar supports the mesh net. Fixed cages have a net bag supported by posts driven into the lake or pond bottom; they are inexpensive and commonly used in some developing countries. Submerged cages remain permanently below the water while submersible cages can take advantage of prevailing environmental conditions by moving vertically in the water (e.g., submerged during storms or harmful algae blooms). Many types of fish are grown in cages. Fish such as tilapia and carp are commonly raised in freshwater, while in the marine environment, they primarily are used to raise salmonids, especially Atlantic salmon, as well as sea bass and sea bream. For more details, see Beveridge (2004).

CHAPTER 3

The Clinical Workup

EQUIPPING A FISH DISEASE DIAGNOSTIC FACILITY

Hospitalization Systems

General guidelines and needed equipment for setting up a hospitalization/quarantine system are described in "**Clinic Hospitalization**" (**p. 372**).

Basic Diagnostic Tools

Most of the equipment required for fish disease diagnosis is inexpensively available (excluding materials needed for specialized procedures, which are described in detail starting on **p. 35**). The only major piece of equipment that is absolutely needed is a high-quality microscope having 10X, 40X, and 100X (oil immersion) objectives (giving final magnifications of 100X, 400X, and 1,000X with a 10X ocular). Other basic, required equipment includes disposable latex gloves; simple surgical instruments (scalpel, fine and coarse forceps, and fine and coarse scissors); 10% neutral, buffered formalin; microscope slides; and coverslips (all available from companies such as Baxter Diagnostics, Inc., Carolina Biological Supply Company, or Fisher Scientific).

In addition, water testing kits are available from a number of companies such as Chemetrics, Inc., Hach Company, LaMotte Company, Marine Enterprises International, Ltd., or Tetra Sales, USA. Simple colorimetric kits (that visually compare the color intensity of the sample to a color chart) are perfectly adequate for most routine diagnostic workups. More sophisticated, highly quantitative instruments are also available, especially if large numbers of samples are to be measured (e.g., dissolved oxygen meter [YSI, Inc.]) or more accurate measurements are needed, such as for research protocols. Other required materials include disinfectant/antiseptic (see "**Pharmacopoeia**"), anesthetic (see "**Pharmacopoeia**"), several clean 20 and 40 liter (5 and 10 gallon) plastic buckets, a supply of various-sized aquarium bags, various-sized nets, and several airstones connected to a small air pump (available from companies such as Aquatic Ecosystems, Inc., Argent Chemical Laboratories, or Aquacenter, or from a local pet shop).

It is also helpful to have media available for bacterial culture (sold by companies such as Baxter Diagnostics, Inc., or Fisher Scientific), depending on the number and types of cases seen. Addresses for suppliers are listed in **appendix II**. Details about choosing specific items for the clinic are described in "**The Clinical Workup**." See "**chapter 16**" for items needed for treating diseases.

Other, more specialized instruments (e.g., otoscope, ophthalmoscope, ultrasound imaging equipment, radiography equipment and surgical equipment), while not used routinely, can be useful for some cases. Their use is described under specific sections below.

CASE SUBMISSIONS

Submissions to the Clinic

The basic steps that should be followed in the clinical workup of a fish disease case are illustrated in Figure I-1. If fish are submitted to the clinic, virtually all procedures can be handled on an outpatient basis, eliminating the need to keep fish overnight. Most cases will be initiated by a telephone call from an owner who is having a problem. The owner should be asked to bring in one to several representative fish for examination. It is important to determine whether the owner is amenable to the euthanization of any fish for the determination of a diagnosis. Hobbyists who are breeders are usually willing to sacrifice some fish, unless the fish are rare or expensive brood stock. While a complete postmortem examination is superior to performing only biopsies, this will not be possible in many pet fish cases; this can usually be discerned during the conversation with the owner.

If the client is submitting the fish to the clinic, the owner should be advised to bring both the fish and a water sample in separate clean containers. The best containers are a clean plastic bucket (never exposed to soap or other toxic chemical), plastic-lined cooler, styrofoam cooler, or a plastic aquarium bag. However, a well-washed and rinsed glass food container is also acceptable.

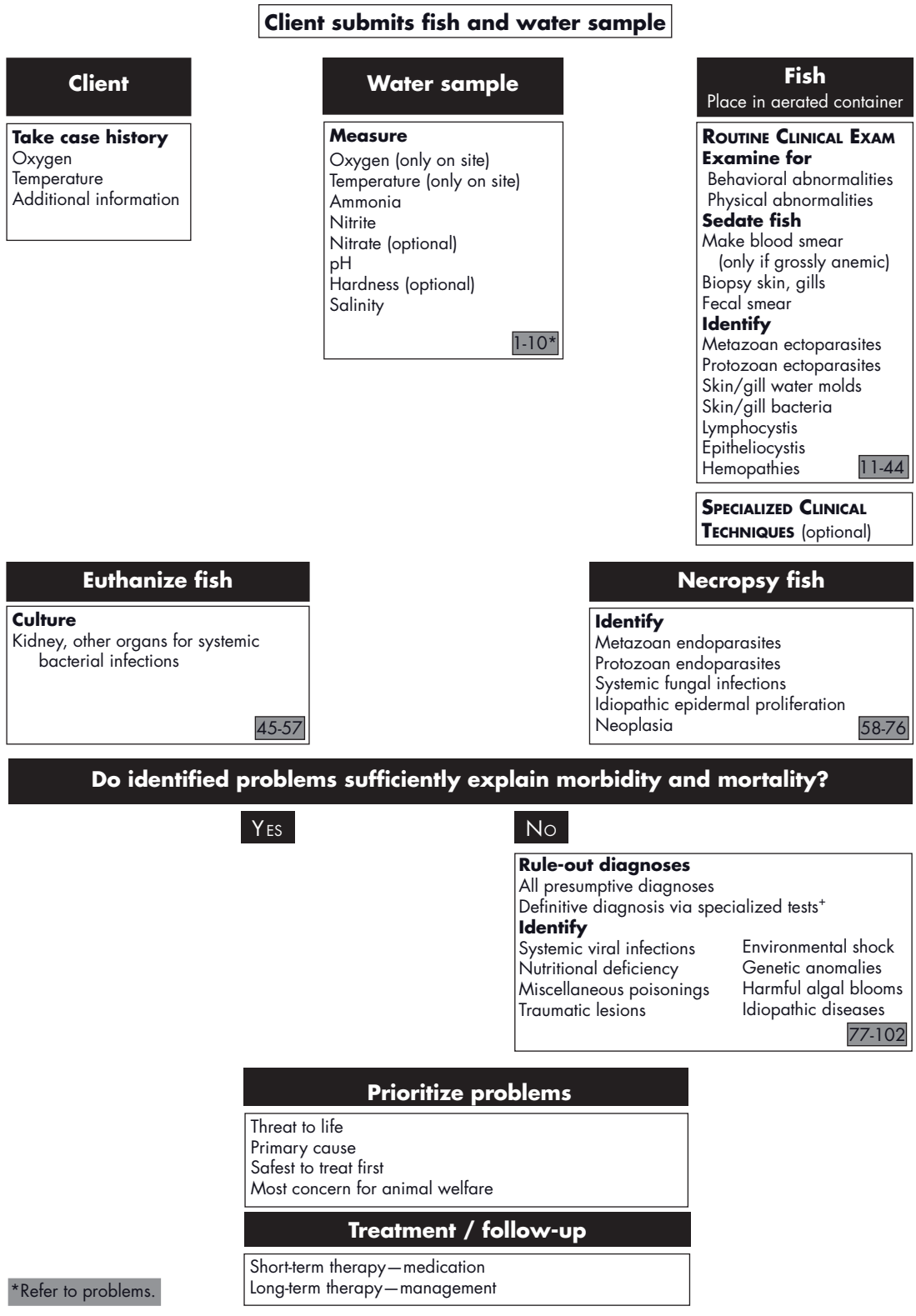


Fig. I-1. Steps in working up a fish disease case.

Half a liter (1 pint) of water is adequate for core water-quality analysis (**see p. 16**).

An owner submitting samples from a pond should be advised to walk the pond bank with a dip net or cast net and selectively remove fish that are either at the surface, at the water's edge, or otherwise appear abnormal. This is far preferable to randomly seining fish out of the pond, because they are less likely to be sick or display the most important clinical signs.

To transport the fish (assuming the trip to the clinic will be less than 30 minutes), a good rule of thumb is to have about 1 liter of water for every 1 cm of fish (or 1/2 gallon of water for every 1 inch of fish) to be transported. Much higher densities can be used if supplemental oxygenation is provided. It is best to place the container of fish in a cooler to prevent temperature shock. Fish may also be transported directly in the cooler. For longer journeys, it is best to provide supplemental oxygen during transport. Oxygen cylinders or portable aerators (Bait-Saver® [Save My Bait] or equivalent) can be used to provide oxygenation. Alternatively, small fish can be shipped in a sealed plastic aquarium bag that has an oxygen-enriched atmosphere. The **Fish Disease Diagnosis Form** (**appendix I**) provides details on various methods of shipment.

The ability to diagnose a problem is directly related to the quality of the samples submitted. Live fish that show typical clinical signs of the problem provide the best samples. Preserved material is least useful for most, but not all, diagnoses. Different methods of tissue storage are more useful for certain problems. Water samples also have a finite storage time (Table I-3).

Commercial Producers

While most individual pet fish cases are best submitted directly to the clinic, there is increasing justification to make on-site visits, especially as systems become more expensive and complex. It is even more common and often necessary to visit the facilities of commercial growers, such as pet fish breeders, retailers, wholesalers, or commercial food fish producers. A visit allows a more thorough evaluation of the facilities and management, which are often the root cause of a disease complaint. The procedures used for diagnostic workup are the same as for individual aquarium fish (see Fig. I-1).

Because more fish are usually involved in commercial producers' cases, more fish can be examined, which strengthens the diagnosis. Generally, at least four to six fish should be examined during epidemics. Live fish should be used whenever possible. The only exception is when all of the live fish appear healthy; in this case, the freshest dead fish should also be examined.

If fish are being certified for presence or absence of certain diseases, the number examined depends on the total population size, the prevalence of the disease to be surveyed, and the level of confidence desired (see "**Regulatory Issues: Reportable Diseases and Certification of Stocks**," **p. 73**)

Table I-3. Recommended sampling containers and storage procedures for water samples (compiled mainly from Langdon 1988, Hill 1983, and Boyd 1979). Suggested time intervals should be considered liberal estimates. Samples may be less stable under some conditions.

Variable	Container	Vol ml	Handling procedure	Analyze within
All	Clean or new			ASAP
Oxygen (via Winkler technique)	Glass-stoppered glass	300	Fill totally, 4°C in dark	6 hours
Temperature	N/A	N/A	N/A	Must do on site
pH	Polyethylene	100	4°C in dark	6 hours
Ammonia, nitrate, nitrite	Polyethylene or glass lab-washed (*NOT* HNO_3^- washed)	500	Acidify with 1 ml conc. H_2SO_4/L (to pH < 2.0); on ice or freeze	24 hours
Metals	Polyethylene, HNO_3^- washed	500	Acidify with analytical HNO_3^- to pH < 2.0; freeze if analysis delayed	24 hours
Pesticides, other organochemicals	Glass- or Teflon-stoppered glass, hexane-washed (*no* plastics)	500	Fill totally	24 hours
Solids (dissolved, suspended, settleable)	Glass or plastic	500	4°C	
Cyanides	Glass or plastic	100	Add 0.2 ml of 10 M NaOH, to pH 12	24 hours
Algae	Glass or plastic	100	Fresh chilled, or add Lugol's iodine to color of weak tea or 10% formalin 1:1	24 hours (fresh)
SUMMARY (agents unknown)	Polyethylene, HNO_3^- washed	500	Add HNO_3^- to pH < 2.0	24 hours
	Glass or plastic, ×2	500	Freeze one	24 hours
	Glass, hexane-washed	500	Fill totally	24 hours
	Glass, hexane-washed	500	Fill totally, 4°C in dark	6 hours

WATER-QUALITY ANALYSIS

Core Water-Quality Parameters

Core water-quality parameters are tests that should be run when any fish disease case is submitted. They include ammonia, nitrite, and pH (and salinity in a marine or brackish water system). Oxygen and temperature are also part of this core list, but to obtain an accurate measurement, the water must be measured on site (i.e., at the pond, aquarium); this can be done only if the clinician visits the site. There are chemical methods available to preserve a water sample for later measurement of oxygen, but this method is mainly used as a research tool. Thus, oxygen and temperature must usually be assessed from the history (i.e., the client has measured the oxygen or temperature on site with a meter; or, a problem with oxygen or temperature is discerned from the client interview).

While it is not always part of the core list, it is often advisable to measure alkalinity and hardness in commercial ponds and nitrate in aquaria (especially marine aquaria). Chloride should also be measured in commercial ponds when nitrite levels are high (see PROBLEM 5). All of these core water-quality factors are discussed in more detail in the problem list.

Special (Noncore) Water-Quality Sampling

Many other water-quality changes besides the core list can affect fish health (see PROBLEMS 90 through 96). While not routinely measured, some cases may warrant examining these other factors (see RULE-OUT DIAGNOSES [chapter 13] and Fig. I-1). Note that in some areas, certain water-quality factors may be part of the core list because they are a common problem (e.g., hydrogen sulfide in many parts of China). Specific recommendations for sample collection vary with the type of substance being measured and with how quickly the sample can be submitted (i.e., will preservative be added?). Also, different types of samples need to be collected in different types of sample containers (plastic, glass). After determining that certain measurements should be taken, the clinician should contact the laboratory where the samples are to be submitted to obtain specific information on methods of collection. The American Public Health Association (APHA 1992, 2005) also provides extensive details on water sampling.

Water-Quality Testing

Many manufacturers produce simple test kits for measuring core water-quality parameters (see above) and other water-quality variables. Most tests are based on adding a known amount of the water sample to a vial and then adding chemicals, which react with the substance to be measured, producing a colored reaction. The amount of substance present is proportional to the intensity of the color change. Most tests take less than 15 minutes to run. It is important to realize that special procedures are sometimes required to test substances in seawater; thus, while most kits for measurements in seawater are also usable for freshwater samples, the converse is not always true.

The accuracy of commercial water test kits is related to the cost of the kit. Inexpensive kits that use a color chart for measurement are available from aquarium wholesalers or retailers (e.g., Marine Enterprises). These water test kits are only semiquantitative but give a general indication of water quality and are often sensitive enough to diagnose most water-quality problems encountered in routine clinical cases. More expensive kits designed specifically for water-quality testing on commercial farms (e.g., FF-1A Kit [approximately $250]; FF-2 Kit [approximately $450]; Hach Company) are more accurate and acceptable for all routine diagnostic procedures; these also have the advantage of combining most routine tests into one kit. Even more sophisticated colorimetric kits use a spectrophotometer for measurements (e.g., Hach DREL 2000, approximately $4,000) and are usually accurate to within 20% of the so-called standard methods (Boyd 1979).

The most accurate methods for water-quality analyses are the standard methods. In the United States, most standard methods are developed and sanctioned under the auspices of either the American Public Health Association (APHA 1992, 2005) or the U.S. Environmental Protection Agency (USEPA 1979). Standard methods of analytical accuracy are not needed for clinical diagnoses unless a particular case may eventually involve litigation or is involved in certain research protocols. Samples taken for regulatory compliance monitoring or collected as evidence during enforcement investigations must also conform to well-defined procedures regarding sample handling, shipment, and chain-of-custody documentation. The clinician should refer to EPA guidelines or contact the appropriate environmental agency (e.g., USEPA or regional or state environmental agency) for assistance in collecting such samples.

If frequent visits to culture facilities are anticipated, it is also advisable to purchase a dissolved oxygen meter (e.g., YSI, about $1,500). Electronic probes are also available for measuring temperature, pH, ammonia, nitrite, chloride, and conductivity (salinity). The major advantage of electronic probes is that measurements can be taken quickly and accurately. However, probes are expensive, must be calibrated regularly, and are subject

to failure if they are not maintained properly. It is also desirable that probes withstand disinfection, reducing the potential transmission of disease. For example, YSI dissolved oxygen probes can be left in disinfectant indefinitely, including 70% ethanol, povidone iodine, quaternary ammonium, or just about any chemical that does not damage the housing (e.g., does not chemically react with the plastic housing; the probe itself is inert, being Teflon). Details of various water sampling devices are described with specific water-quality problems.

Water Samples Submitted to the Clinic

The water sample should be immediately examined for core water-quality parameters because changes can occur within a short time after collection (see Table I-3). If it cannot be examined immediately but will be examined within 1 hour, it should be left at room temperature. If it will not be examined for over an hour, it should be refrigerated but should be tested for ammonia, nitrite, and pH within 24 hours. The water should be allowed to come back to room temperature before doing any measurements.

Water Sampling on Site

Water samples may vary tremendously from one part of a culture system to the other. For example, oxygen and pH are highest, while carbon dioxide and ammonia are lowest, at the inflow of a flow-through system. The opposite is true at the outflow. Thus, flow-through systems should be sampled for oxygen, pH, and ammonia at both the inflow and the effluent.

Ponds should be sampled for dissolved oxygen and temperature at both the windward and leeward sides to account for wind-induced mixing (Boyd 1990). Samples should be taken at 0.5–1.0 m (1.5–3 feet) in waters less than 2.0 m (6 feet) deep. Both surface and water samples should be taken to assess variability. Different bodies of water can have markedly different water-quality characteristics, even with identical stocking densities, feeding rates, and so forth (Noga and Francis-Floyd 1991). Thus, each system should be treated as an individual unit in terms of water-quality sampling.

TAKING THE HISTORY

When ready to see the client, a thorough history should be taken (see **Fish Disease Diagnosis Form, Appendix I**) (Stoskopf 1988). It can be useful to try to determine whether the problem is acute or chronic, since this can help to eliminate some differentials (Fig. I-2). Acute problems are typically those that have developed within a matter of only a few days and have resulted in considerable morbidity and/or mortality within that time. Conversely, chronic problems typically develop over several weeks or more and may only result in an occasional mortality. Also, such fish are often in poor condition and may be anorexic.

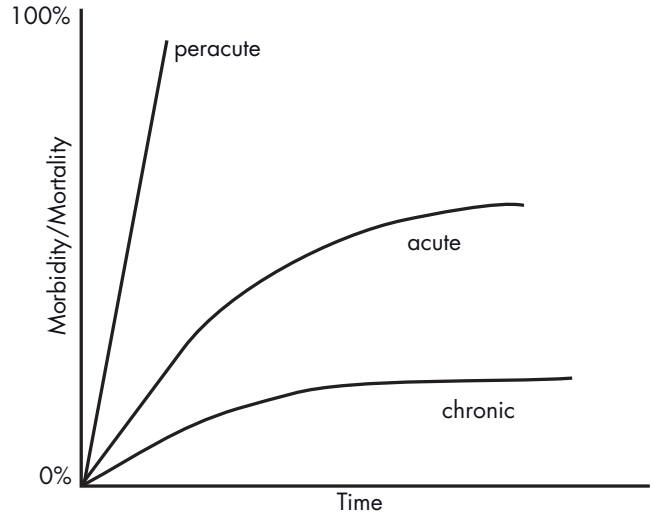


Fig. I-2. Typical morbidity and/or mortality rates with peracute, acute, and chronic disease.

Important questions to ask include the following:

- How long has the culture system (aquarium, pond, etc.) held fish?
- Are all fish affected?
- If not, which are not?
- Do the fish display any behavioral signs, such as *flashing* (rubbing against objects suggesting skin problems) or *piping* (staying near the air-water interface to obtain more oxygen, suggesting gill problems)?

Low oxygen is common; unfortunately, oxygen can only be accurately measured on site (i.e., at the pond), so the history may be crucial to assessment.

It is often best to ask the client to describe the usual routine for feeding, water changes, and other management procedures to discern an accurate history. It is also important to determine what prior treatments, including medications, have been given.

THE PHYSICAL EXAM

Humane Care of Clinical Cases

General guidelines on providing hu mane care to fish undergoing diagnosis or treatment is based upon the criteria mentioned in "**Animal Welfare**" (**p.** 77). These criteria should be reviewed before undertaking any clinical cases. Specific details of the humane issues mentioned in those guidelines are provided in appropriate sections of "**The Clinical Workup**."

Behavioral Examination

If a client submits live fish to the clinic, aeration should be immediately placed into the container that holds the fish. Once a thorough history has been taken, the fish should be closely examined for behavioral abnormalities. Note that behaviors seen in their natural setting are not always displayed when fish are removed from their normal environment.

Sick fish often congregate together, separating themselves from their healthier cohorts (Fig. I-3, A). Weak fish in raceways or other systems with flowing water will often be found near the water outlet. Different fish species inhabit different parts of the water column (surface, bottom, shoreline, etc.), and this position often changes with sickness. Extremely sick fish may be in dorsal or lateral recumbency. Sick fish may also exhibit other behavioral signs, including staying near the surface of the water because of hypoxia (e.g., PROBLEM 1), scraping the body or holding the fins close to the body ("clamped") because of parasite irritation, or showing various behavioral abnormalities because of nervous system involvement (e.g., PROBLEM 77). Increased ventilation (indicated by wider opening and faster opening and closing of the opercula) suggests gill pathology or a respiratory poison (Francis-Floyd 1988).

External Lesions

Color Change

The melanin pigmentation in fish's skin is under neuroendocrine control and is thus affected by hormones, such as epinephrine. When fish are sick, maintenance of a normal pigmentation pattern presumably takes less precedence than homeostasis of more vital body functions. Thus, sick fish are often abnormally colored, compared with the healthier cohorts. This is a common response of salmonids to disease, with sick fish being typically darker than normal. A color change can also be caused by blindness, which eliminates the normal visual cues that are needed to maintain a normal color pattern in daylight (Fig. I-3, A). Fish in breeding condition often have more brilliant colors than nonbreeding fish (Axelrod et al. 1980). Since the chemical signals that control pigmentation are transmitted via the nerves, peripheral nerve damage, such as from vertebral instability, can cause a focal change in pigmentation pattern (see PROBLEM 68). Focal color change can also be caused by local tissue irritation/damage, such as parasite feeding, chronic wounds, or healing wounds, which cause a change in the pigment cell distribution at that site (see PROBLEMS 55 and 58).

Reddening of the body is usually caused by hemorrhage, which can result from systemic bacterial or viral infections (see PROBLEMS 45 and 77) or skin wounds (e.g., ulcers). Parasites or other irritating conditions may also elicit a thickening of the skin, leading to a whitish or bluish skin color. This change might be highly localized (pinpoint to larger foci) or cover nearly the entire body (see PROBLEM 20 and Fig. I-3, G). See "**Evaluation of Skin Biopsies**," **p. 26**, for a further discussion of gross lesions affecting the skin. Observations of color pattern are best made while the fish is in its culture system, since the pattern can also be affected by acute stress (e.g., confinement, transport).

Other Common Gross Signs of Disease

Loss of fin tissue, resulting in eroded or irregular fins, most often results from poor water quality (see PROBLEM 37). However, be aware that acute confinement can quickly lead to iatrogenic skin erosion and ulceration (PROBLEM 97). Thus, one must be certain that such changes were not caused by the acute stress of capturing and transporting the fish.

Trauma to the eyes or mouth is often present in large fish in aquaria or in any fish that exhibit a strong pressing behavior against the sides of a tank (e.g., pelagic fish such as Atlantic menhaden that are kept in aquaria with corners rather than round aquaria). Masses on the body may be due to parasite cysts or neoplasia.

Abdominal swelling (Fig. I-3, B) is most commonly caused by an infectious peritonitis (viral, bacterial, or parasitic) but can also be caused by a metabolic disturbance (e.g., renal failure), neoplasia, obesity, or egg retention ("egg bound"). This clinical sign is often referred to as dropsy in the aquarium literature and may

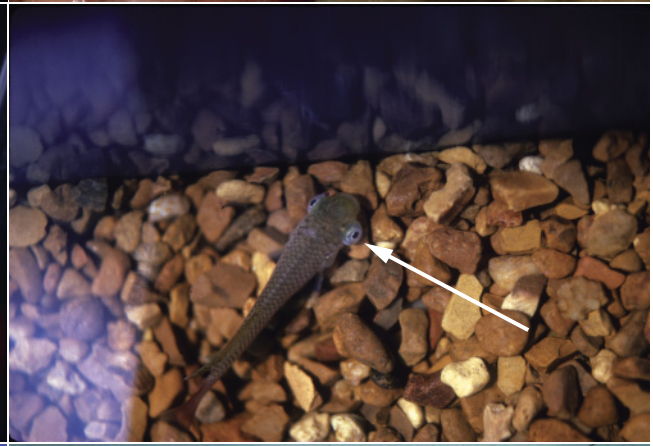
Fig. I-3. Common gross signs of disease in fish. A. Salmonids congregating near the outlet screen of a raceway. In this case the dark color is caused by blindness. But color change is a general indicator of ill health. The segregation of these fish away from the rest of the fish population is also characteristic of sick fish. B. Massive swelling in a channel catfish caused by fluid accumulation in the peritoneal cavity. C. Abdominal swelling in a goldfish. Note that the scales are also protruding. D. Exophthalmos (*arrow*) in a killifish. E. Exophthalmos (*arrow*) in juvenile flounder. F. Spinal curvature, including scoliosis (*lateral curvature*) and lordosis (*forward curvature*). G. Severe epidermal thickening, as indicated by white patches on the skin (*circle*) due to ectoparasite (*Trichodina*) infestation of a largemouth bass. Note that this is an extreme example and much milder white foci are usually observed. (*A* photograph courtesy of C.L. Davis, Foundation for Veterinary Pathology; *B* and *D* photographs courtesy of T. Wenzel; *E* photograph by S. Wada and E. Noga; *F* photograph courtesy of A. Mitchell; *G* photograph by M.-D. Huh, P. Udomkusonsri, and E. Noga.)

A

B

C

D



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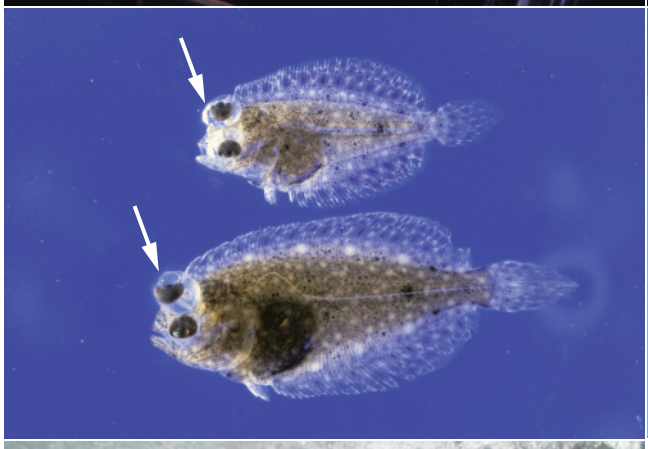

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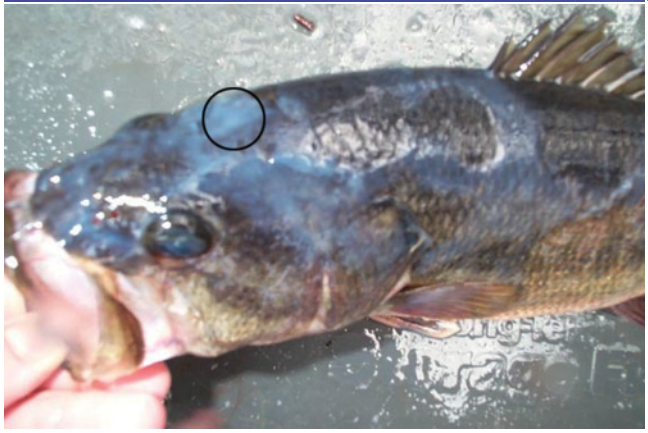
G



also include protrusion of the scales (Fig. I-3, C). Abdominal swelling may also be a normal sign of sexual maturity in female fish that are ready to spawn. Overinflation of the swim bladder is common in fancy goldfish and may cause an inability to remain upright.

Chronically ill fish are often emaciated. This is evident by loss of dorsal (back) muscle, a concave abdomen, and enophthalmos. Eye lesions, such as exophthalmos (Fig. I-3, D, E) are common in several infectious diseases, including several viral and bacterial infections. Unilateral eye lesions often indicate a possible traumatic cause, especially in large fish. Many nutritional deficiencies are also associated with ocular pathology.

Skeletal deformities (Fig. I-3, F), especially of the vertebral column, can have many causes, including hereditary factors, defective embryonic development, unsuitable water temperature, salinity fluctuation, environmental hypoxia, x-irradiation, ultraviolet radiation, ascorbate deficiency, parasitic infection, electric current, and certain toxins (Bengtsson 1975).

Gills may also exhibit gross lesions. Examining gills is most easily done when taking biopsies. See "**Gill Biopsy**," **p. 28**, and "**Common Lesions Found in the Viscera**," **p. 60**, for other gross signs of disease.

Dangerous Fish

It is rare that a practitioner is in any physical danger from performing a clinical workup. However, one should be aware that a few species are dangerous. Surgeonfish have a scalpel-like scale on the caudal peduncle, but it is nonvenomous. The greatest potential for harm is from venomous species. The most dangerous catfish are members of the freshwater family Clariidae (Asian stinging catfish and related species), the marine family Plotosidae (saltwater catfish and relatives), and the family Ariidae (both freshwater and marine species) that have venom associated with their sharp dorsal and pectoral spines or can inflict a locally painful wound. Some members of the freshwater family Ictaluridae (madtoms) are also venomous. Rabbitfish (Siganidae) fins have venom glands; although not life-threatening, envenomation can be very painful.

Of more serious concern are members of the family Scorpaenidae (scorpionfish, lionfish, stonefish, and weaverfish), which can inflict painful wounds that, depending on the species and severity, may require medical attention. The most commonly maintained members of this group are the lionfish, which have numerous specialized fin spines capable of delivering venom. The most dangerous scorpionfish is the stonefish, which has a dangerous and rapidly lethal toxin. Fortunately, it is rarely seen in hobbyists' tanks. Some fish can inflict painful bites if they are not handled carefully, such as moray eels, anguillid eels, large triggerfish, large pufferfish, or sharks. Some freshwater fish can produce a powerful electrical current. The electric catfish can produce a mild jolt, but the electric eel can produce a powerful surge. Freshwater and marine stingrays (family Dasyatidae and family Pomatotrygonidae) have barbs on their tail fins that can be whipped into an unsuspecting handler. The cownose ray and the southern eagle ray (family Myliobatidae) have a venomous spine at the base of the tail. For treating envenomations, see Meier and White (1995), Auerbach (1996), and Williamson et al. (1996). Nonvenomous spines can also be painful and can inoculate human-pathogenic bacteria.

CLINICAL TECHNIQUES: ROUTINE METHODS

Skin and Gill Biopsies

Preparing Fish for Biopsy

Latex gloves should be worn when handling fish for disease diagnosis. Fish skin is not keratinized and thus is susceptible to iatrogenic damage. A dry paper towel should never be used to grab a fish for biopsy! Latex or nitrile gloves are soft and slippery when wet, reducing possible skin damage and preventing the loss of surface-dwelling parasites when handling the fish. Also, some zoonotic pathogens can be contracted by handling infected fish (see PROBLEMS 46, 49, 50, and 55). If disposable gloves are coated with talc, gloved hands should be rinsed in water before handling the fish to prevent talc crystals from contaminating biopsies (see Fig. II-43, C) and possibly irritating skin and gills (C. Harms, personal communication).

After the visual examination, the skin and gills should be biopsied to look for pathogens. Skin biopsies usually can be taken from any fish larger than 25 mm (1 inch), and gill biopsies usually can be taken from any fish larger than 50 mm (2 inches). These techniques are valuable because many of the diseases that affect fish are confined to the skin or gills.

Sedation/Anesthesia

For a cursory physical exam, teleost fish can often be examined without sedation by gently handling them while they are under water. Some fish can be examined briefly (up to about a minute) while out of water. Careful handling may also allow the stripping of sperm and eggs or the administration of an injection. Nonsedated handling is improved by covering the eyes and minimizing noise (Ross 2001). Sharks and other elasmobranchs can often be successfully restrained without drugs via a process called tonic immobility (see "**Pharmacopoeia**").

For other procedures, sedation or anesthesia is usually needed. The same drugs can be used for both sedation and anesthesia in fish. The only difference between sedation and anesthesia is the dosage of drug and/or the

length of time that the fish is exposed. Since most of these drugs are administered through the water, the dosage is directly proportional to both the amount of drug in the water and how long the fish has been left in the solution.

Whereas sedation is routinely used to clinically examine fish, it has the potential to compromise the diagnosis of skin and gill pathogens. Sedation or anesthesia may cause some loosely attached ectoparasites, such as monogeneans or leeches, to detach from the fish (Noga et al. 1990a; Svendsen and Haug 1991). In some cases, this effect can be quite dramatic (Fig. I-5). Thus, anesthetic use might interfere with diagnosis of these problems by biasing the number of organisms observed on wet mounts. However, the importance of parasite narcotization on making a clinical diagnosis has not been well studied. With practice, many fish can be biopsied without sedation, although this is less humane. If the fish can be euthanized, pithing or cervical severance can be used for immobilization rather than chemical overdose (see "**Pharmacopoeia**"); this is the only alternative if one wants to be absolutely certain that the sampling has not been compromised.

For biopsy, a portion of the water used to transport the fish is placed into an aquarium bag or other suitable container and a small amount of anesthetic (and buffer, if necessary) is added (see "**Pharmacopoeia**" for types of anesthetics available) (Fig. I-4). The fish is then placed in the anesthetic bath and watched carefully. The "**Pharmacopoeia**" provides a range of doses that have been used for various fish species. Response to a given dosage varies considerably, depending on fish species and environmental conditions. When these drugs are used on a fish species with unknown susceptibility, start with the lower recommended dose and gradually add more if needed, until the desired effect is reached.

Fish exhibit planes of anesthesia that are similar to mammals (Table I-6). The first response is excitation; some fish, such as eels, may struggle violently during this stage and may attempt to escape. Other fish species may exhibit a similar response, but individual animals vary greatly in their response. The container that holds such fish should be well covered. After excitation the fish becomes depressed (less response to touch), loses equilibrium (lies increasingly on its side), and ventilation slows (the opening and closing of the gill covers becomes slower and weaker). If the fish is left in the anesthetic bath long enough, breathing will stop. Fish should not be left in anesthetic long enough to stop breathing; however, many fish will recover even after breathing has stopped for several minutes.

If the proper amount of anesthetic is added, the fish should be immobilized in less than 5 minutes. If the fish remains alert after this time, a bit more anesthetic should be added. Once the fish has ceased to struggle and can be handled, a fin clip should be taken with fine forceps and a skin scrape should be taken with a scalpel. These biopsies should be placed immediately on a slide with a drop of aquarium water, a coverslip should be added, and then the specimen may be examined. A gill biopsy should then be taken, using fine scissors.

Anesthesia often causes involuntary defecation, allowing the collection of a fecal sample (see "**Fecal Exam**," **p. 28**). It is often advisable to weigh the fish while it is sedated to determine body condition, or if the clinician anticipates administration of a medication that is based upon body weight (e.g., injection or medicated feed).

Using the Microscope

Next to water testing, examining tissues by wet mount is the most informative technique in fish disease diagnosis. In fact, the majority of fish disease cases can be diagnosed by using just the water-quality tests outlined and by an examination of skin and gill wet mounts.

The microscope used for diagnosis should have a range of objectives, including at least 10X, 40X (low and high dry), and 100X (oil immersion, high power). A low-power (4X) objective is also useful for rapidly scanning a sample. Close down the iris diaphragm to exclude much of the light and increase contrast. Alternatively, if the microscope has a movable condenser, lower it to increase contrast. A small portable microscope (e.g., Swift Model FM31, Swift Optical Instruments) can be used to evaluate biopsies in the field. When wet mounts are examined, it is important to determine the size of various objects in the microscope's field because the proper identification of a parasite or other organism is much easier when its size is known. The most accurate

Table I-6. Stages of anesthesia (modified from Summerfelt and Smith 1990).

Stage	Plane	Description	Signs
I	1	Light sedation	Responsive; decreased movement and ventilation
	2	Deep sedation	As above; some analgesia; responsive to strong stimulation
II	1	Light anesthesia	Partial loss of equilibrium; good analgesia
	2	Deeper anesthesia	Total loss of muscle tone and equilibrium; greatly reduced ventilation
III		Surgical anesthesia	As above; total loss or reaction to even very strong stimulation
IV		Medullary collapse	Ventilation ceases; cardiac arrest, eventual death; overdose

Fig. I-4. Method for sedating fish before a clinical procedure. A. A simple device for providing aeration during a clinical visit. An electrical air pump is attached to a gang valve having five outlets, so that up to five containers can be aerated at once. B. Adding some water from the container in which the fish was submitted to another container to be used for sedation. C. Mixing the tranquilizer/anesthetic. D. The fish is responding to sedation (losing balance). E. The fish is being removed for a clinical procedure. F. The fish is placed in aerated water after completion of the clinical procedure.

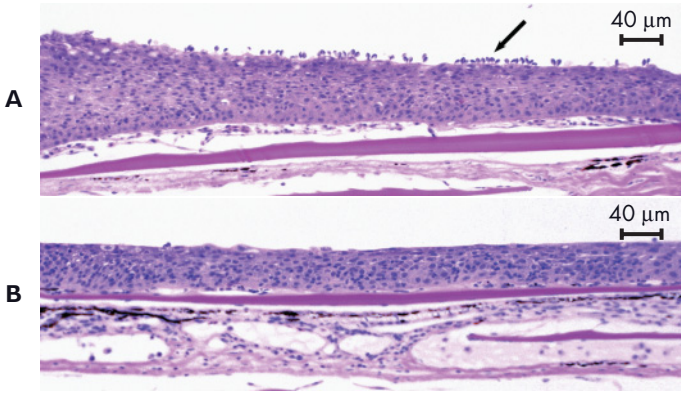


Fig. I-5. The effect of unbuffered tricaine on the ability to detect protozoan ectoparasites. A. Histological section of skin from a hybrid striped bass that was euthanized in tricaine (200 mg/l) buffered with 400 mg/l sodium bicarbonate. Note the large number of parasites present (*arrow*). B. Histological section of skin from a hybrid striped bass that was euthanized in unbuffered tricaine (200 mg/l). Note the total absence of parasites. (*A* and *B* photographs from Callahan and Noga 2002.)

way to measure an object's size is to use an ocular micrometer. This micrometer is placed into the eyepiece of the microscope and can then be superimposed over the organism in question to measure its size. Note that an ocular micrometer must first be calibrated with a stage micrometer to determine an accurate measurement. Another way to measure the size of an object is to compare it to the size of a red blood cell (RBC) in the same field. Fish RBCs usually range from about 6 to 9 µm on the long axis. They can be identified on a wet mount by their platter-shaped or fried-egg appearance (see Fig. I-12). Because the RBCs are fairly consistent in size, they can be used to estimate the dimensions of an object. Latex beads can also be used for size estimation.

Biopsy Procedures: Preparing Slides

Immediately before performing any biopsies, a drop of water (seawater, if it is a marine fish) is added to a slide for every biopsy that is to be performed on the fish. Water from the container that holds the fish can be used (Fig. I-6, A). One of the quickest ways to transfer the water is to dip the tip of your finger into it and then touch your finger to the slide. This will leave a small drop of water on the slide. A pipet can also be used. The biopsy should be placed immediately in the water drop to prevent any organisms in the sample from drying out and thus dying.

Skin Biopsy

Skin biopsy is the single most useful tool available for diagnosing diseases in fish because the skin is a primary target organ for a number of common infectious agents. The skin of fish has layers analogous to those present in mammals, including the hypodermis, dermis, basement membrane, epidermis, and cuticle (Fig. I-7). The dermis contains pigment cells and the scales, which are embedded in connective tissue and overlap one another like shingles on a roof. Some species, such as catfish, lack scales, while others, such as eels, have small scales. Covering the scales is the epidermis, a stratified squamous epithelium with goblet (mucus-producing) cells. The epidermis is covered by the cuticle, a thin layer of mucus that contains sloughed epithelial cells and many protective substances, such as antibody, lysozyme, and C-reactive protein (Alexander and Ingram 1992). In almost all fish, the epithelium is not keratinized, and living cells are present in all layers. This makes fish skin susceptible to both acute and chronic injury.

The skin performs the following three functions in all fish: (1) it reduces drag by providing a smooth friction-free surface for locomotion; (2) it acts as a first line of defense against the invasion of infectious agents; and (3) it makes an impermeable barrier to the movement of fluids and salts. In some species (e.g., eels, catfish), it also acts as an accessory site for respiration. Its critical importance in maintaining internal homeostasis is a major reason why skin damage, exclusive of other organ involvement, can kill fish.

Skin Biopsy Procedures: Scraping

Two major methods are used to obtain skin biopsies: skin scraping and fin clipping. Skin scraping is performed by taking a spatula or a scalpel and gently scraping along the side of the body or fins while the fish is adequately restrained (Fig. I-6, B through G). Alternatively, a plastic coverslip can be used for scraping. Lightly sedated fish can usually be prevented from struggling by enclosing the body with a loosely clasped hand. Avoid damaging the skin when performing any procedures by not exposing the fish to dry or rough surfaces. For example, fish should not be held with paper towels, even if the towels are moistened. This rough surface can easily remove the cuticle. Latex or nitrile gloves moistened with water are especially good for handling fish.

Only gentle pressure is necessary when taking a scraping because most pathogens are found near the surface. Much less pressure is required than that used in performing skin scrapings of mammals. Overzealous sampling does more harm than good. Even light scrapings usually remove the epidermis and dermis from small fish (see Figs. I-6, C, and I-7, A). Large areas of skin should not be scraped because the resulting open wound may become secondarily infected or cause serious fluid imbalance. Scraping should be done in a cranial to caudal direction. A satisfactory scraping should always have mucus and numerous epithelial cells. In smaller fish, a

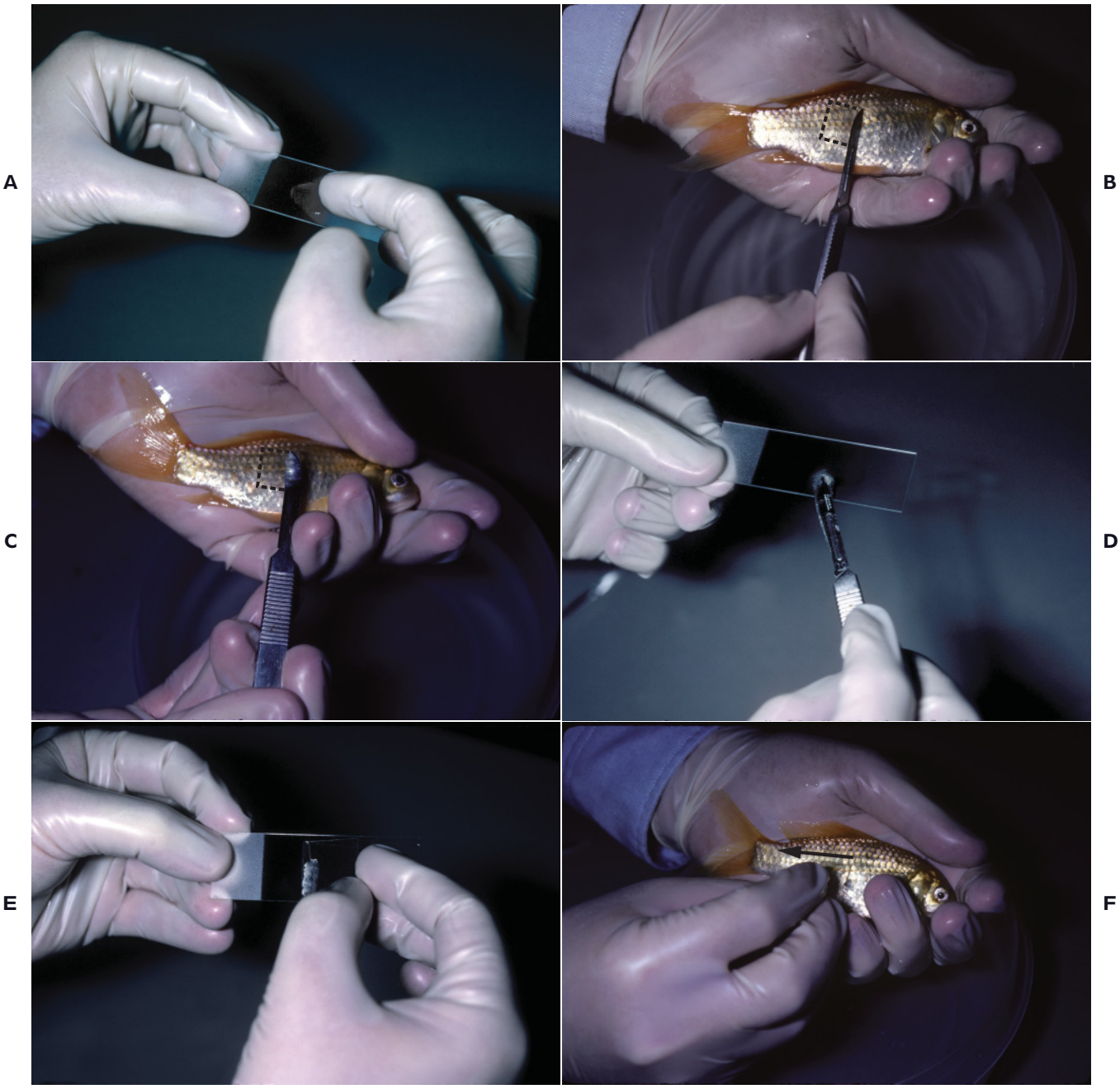


Fig. I-6. The skin scraping. A. Adding a drop of water to a slide before performing the biopsy. Dip a finger in water, then touch the finger to the slide. B. Scraping the skin with a scalpel to obtain a biopsy sample. Note that the back side of the blade is used for scraping. Only a relatively small area (*dashed line*) should be scraped. C. Biopsy material on the scalpel blade. Note that scales (flat, refractile) have been included in the biopsy, indicating that the entire epithelial layer has been removed. D. Scraping the biopsy material onto the slide. E. Covering the sample with a coverslip. F. Using a coverslip to make a skin scraping. While it is easiest to prepare a tissue squash with a plastic coverslip (unbreakable), a glass coverslip is more rigid; however, it is also more prone to breaking. The arrow points in the direction of the scraping.

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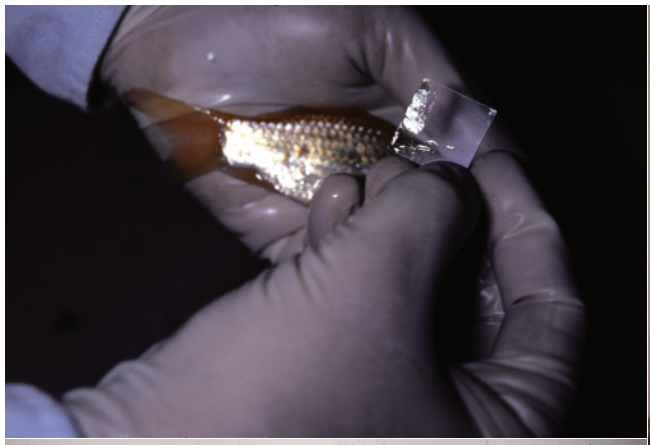

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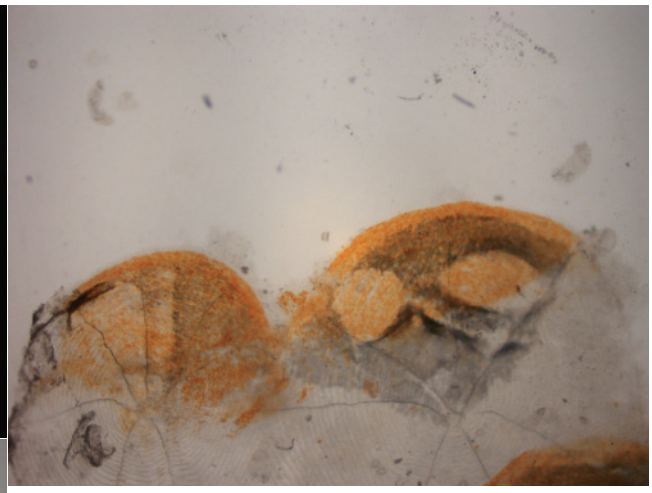

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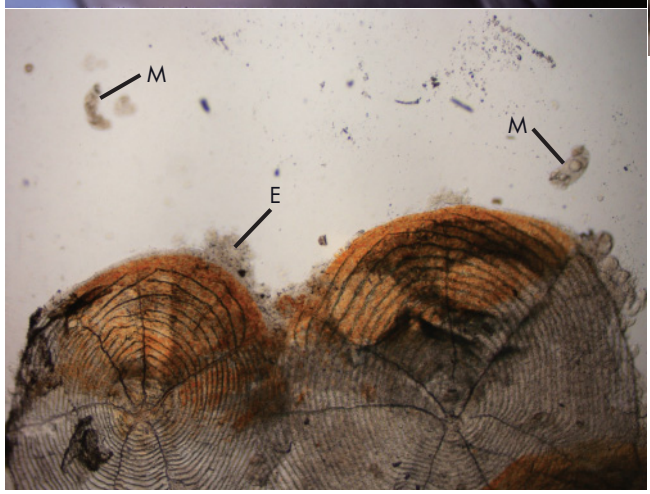

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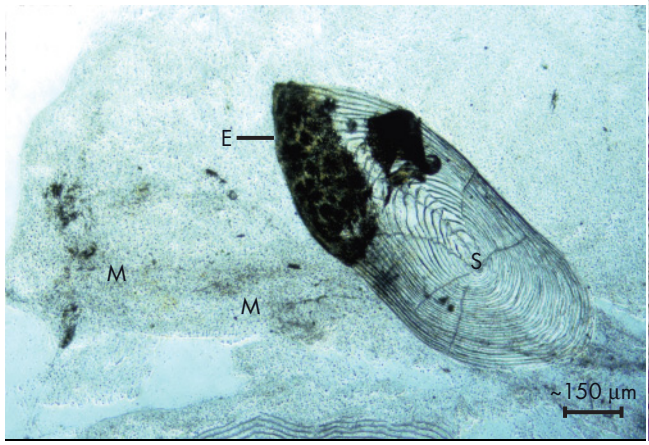
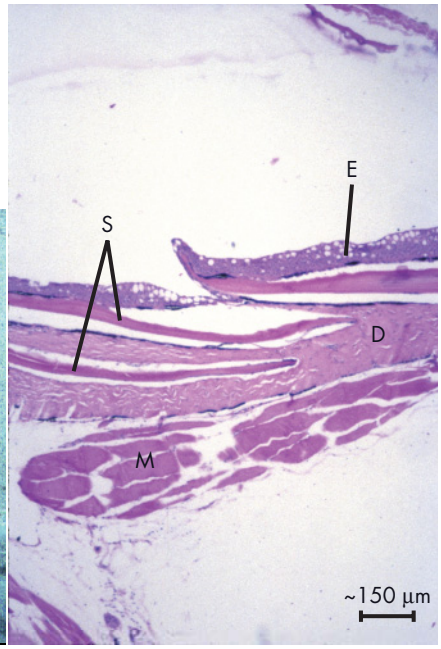
Fig. I-6.—cont'd. G. Biopsy material, including scales, on the coverslip. H. Microscopic view of a biopsy, with the microscope's condenser wide open. Note the lack of contrast compared to that in Fig. I-6, I. I. Same view as Fig. H, with condenser closed. Structures are much more visible, such as the epithelium (*E*), monogenean parasites (*M*) and scales.

A





B

Fig. I-7. A. Low-power photomicrograph of a scraping from normal skin of a black-pigmented fish showing a scale (*S*), dark epithelium (*E*) covering the posterior part of the scale and mucus, and epithelium (*M*) scraped from the skin. B. Histological section of normal skin. The space between the scale and dermis is an artifact caused by shrinkage during histological preparation. *S* = scale; *E* = epithelium; *D* = dermis; *M* = muscle. Hematoxylin and eosin.

few scales can be present, as well as a few blood cells. However, large amounts of scale removal and extensive hemorrhage should be avoided.

Scrapings should be taken where obvious lesions are present. The smaller wounds should be examined carefully since older lesions are often overgrown by opportunistic bacteria (e.g., *Aeromonas hydrophila;* see PROBLEM 46) or water molds (see PROBLEM 34). The leading edge of a lesion should always be examined because this area is most likely to harbor the responsible pathogen(s). To determine the initiating etiological agent may require sampling sites other than obvious lesions to discover which pathogens are present and also examining other fish in the same group. When lesions are absent, scrapings behind the pectoral or pelvic fins may detect parasites since these areas shelter the parasites from shearing water flow over the fish (C. Harms, personal communication). When pathogens are not detected by wet mount, bacterial culture of lesions is warranted.

The scraping should be immediately transferred to a glass slide, applying a drop of water (seawater, if a marine fish) and a plastic coverslip. Plastic coverslips are preferred to glass, since they are inexpensive and are less easily broken when crushing wet mounts from viscera. If a plastic coverslip has been used for scraping, it can simply be inverted and placed on the drop of water.

The wet mount of the skin scrape (and all other skin and gill biopsies) should be examined immediately, because many parasites, especially the protozoa, will die soon after being removed from their hosts. Most parasites are difficult to identify when dead. It can be helpful to apply a drop of methylcellulose solution (Carolina Biological Supply Company) to slow the movement of protozoa, but this is almost never needed for identification of parasites.

Hyphae (of water molds and true fungi), granulomas, and most protozoa are visible at low (40X–100X) magnification under the microscope. The definitive identification of protozoa and bacteria usually requires high dry magnification (400X) and sometimes oil immersion (1,000X).

Skin Biopsy Procedures: Fin Clip

To fin clip (Fig. I-8), simply snip a small piece of one of the fins (the tail fin is usually the easiest) and prepare it as described for the skin scraping. This procedure is less traumatic than skin scraping because a cleaner and much smaller wound is produced; however, it is usually not as useful as a skin scraping. It may be difficult to see small pathogens such as *Ichthyobodo,* because the thick, hard fin rays prevent the preparation of a thin smear. The thinner parts of the biopsy should be searched.

Evaluation of Skin Biopsies

Like all organ systems, the skin has a characteristic repertoire of reactions to injury. These can include hemorrhage, erosion, and ulceration (Fig. I-9, A and B). Fin ulceration (often termed fin erosion or fin rot) is actually a gangrenous loss of tissue. It usually presents as a progressive necrosis starting at the tip of the fin. The leading edge of the lesion is often hyperemic or hemorrhagic. The necrotic tissue loses its normal color and often becomes white. Fragments of the fin rays may remain after the epithelium has sloughed, leaving a ragged appearance to the fin. Proliferation of epithelium may also occur concurrently with the progressive necrosis.

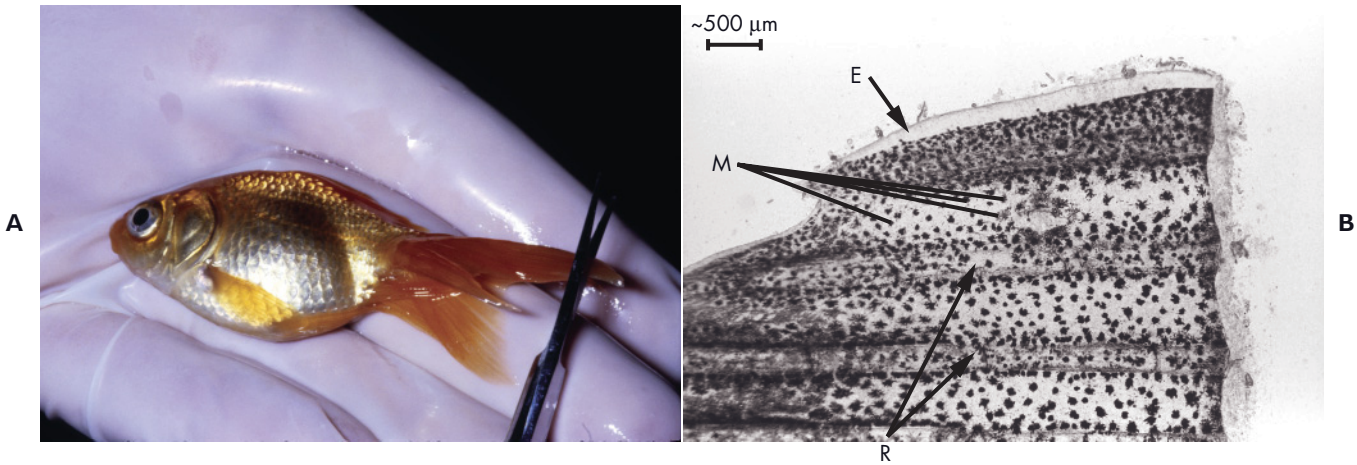


Fig. I-8. The fin clip. A. Clipping the fin. B. Microscopic features in normal, black-pigmented fin, including fin rays (*R*) and epithelium (*E*), which covers the entire fin but is most easily seen on the edge of the fin. Numerous melanocytes (*M*) are also present. The cut edge of the fin is on the right.

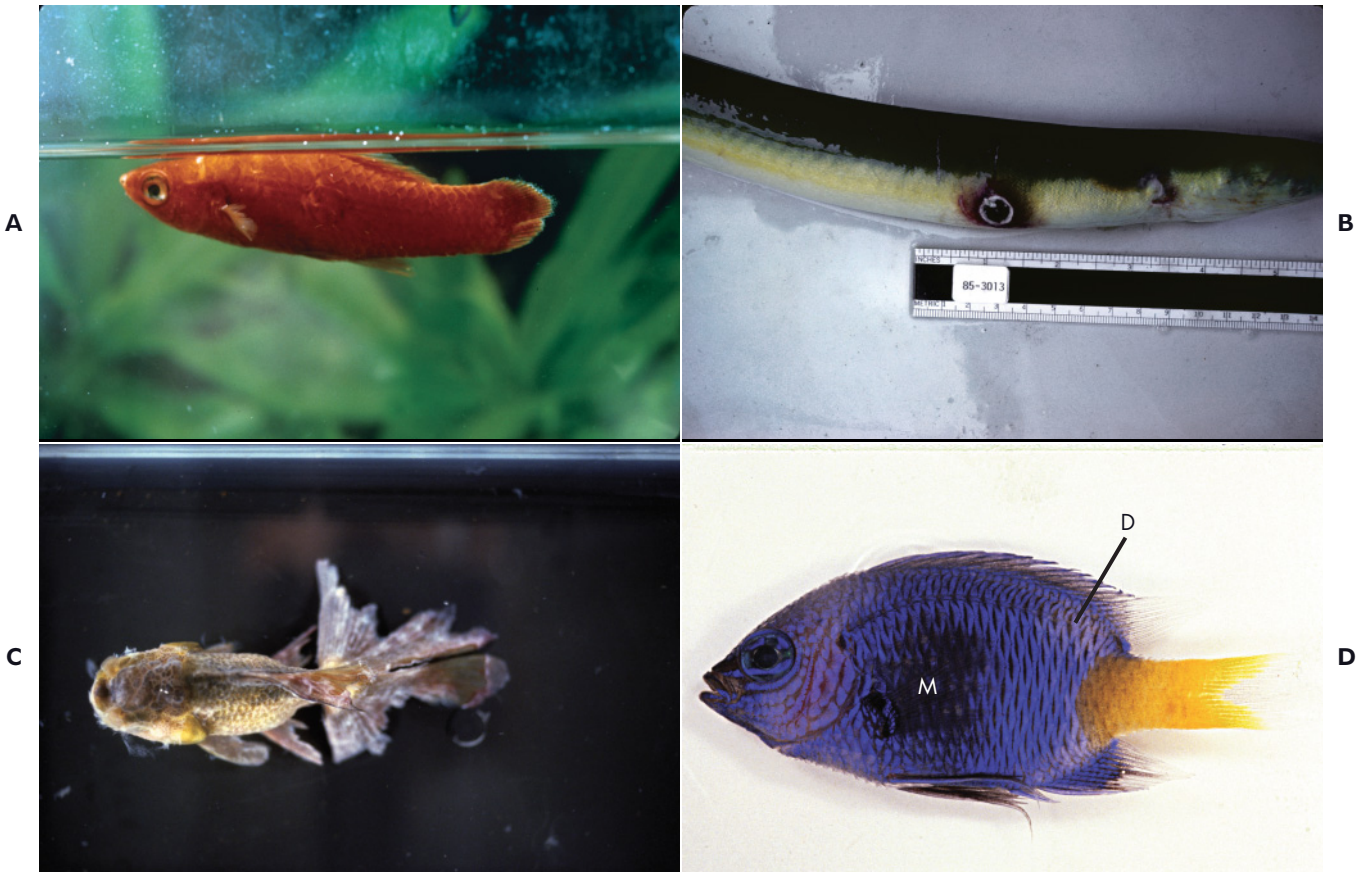


Fig. I-9. Common responses of the skin to damage. All of these responses are nonspecific and are thus only suggestive of certain problems. A. Caudal fin erosion and ulceration (fin rot). B. Skin ulcer. Note the hemorrhage around the ulcer. C. Cloudy appearance of the skin, with white flecks of detaching tissue; this may be due to epithelial hyperplasia and/or increased mucus production. D. Depigmentation (*D*) and melanization (*M*).

Another common response of the skin is hyperactivity of epithelium and goblet cells, which results in a thickening of the epithelium or increased mucus production that can give a cloudy appearance to the skin (Fig. I-3, G and Fig. I-9, C). Also, because the epidermis is not vascularized in small fish, there can be extensive epidermal damage without any bleeding. This may appear as depigmentation (Fig. I-9, D).

Numerous ectoparasites, bacteria, and other agents can elicit these responses and often act together to produce lesions. Thus, the diagnosis of skin lesions can be complicated by the presence of several agents. Most ectoparasites can be present in low numbers on fish without causing disease. For example, clinically healthy channel catfish frequently have one or two trichodinids per low power field (MacMillan 1985). Even such virulent pathogens as *Ichthyophthirius* (see PROBLEM 20) and *Amyloodinium* (see PROBLEM 27) can be carried asymptomatically. Conversely, heavy ectoparasitemia may be associated with systemic bacterial infections or other debilitating conditions. Thus, their significance depends on their concentration relative to other clinical findings.

It is important to determine the agent responsible for initiating a skin lesion to provide proper treatment. For example, water molds can colonize open skin wounds, and chronic ulcers often have many bacteria, especially motile rods, regardless of their primary etiology. However, even opportunists can kill fish, so treatment of secondary infections also is often advisable.

Many systemic diseases can have dermatological manifestations, although the etiological agents will often not be detectable in skin lesions. Reddening of the fins and body (caused by congestion or hemorrhage) can be caused by Gram-negative bacteremias/septicemias, virus infections, or stressful environmental conditions (Smith and Ramos 1976). Fish with mycobacteriosis (see PROBLEM 55) often have fin ulceration and faded coloration (Fig. I-9, D) (Reichenbach-Klinke 1973).

Acute skin loss, such as that caused by acute confinement stress (PROBLEM 97) or aggressive tankmates

(see PROBLEM 98), may mimic an infectious fin ulceration. Trauma is more likely to affect the more submissive members of a tank. Infectious agents are not present in purely traumatic lesions, although these may become secondarily infected.

Abnormal pigmentation may arise because of metacercarial infections (see PROBLEM 58) or other inflammatory lesions (e.g., *Ichthyophonus;* see PROBLEM 71) or *Mycobacterium* (see PROBLEM 55), or it may be a healing response to injury (Fig. I-9, D). Chronic inflammatory lesions often have large numbers of melanin-containing cells, including normal pigment cells (melanocytes) and inflammatory cells (melanomacrophages). These lesions should not be mistaken for melanotic cancers, which are much less common in fish.

Gill Biopsy

Gill biopsy is a useful diagnostic tool in fish medicine. Many infectious agents that affect the skin can also infect the gills. Like the skin, the gill is a multifunctional organ; it is the major respiratory organ, is the primary site of nitrogenous waste excretion, and plays an important role in ionic balance. The complexity of the gill is reflected in its anatomical structure. Each gill arch has rows of macroscopically visible finger-like processes—the primary lamellae (Fig. I-10). Each primary lamella has rows of microscopic secondary lamellae. A capillary-like network of vessels in the secondary lamellae moves blood countercurrent to the water flow, facilitating gas exchange and nitrogenous waste excretion.

Gill Biopsy Procedure

Immediately before biopsy the gills should be examined grossly. Healthy gills are bright red. Pale pink gills suggest anemia, while pale tan gills suggest methemoglobin formation (see PROBLEM 5). Do not confuse anemia with postmortem change (gills might quickly become pale pink after death because of passive drainage of blood from the gills). Debris may sometimes be lodged in the gill, especially if the fish has been lying in sediment. This material is easily washed away by gentle rinsing and should not be confused with gill necrosis. Because the thymus is grossly visible in the gill chamber, it can also be evaluated at the same time. It should be glistening white (see Fig. I-44). Thymic hemorrhage has been associated with stress in salmonids (Goede and Barton 1990).

Gill biopsy (Figs. I-11 and I-12*)* is performed by inserting the tip of a pair of fine (e.g., iridectomy) scissors into the gill chamber. The scissors are then gently opened, lifting the operculum until the gill arches can be seen. The tips of several primary lamellae are then cut and transferred to a slide; a coverslip is then applied. Only the tips of the lamellae should be cut; bleeding should be minimal. However, if done in front of a client, they should be forewarned that some bleeding will occur.

Evaluation of Gill Biopsies

The most common response of the gill to damage is hyperplasia and hypertrophy of epithelial cells, which can eventually lead to fusion of adjacent secondary or even primary lamellae. This severely reduces gas exchange at the lesion site and can lead to respiratory distress. This can occur because of injury from bacteria or parasites or from poor water quality. Hyperplasia and hypertrophy can result from the feeding activity of protozoa such as *Trichodina* (see PROBLEM 22), *Chilodonella* (see PROBLEM 23), or *Ichthyobodo* (see PROBLEM 29) (Wootten 1989). Some parasites, such as *Ichthyophthirius* (see PROBLEM 20) and *Amyloodinium* (see PROBLEM 27), induce focal hyperplasia at their attachment sites (see Fig. II-20, D). Some bacterial pathogens produce substances that stimulate epithelial proliferation. Epithelial hyperplasia and lamellar fusion have also been documented in vitamin deficiencies (see PROBLEM 89). Changes in gill structure are most easily recognized in histological sections, but if gill hyperplasia is detected on a wet mount it indicates that serious damage is present (Fig. I-13, A and B).

As on the skin, many pathogens may be present in low numbers on the gill without causing clinical disease; thus, interpretation of their significance depends upon other clinical findings.

A common sequela of gill infections is telangiectasis, or the dilatation of groups of small blood vessels on the secondary lamellae (Fig. I-13, C). This condition can also result from a number of environmental toxins. Telangiectasis can also be iatrogenically induced in some fish by cranial concussion (Herman and Meade 1985) or improper gill biopsy procedure (dull scissors) (L. Khoo, personal communication). Frank necrosis of gill tissue (gill rot) is characterized by the destruction of secondary lamellae and, in severe cases, the stripping of gill tissue down to the cartilaginous skeleton of the primary lamellae. It can be caused by pigmented bacteria and various toxins.

Because the gill is highly vascularized, lamellar biopsy can also be used to examine the blood in fish that are too small to be bled by conventional means (Fig. I-12), allowing the detection of hemoparasites or other pathogens.

Fecal Exam

A fecal exam may identify helminth ova (especially nematodes and also digeneans) and some protozoans (e.g., diplomonad flagellates). Fecal material can be obtained by siphoning debris from the bottom of the tank. This is not stressful to the fish; however, it is least sensitive

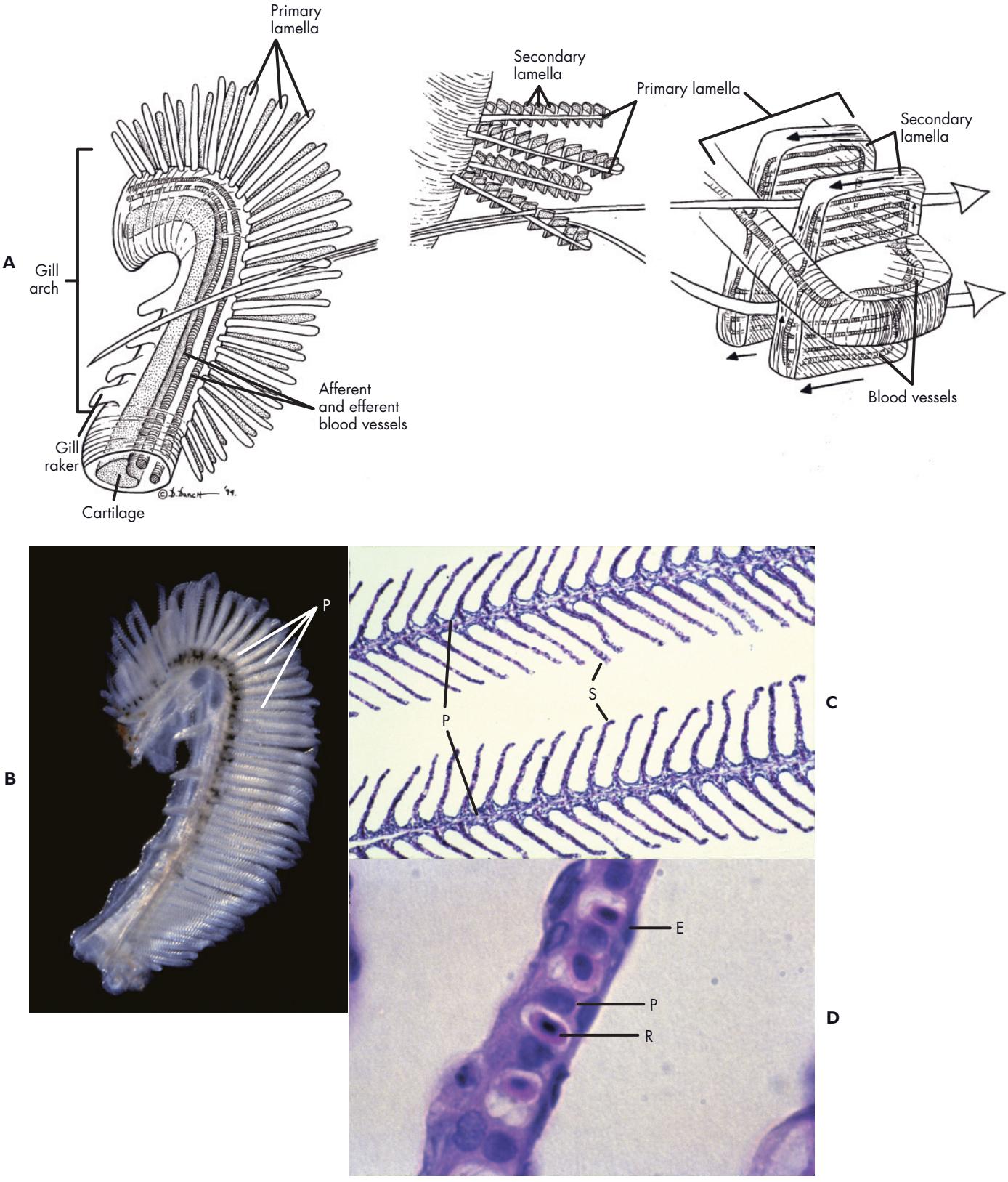


Fig. I-10. A. Diagram of normal gill. *Light arrows* indicate direction of water flow; *dark arrows* indicate blood flow. B. Low magnification microscopic view of a formalin-fixed gill arch, showing primary lamellae (*P*), each having rows of secondary lamellae. (Compare with Fig. I-10, *C*.) C. Low magnification histological section of normal gill. *P* = primary lamella; *S* = secondary lamella. Hematoxylin and eosin. D. High magnification histological section of normal gill secondary lamella. *R* = red blood cell; *P* = pillar cell; *E* = epithelial cell. Hematoxylin and eosin.

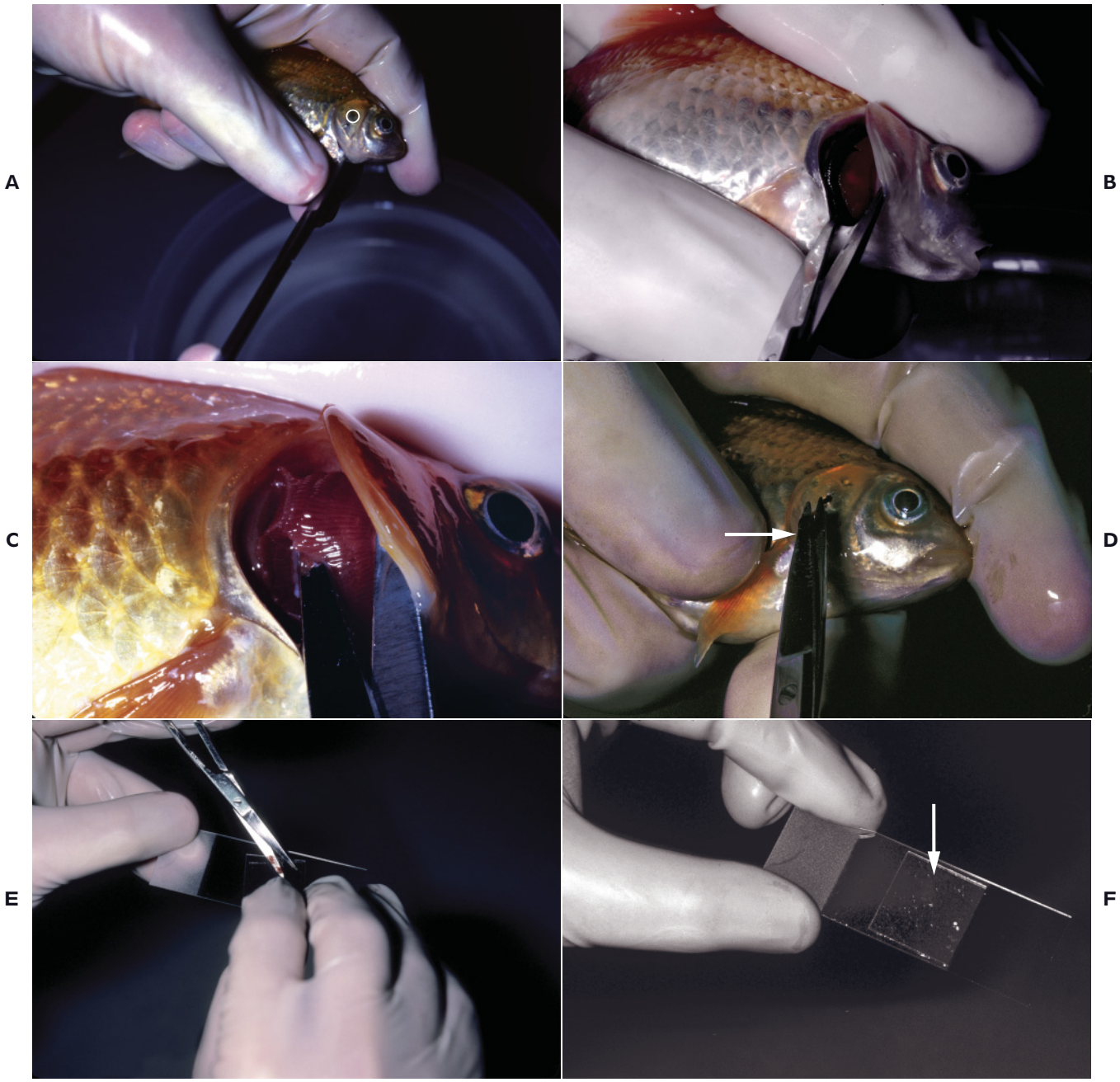


Fig. I-11. The gill biopsy. A. Using a fine pair of scissors to pry open the gill cover, or operculum (*O*). B. Inserting the scissors under the tips of the gills just before cutting the tips. C. Close-up of Fig. I-10, *B*. Each horizontal, finger-like strip of tissue is a primary lamella. Only the distal tips of the primary lamella should be cut. D. Gill tissue on the scissor (*arrow*) after being excised from the gill. E. Scraping the biopsy material onto the slide with a coverslip. F. Gill tissue (light material at the *arrow*) covered with the coverslip.

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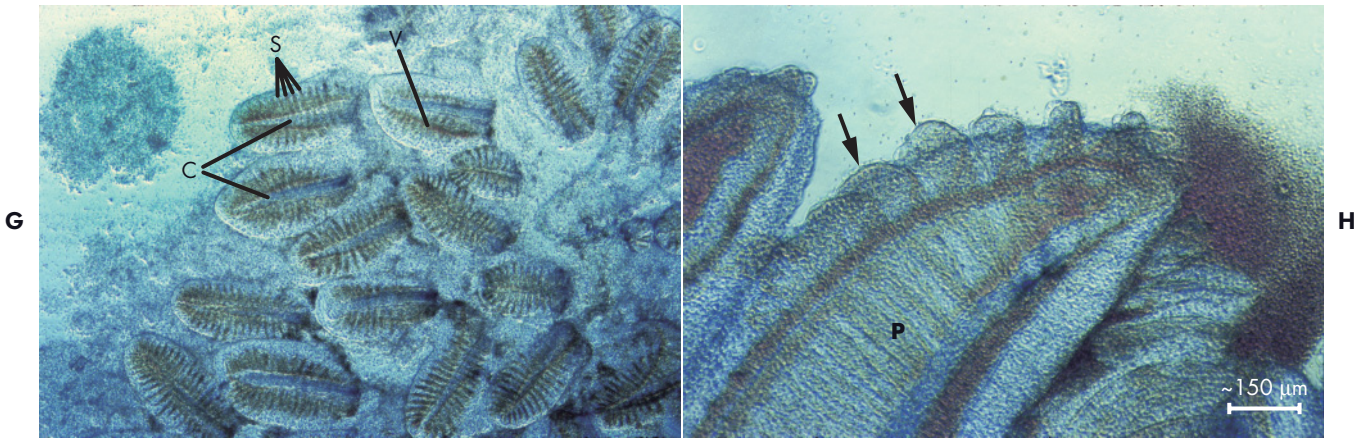
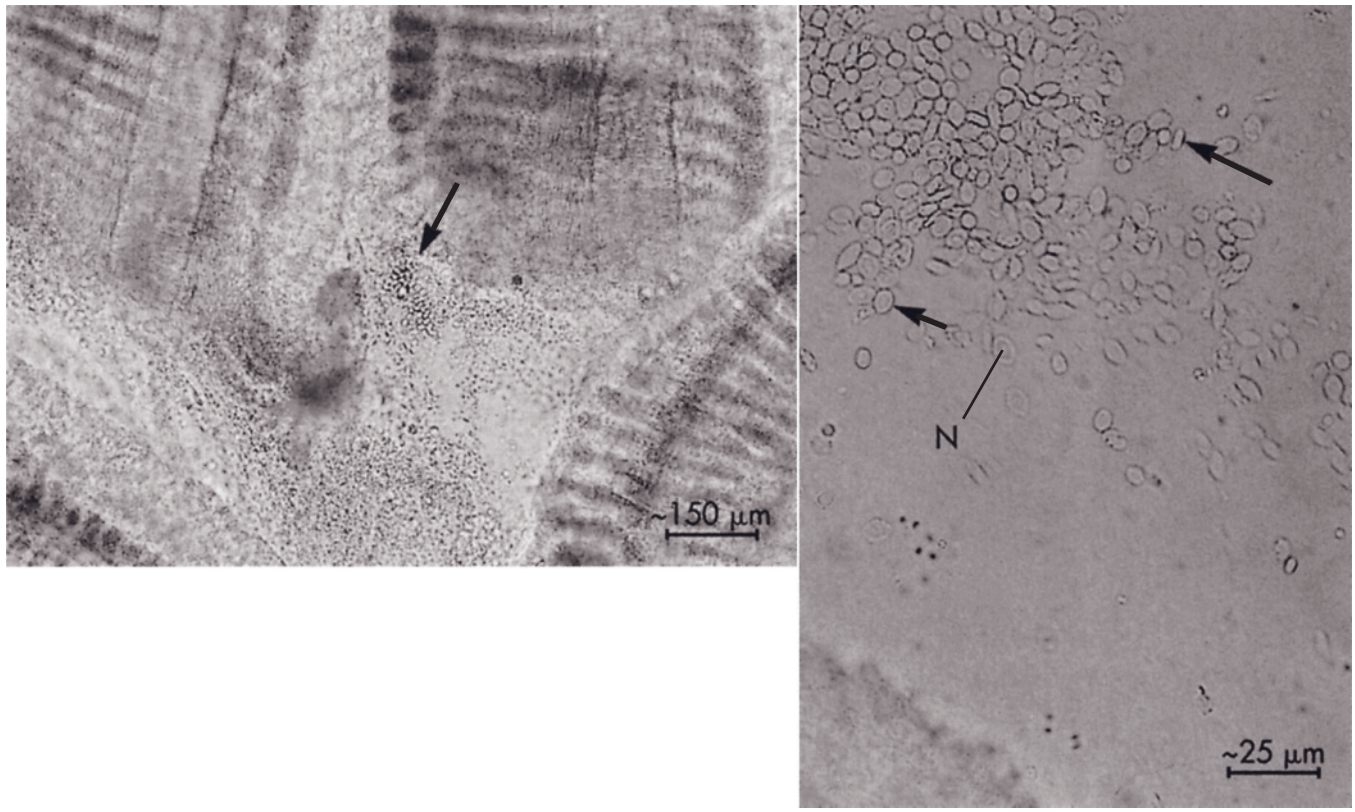


G H

Fig. I-11.—cont'd. G. Low magnification photomicrograph of biopsy of a normal gill. The large finger-like structures are primary lamellae. *S* = secondary lamellae; *C* = cartilage support of primary lamella; *V* = blood vessels. H. High magnifiction view of normal gill, showing secondary lamellae (*arrows*). Individual secondary lamellae may not be visible in some squashes of normal gill, depending on how the tissue lies. *P* = primary lamella.



A B

Fig. I-12. Blood cells in a wet mount of gill. A. The cells (*arrow*) are streaming from the cut surface of the gill. B. High magnification view showing individual red blood cells. Key characteristics include oval shape in top view (*small arrow*) and laterally compressed in side view (*large arrow*). Nucleus (*N*) gives cells a fried-egg appearance. (*A* and *B* photographs by L. Khoo and E. Noga.)

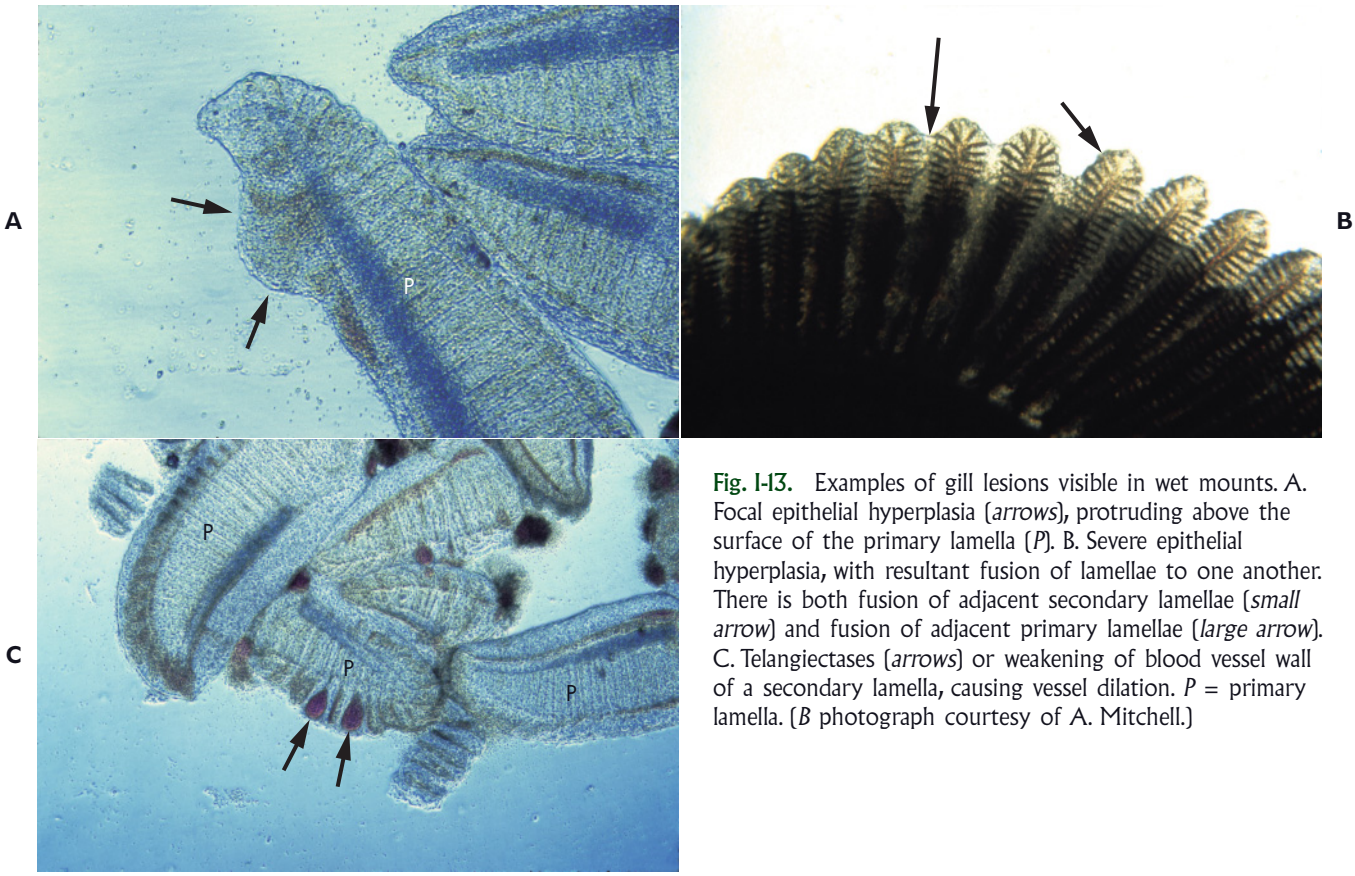


Fig. I-13. Examples of gill lesions visible in wet mounts. A. Focal epithelial hyperplasia (*arrows*), protruding above the surface of the primary lamella (*P*). B. Severe epithelial hyperplasia, with resultant fusion of lamellae to one another. There is both fusion of adjacent secondary lamellae (*small arrow*) and fusion of adjacent primary lamellae (*large arrow*). C. Telangiectases (*arrows*) or weakening of blood vessel wall of a secondary lamella, causing vessel dilation. *P* = primary lamella. (*B* photograph courtesy of A. Mitchell.)

for diagnosis. Samples are also contaminated with many nonpathogenic organisms. A fecal sample can often be obtained by anesthetizing a fish. Standard sodium nitrate flotation can be used for concentrating samples from fecal matter or aquarium debris (Langdon 1992a), but this cannot be used for some parasites; for example, the cell walls of piscine coccidia are too fragile to be separated via fecal flotation (see PROBLEM 74). A direct examination is useful for fragile parasites, digenean trematode ova, and when the amount of feces is insufficient for a flotation.

Bleeding Fish

Hematology and clinical chemistry are not routinely used for fish disease diagnosis, although they can be useful in some circumstances. Anemia in fish is often easily detected by examining the gills, which are a pale pink color (rather than a normally bright red color) if anemia is present. Blood samples should always be taken if fish are anemic.

Fish that are less than 8 cm (3 inches) usually cannot be bled without risk of killing them, so this technique is not feasible for small fish that cannot be sacrificed.

Anticoagulants

If blood is to be obtained simply for determining hematocrit or for making routine blood smears to look for hemoparasites or bacteremia, standard mammalian blood collection procedures are satisfactory. Heparin is usually an effective anticoagulant when used at ~50–100 USP units/mL. However, heparin, which inhibits thrombin, will not prevent coagulation if clotting has begun (i.e., if a small clot is present in the sample because of blood vessel damage during sampling), because coagulation can proceed via an alternate pathway; this is a common problem in fish because of their small vessels. Ethylenediamine tetraacetic acid (EDTA) at 4–5 mg/ml final concentration will totally prevent clotting by chelating required divalent cations. However, using EDTA in combination with tricaine sedation is not recommended because it causes hemolysis in many cases. This might be due to the swelling of erythrocytes that occurs with tricaine anesthesia. This hemolysis problem can be reduced by cooling the blood to 4°C and/or rapidly preparing smears and separating plasma from the cells.

Blood Separation and Analysis

If detailed cellular or chemical analyses are to be performed (e.g., differential counts, enzyme measurements,

❧ Box I-1 ❧

METHOD FOR STAINING BLOOD FOR WHITE BLOOD CELL COUNTING. MODIFIED FROM THE PROCEDURE OF T. LAWS (PERSONAL COMMUNICATION).

Step 1. Prepare Natt-Herrick's stain (Natt and Herrick 1952) as follows:

- Sodium chloride (NaCl) 3.88 g
- Sodium sulfate ($NaSO_4$) 2.50 g
- Sodium phosphate (Na_2HPO_4) 1.74 g
- Potassium phosphate (KH_2PO_4) 0.25 g
- Formalin (37%) 7.50 ml
- Methyl violet 0.10 g

Bring to 1,000 ml with distilled water and filter through Whatman #10 medium filter paper.

Step 2. Prepare a 1:200 dilution of blood with Natt-Herrick's stain by adding 20 µl of blood to 4 ml of Natt-Herrick's stain. Alternatively, using a red blood cell diluting pipet, draw whole blood to the 0.5 mark on the pipet, and then draw the Natt-Herrick's stain to the 101 mark to obtain a 1:200 dilution.

Step 3. Mix well and leave at room temperature for 5 minutes; then fill both sides of a Neubauer hemocytometer with the stained blood.

Step 4. After 5 more minutes (allowing the cells to settle), perform a white blood cell count, using the 10X objective. That is, count all white blood cells in the four large corner squares on both sides of the hemocytometer chamber (the counts within each square should be within 10% of each other). Add all eight counts together and use this total count to calculate:

$$\frac{\text{Total \# WBCs counted}}{8 \times 2{,}000} = \text{\# WBCs/µl of blood}.$$

All white blood cells (leukocytes + thrombocytes) will stain dark violet, distinguishing them from red blood cells, which stain more lightly. It is usually not possible to easily distinguish thrombocytes from leukocytes (especially small mature lymphocytes); staining for 60 minutes rather than 5 minutes may improve the ability to distinguish them (Campbell 2004b).

etc.), the clinician should standardize the conditions for the fish species that are being examined because various researchers have noted problems under a wide range of conditions that are routinely used in mammalian hematology. The most important variables are type and concentration of anticoagulant and type of anesthetic. It is preferable to avoid using chemical anesthesia. Stunning fish avoids potential complications of anesthesia.

Samples should be analyzed as quickly as possible. Significant changes often occur in whole blood after 1-hour storage at room temperature and can occur 1–3 hours after refrigeration (Houston 1990).

Plasma and/or serum should be rapidly separated from cells and frozen at the lowest possible temperature. For serum from teleost fish, it is usually best to allow the sample to clot at room temperature for 5 minutes, refrigerate it for 1–2 hours, rim the clot, and then centrifuge to separate the serum from cells. Elasmobranch (e.g., shark) blood typically takes longer to clot, often 20 minutes or more (Campbell 2004b). Pediatric serum separator tubes (Becton-Dickinson) are useful because they handle small volumes. For information on interpreting clinical chemistry responses, see Campbell (2004a).

All fish blood cells, including erythrocytes and thrombocytes (platelet analogue), are nucleated, which prevents the use of automated white cell counting or differentiation. Total white cell counts must be done by staining the white cells and then counting them with a hemacytometer (see Box I-1). Differential counts are obtained from blood smears. For information on interpreting leukocyte responses, see Noga (2000a) and Campbell (2004b).

Preparing Blood Smears

Blood smears are prepared as are routinely done for mammals. Smears should be made quickly and dried rapidly; a hair dryer can speed up the process. Commercial differential stains (e.g., Diff-Quik, Baxter Diagnostics, Inc.) are suitable for most diagnostic purposes (Fig. I-14). White blood cell morphology varies greatly between different fish species. Heinz bodies in erythrocytes, which have been observed in some toxicities (PROBLEM 92) can be stained with new methylene blue, brilliant cresyl blue, or methyl violet (Heinz bodies do not stain with Wright-Giemsa).

Bleeding with Needle and Syringe

For larger teleost fish, a needle and syringe with anticoagulant can be used to bleed the fish from one of several sites. One of the least traumatic sites for collecting blood is the caudal vessel. This site can be approached laterally or ventrally. After the fish is sedated, the needle is gently pushed through the skin near the base of the caudal peduncle. After contact is made with the vertebral column, which is felt as firm resistance, the needle is directed slightly ventrally and laterally to the vertebral

column, while the plunger of the syringe is gently and slowly pulled, aspirating the blood into the syringe (Fig. I-15, A and B). It may be necessary to slowly rotate the needle before blood can be withdrawn. When one of the caudal vessels is entered (either artery or vein; they run closely together), blood is aspirated. Filling the hub of the needle is a sufficient amount for making a blood smear.

Larger teleost fish may also be bled from the heart. The heart is usually located near the posterior edge of the gill chambers (Fig. I-16). The heart may also be approached dorsally by directing the needle into the posterior portion of the gill chamber. Bleeding the heart is probably more traumatic and potentially more dangerous than bleeding the caudal vessels. Less commonly used anatomical approaches for teleosts are discussed by Houston (1990).

Large sharks can be bled using the vein that traverses caudal and slightly ventral to the dorsal fins. With the fish restrained in ventral recumbency or in a sling with its back exposed, a needle is inserted through the soft skin just under the caudal aspect of a dorsal fin as it is lifted dorsally (Fig. I-17). The needle is then directed under the dorsal fin but is kept to the back and slightly off the midline. Use of a needle with an extension tube can help to keep the needle in position if the shark moves during the procedure. The advantage of this method when bleeding large sharks compared with bleeding the caudal vessels is the ease of access to the vessel and more secure restraint of the fish (Campbell 2004b). However, the PCV tends to be lower from this site (Mylniczenko et al. 2006).

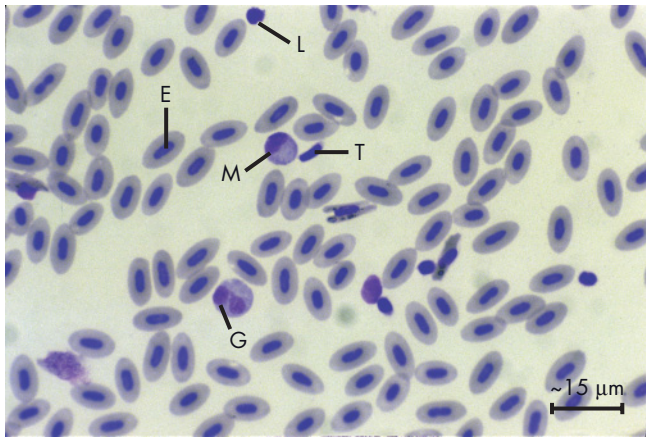


Fig. I-14. Blood smear from a normal goldfish. Blood cell morphology varies greatly among different species. If used, smears must be compared with those from known, healthy individuals. Erythrocyte (*E*); thrombocyte (*T*); lymphocyte (*L*); monocyte (*M*); granulocyte (*G*). Modified Wright's stain. (Photograph by L. Khoo and E. Noga.)

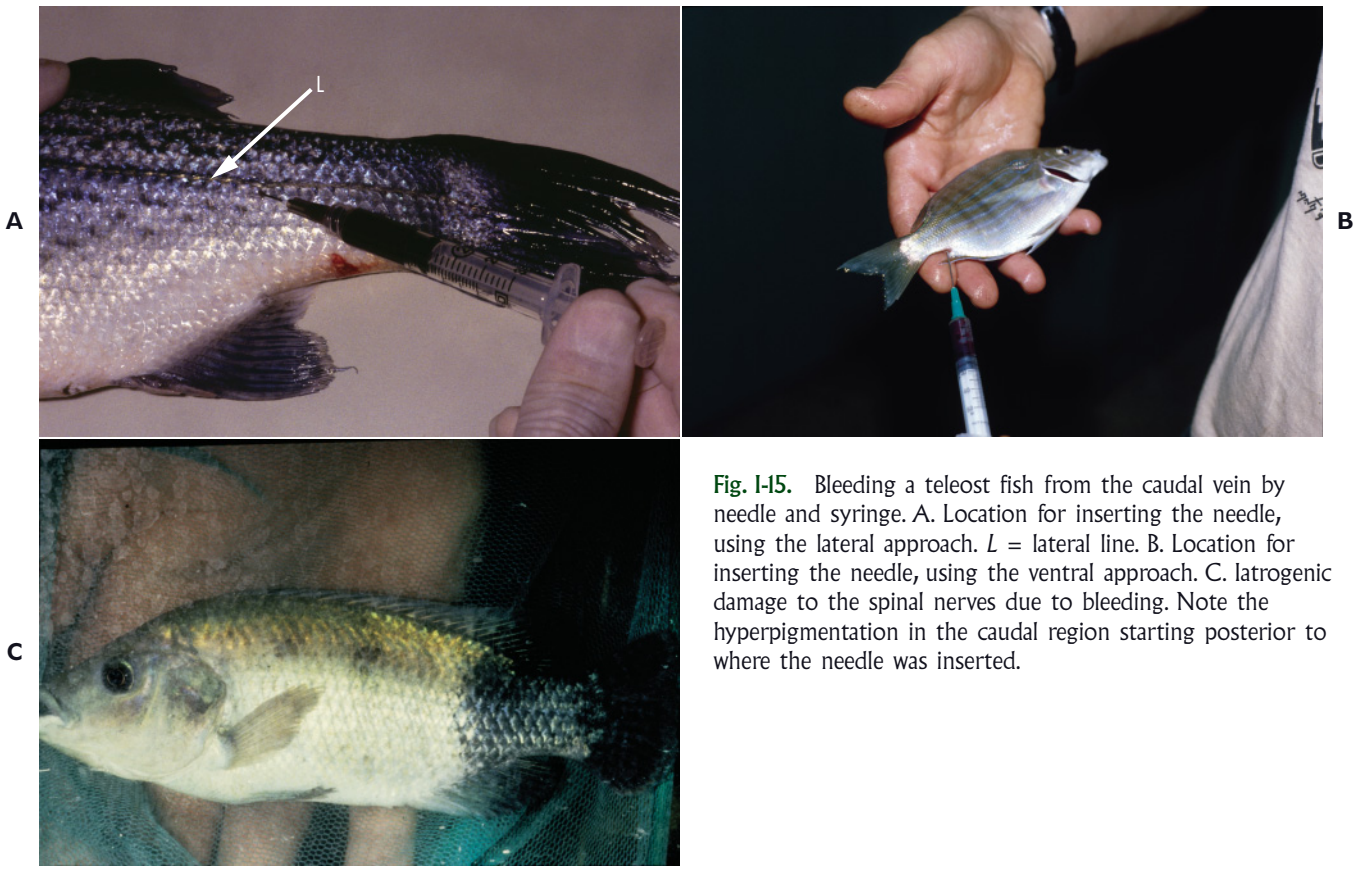


Fig. I-15. Bleeding a teleost fish from the caudal vein by needle and syringe. A. Location for inserting the needle, using the lateral approach. *L* = lateral line. B. Location for inserting the needle, using the ventral approach. C. Iatrogenic damage to the spinal nerves due to bleeding. Note the hyperpigmentation in the caudal region starting posterior to where the needle was inserted.

Bleeding by Capillary Tube

This method is used to bleed small fish (less than 8 cm or 3 inches). The fish is anesthetized and then placed on a smooth, flat surface. The base of the tail is then severed with a scalpel blade (Fig. I-18). A heparinized capillary tube is quickly applied to the caudal vessel, and the blood is collected in the tube by capillary action. A blood smear is then made immediately and is stained, using standard methods. This method probably results in significant tissue fluid contamination, which should be considered if samples are used for clinical chemistry. The fish should be euthanized immediately.

Fig. I-16. Bleeding a teleost fish (rainbow trout) from the heart.

Small volumes of blood can also be collected via capillary tube using the StatSampler™ (StatSpin). This allows collection of up to 200 µl of blood via capillary tube as either an unpreserved sample or as a lithium heparin-treated or EDTA-treated sample. After collection, serum or plasma can be separated from the cells by centrifugation via a gel separator.

CLINICAL TECHNIQUES: SPECIALIZED METHODS

Fluorescein Test

The fluorescein test is not yet used extensively in fish health assessment but it is mentioned here because it has the potential to be a useful addition to the standard clinical workup. Skin ulceration is one of the most common clinical presentations in fish (Noga 2000b). While

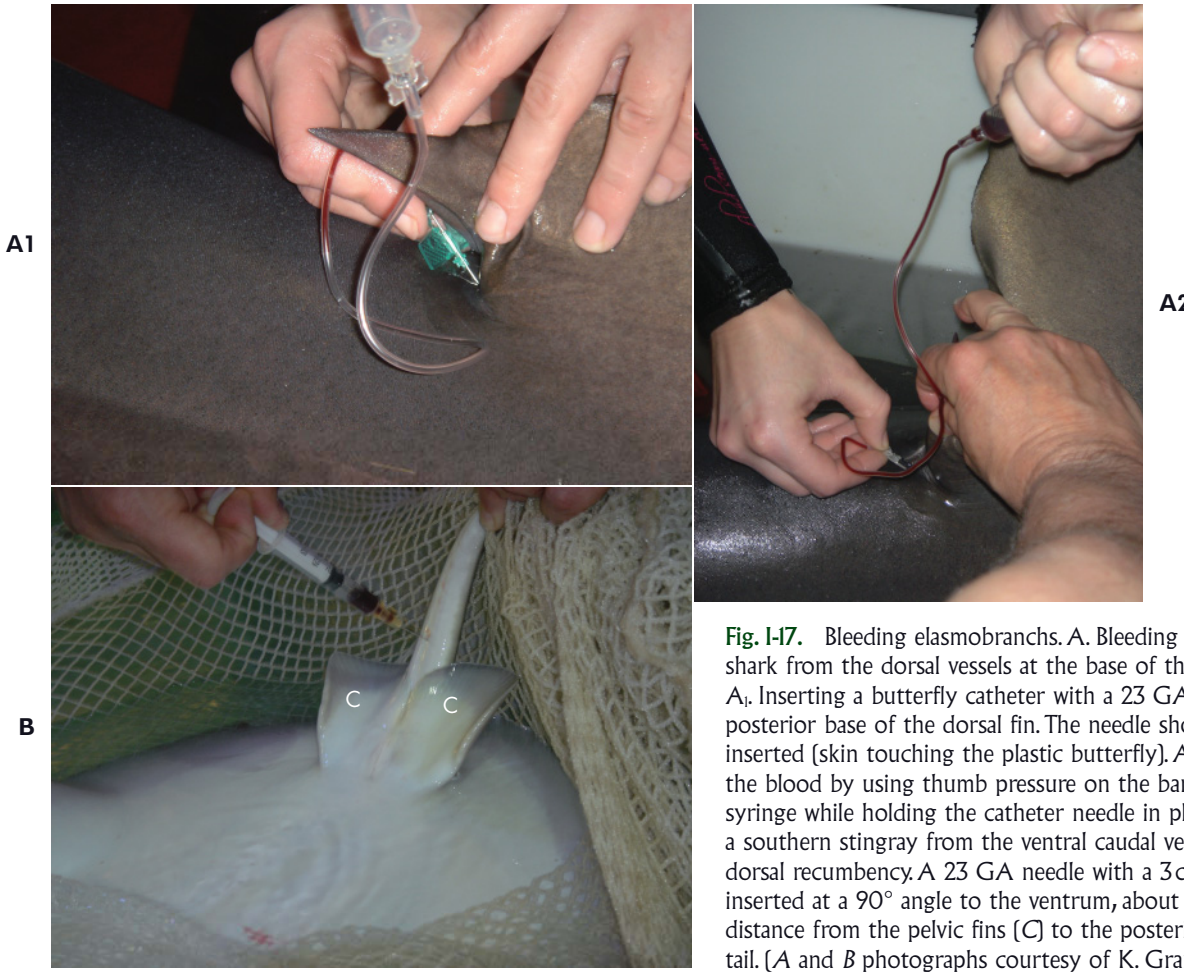


Fig. I-17. Bleeding elasmobranchs. A. Bleeding a sandbar shark from the dorsal vessels at the base of the dorsal fin. A_1. Inserting a butterfly catheter with a 23 GA needle at the posterior base of the dorsal fin. The needle should be fully inserted (skin touching the plastic butterfly). A_2. Aspirating the blood by using thumb pressure on the barrel of the 5 cc syringe while holding the catheter needle in place. B. Bleeding a southern stingray from the ventral caudal vein. The fish is in dorsal recumbency. A 23 GA needle with a 3 cc syringe is inserted at a 90° angle to the ventrum, about 1/4 of the distance from the pelvic fins (*C*) to the posterior end of the tail. (*A* and *B* photographs courtesy of K. Grant.)

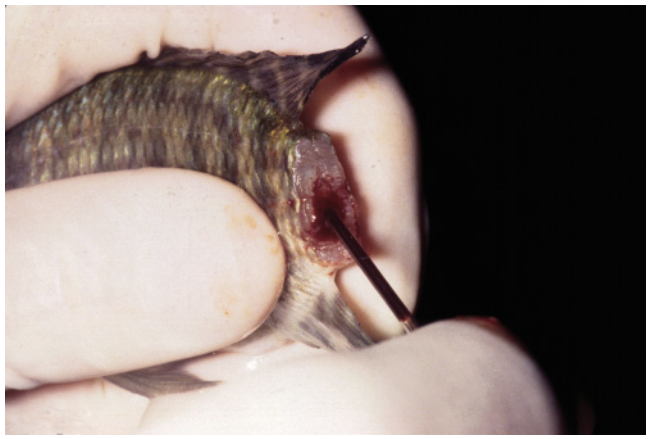


Fig. I-18. Bleeding from the caudal vein by severing the tail. After anesthetization, a sharp scalpel is used to cut off the base of the tail. A heparinized capillary tube is immediately applied to the vessel until sufficient blood is obtained.

❧ Box I-2 ❧

FLUORESCEIN TEST METHOD

- Prepare a fluorescein bath in a bucket or tank. The bath should be large enough to comfortably place the fish to be tested. Add ~0.2 milligrams fluorescein (Sigma-Aldrich Corp.) per ml of water (~1 g/gal). The cost of 1 liter of a 0.20 mg/ml solution is ~$0.10; this is enough to test a large number of small fish.
- Place fish to be tested in the bath for ~6 minutes.
- Remove the fish from the bath and rinse thoroughly in clean water.
- Examine immediately under an ultraviolet light ("black light"). Ulcers are somewhat visible in daylight but are much more evident in the dark.

advanced skin ulcers are usually grossly visible and thus easily identified, the earliest stage of ulceration is usually difficult if not impossible to detect with the naked eye. For example, fish that develop the acute ulceration response (PROBLEM 97) have no gross evidence of skin damage even when virtually their entire epidermal epithelium is sloughed off. Intervention (i.e., various treatments to prevent microbial infection) is much more successful when initiated as soon as possible after the first evidence of skin damage appears.

Fluorescein (3′,6′-dihydrospiro[isobenzofuran-1(3H),9′-(9H)xanthen]-3-one) sodium ("fluorescein") is a yellow, relatively nontoxic, vital, hydroxyxanthene dye that produces an intense green fluorescence in slightly acid to alkaline (pH > 5) solutions. Fluorescein has commonly been used to detect ophthalmic lesions, such as corneal ulceration, in humans and terrestrial animals. It has also been used as a tracer in clinical studies of ocular blood flow (angiography) (Bartlett et al. 1996), reflecting its low toxicity. Fluorescein exhibits a high degree of ionization at physiologic pH and thus does not penetrate intact epithelium, nor does it form a firm bond with (i.e., stain) vital tissue. However, when there is a break in the epithelial barrier, fluorescein can rapidly penetrate (Bartlett et al. 1996). When exposed to light, fluorescein absorbs light in the blue range of the visible spectrum, with absorption peaking at 480–500 nm. It emits light from 500 to 600 nm, with a maximum intensity at 520–530 nm (Berkow et al. 1991).

Except for a few intertidal species, the skin of fish is not keratinized. Thus, it does not have a dead, horny layer of epithelial cells on its surface but rather is like corneal or mucosal epithelium in that living cells are present throughout (Ferguson 1989). Because fish skin is anatomically so much like a mucosa, fluorescein can be used as a highly rapid, efficient, and sensitive indicator of skin damage in fish (see Box I-2).

Exposure of fish to ~0.10 mg fluorescein per ml of water for 6 minutes can easily identify even pinpoint ulcerations that are not visible to the naked eye (Noga and Udomkusonsri 2002) (Fig. I-19). Note that even presumptively healthy fish often have small focal ulcerations (Fig. I-19); these ulcers might be a consequence of damage during capture but also might suggest that minor skin ulceration may be common in "clinically normal" fish as a consequence of normal daily activities. Because the entire fish is bathed in fluorescein, this test also evaluates corneal health (Fig. I-19).

The fluorescein test has a number of advantages over histology, the standard method of evaluating skin damage (Table I-7). An advantage of histology is that it allows skin evaluation after the fish has been euthanized and it allows a specific identification of a pathogen (e.g., parasite, bacterium, etc.) in the skin ulcer. However, since the fluorescein test can detect skin ulceration before the wound is infected, this is not a serious disadvantage for

Table I-7. Comparison of the fluorescein test with histology in evaluating fish skin ulceration.

Characteristic	Histology	Fluorescein
Sensitivity	Low (very small sample area)	High (examines entire body)
Speed	Days	~15–30 minutes
Cost	High (~$30 minimum)	Low (few cents)
Labor	High	Low
Lethal to fish?	Almost always	Never
Hazardous chemicals?	Yes	No
Specific diagnosis?	Yes	No
Sample storage?	Yes	No

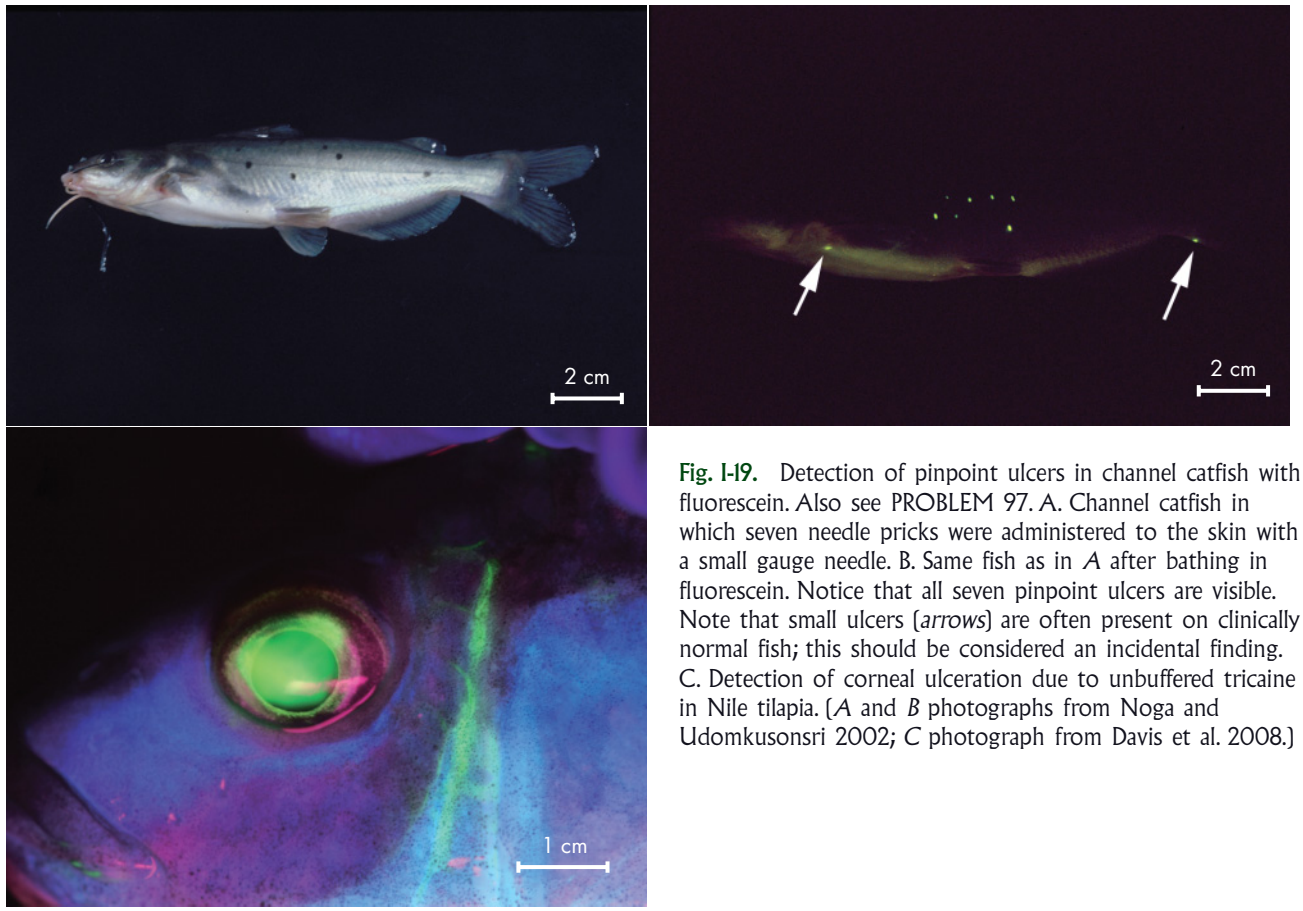


Fig. I-19. Detection of pinpoint ulcers in channel catfish with fluorescein. Also see PROBLEM 97. A. Channel catfish in which seven needle pricks were administered to the skin with a small gauge needle. B. Same fish as in *A* after bathing in fluorescein. Notice that all seven pinpoint ulcers are visible. Note that small ulcers (*arrows*) are often present on clinically normal fish; this should be considered an incidental finding. C. Detection of corneal ulceration due to unbuffered tricaine in Nile tilapia. (*A* and *B* photographs from Noga and Udomkusonsri 2002; *C* photograph from Davis et al. 2008.)

its intended use. Standard skin biopsy (**see p. 23**) is also limited to evaluating only a small portion of the skin; also, presence or absence of ulceration can be difficult to determine.

Eye Examination

Ophthalmic examination is infrequently performed in a clinical workup unless there is grossly visible eye damage or behavioral indication of impaired vision (Nasisse et al. 1989). However ocular pathology has been used as a general indicator of fish health, since the eye is affected by a number of insults, including gas supersaturation, transportation stress, nutritional imbalance, trauma, intoxication, temperature aberration and infections (reviewed in Carrillo et al. 1999). In addition, ophthalmic lesions, especially cataracts, can be a serious problem in some wild or aquacultured fish (see PROBLEMS 58 and 89).

As in mammals, fluorescein can be used to determine if corneal ulceration is present (**see** Fig. I-19). For detailed examination within the eye, an ophthalmoscope (Fig. I-20) is superior to examination with the naked eye and is usually the best instrument option for field examinations, being relatively small, portable, and sufficient to detect relatively small lesions (e.g., grade 1–2 cataracts in Fig. I-21). A hand-held slit lamp biomicroscope is the most sensitive method for detecting eye lesions but is expensive and generally restricted to specialty hospitals. Before ophthalmic examination, fish should be anesthetized; if sequential evaluations are to be made, the same person should do all examinations (Bass and Wall undated; Wall and Bjerkas 1999). The ophthalmoscope should be held as close as possible to the eye without touching the cornea. While the depth of a cataract cannot be determined with an ophthalmoscope, the technique is still useful for field evaluation.

The need to evaluate many individuals at once has prompted the development of rapid assessment methods for grading severity and progression of some ophthalmic lesions. An example of one such method is shown in Figure I-21, where cataract severity is scored on the basis of area of the lens affected. Methods have been developed to estimate the economic costs caused by such lesions (Menzies et al. 2002). Other, highly specialized techniques such as electroretinography can also be performed on fish (Nasisse et al. 1989), but such methods have been restricted to the research laboratory.

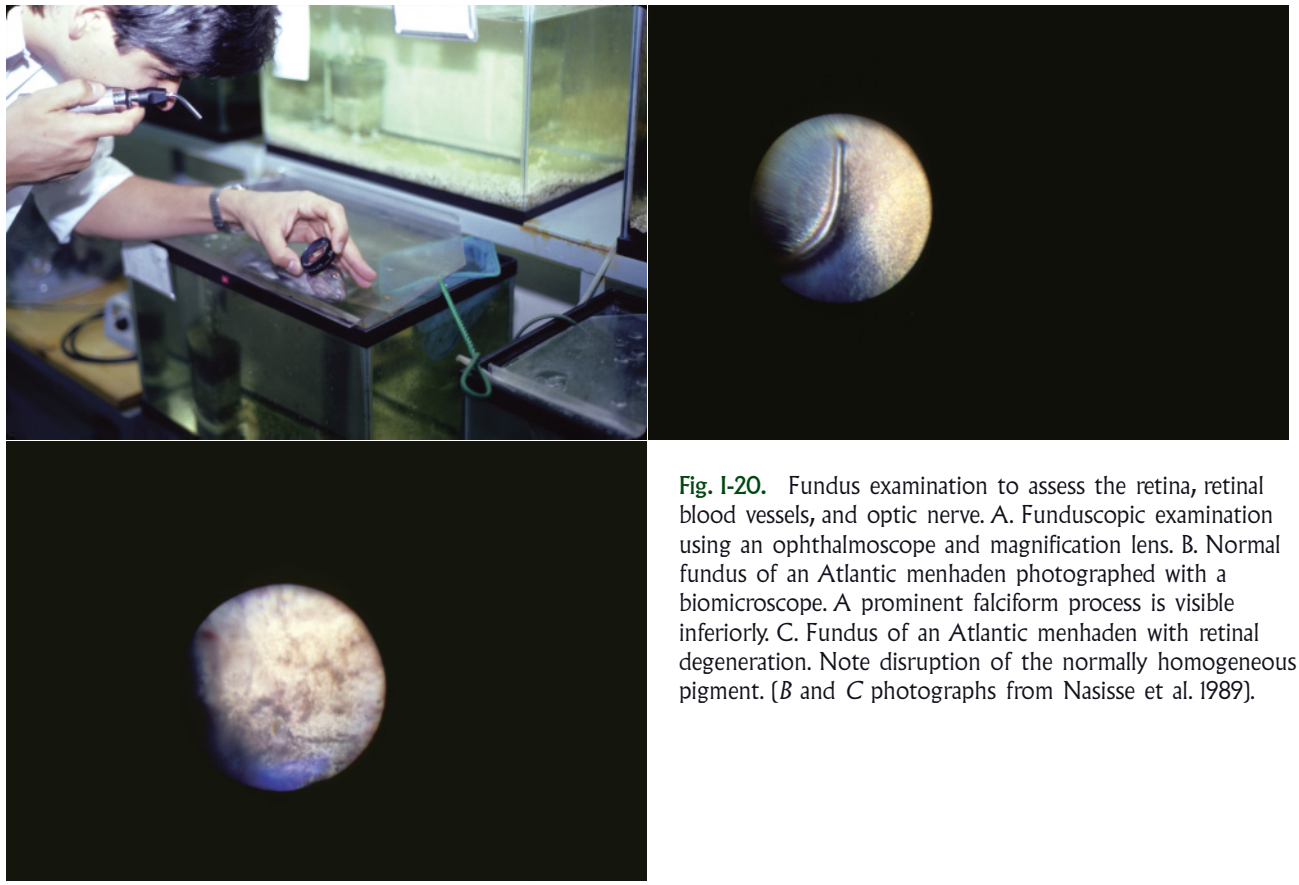


Fig. I-20. Fundus examination to assess the retina, retinal blood vessels, and optic nerve. A. Funduscopic examination using an ophthalmoscope and magnification lens. B. Normal fundus of an Atlantic menhaden photographed with a biomicroscope. A prominent falciform process is visible inferiorly. C. Fundus of an Atlantic menhaden with retinal degeneration. Note disruption of the normally homogeneous pigment. (*B* and *C* photographs from Nasisse et al. 1989).

Diagnostic Imaging

While still not routine, imaging technologies are increasingly used for assessing fish health, especially in pet fish. Fish usually must be sedated or anesthetized to prevent movement during imaging. Correct interpretation of images requires knowledge of the anatomy of that fish species/strain; this may require imaging a normal healthy individual for comparison. Most fish can be held out of water for about 3–4 minutes, during which time the fish is rapidly positioned and the image is taken. If needed, the fish can then be returned to well-aerated water, allowed to respire for at least several minutes, and then another image taken. During imaging, all surfaces in contact with the fish must be smooth and moist to prevent skin damage.

The most common and useful imaging technique is plain radiography. High-definition film with a rare earth intensifying screen should be used; alternatively, digital radiographs can be taken, which allow postexposure manipulation, often eliminating the need to re-shoot. Radiographic cassettes should be enclosed in plastic to prevent water damage. Two views should be taken when possible. The lateral view (in right lateral recumbency) is usually the easiest to do, simply placing the fish on its side. For the dorsoventral view, the fish can be positioned in ventral recumbency; if not possible, it can be radiographed with a horizontal beam while in lateral recumbency (Stetter 2001). Horizontal beam projections can also highlight fluid lines if fluid is present in the swim bladder (C. Harms, personal communication). Many fish will allow plain radiographs to be obtained without restraint or sedation (Bakal et al. 1998). Good quality radiographs will clearly depict all calcified structures (including the skeleton and foreign bodies) and all gas-filled structures (swim bladder, gas in gut, etc.). The relative lack of peritoneal fat in fish prevents clear delineation of viscera (heart, liver, kidney, spleen), but masses can be visualized if they enlarge the abdomen or impinge on the radiolucent swim bladder (Fig. I-22). Thus, plain radiographs are most helpful in diagnosing the cause of skeletal lesions, abdominal swelling, or buoyancy problems (Love and Lewbart 1997). Contrast studies can be used to delineate viscera.

Ultrasonography is complementary to plain radiography because it can delineate soft tissues much better. B-mode ultrasound is most commonly used, with probe size ranging from a 2.5 MHz transducer for large fish to a 10 MHz transducer for small fish. Fish can be directly probed while in water; no acoustic gel is required and

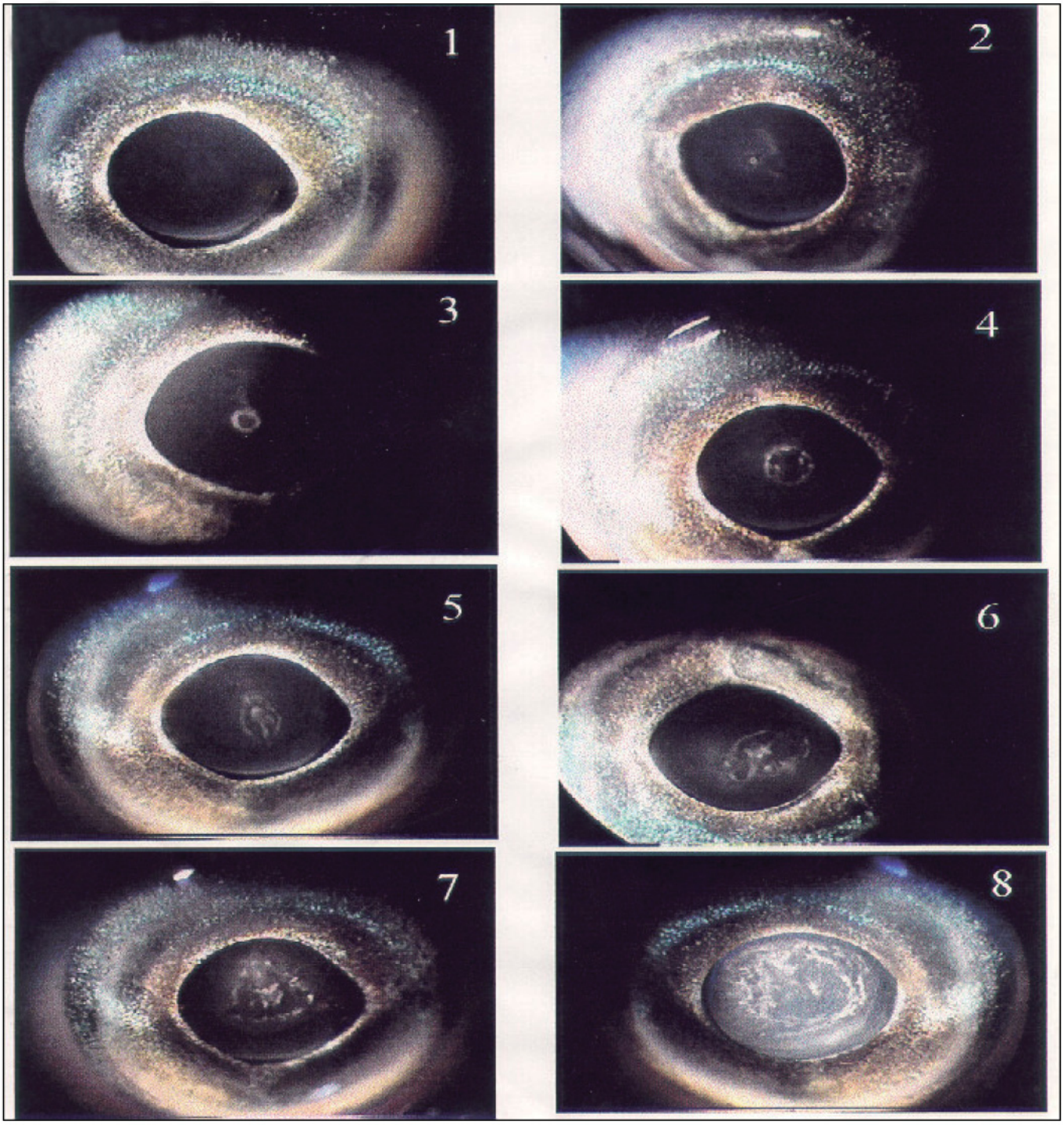


Fig. I-21. Various grades of severity of cataracts in Atlantic salmon, ranging from no cataract (image 1, score 0) to over 75% opacity of the lens (image 8, score 4). Scores correspond to the scoring system as described in Bass and Wall (undated), where severity is based upon the total size of the cataract, not its density: image 1 (score 0), image 2 (score 1), image 4 (score 2), and image 8 (score 4). (Photographs courtesy of P. Campbell.)

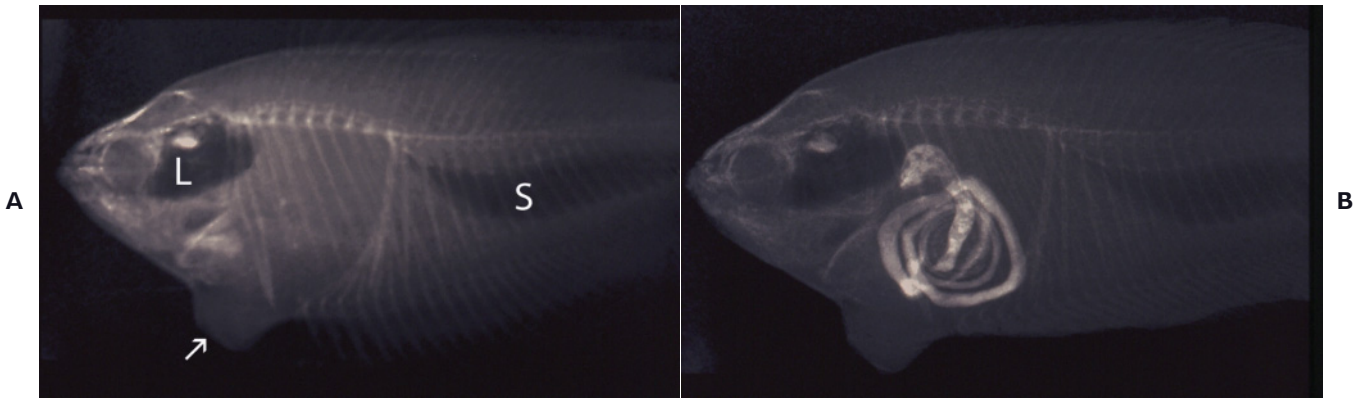


Fig. I-22. Plain radiographs of a sunset, thick-lipped gourami with an abdominal mass. A. Lateral view showing abdominal mass (*arrow*). Radiolucent areas are the labyrinth organ (*L*), an accessory breathing apparatus, and the swim bladder (*S*). B. Lateral view after oral administration of a dye contrast agent (barium sulfate). Note that the dye shows that the mass is not associated with the gastrointestinal tract. (*A* and *B* photographs from Harms et al. 1995.)

the probe does not need to touch the fish (Stetter 2001). Heart, liver, gallbladder, gastrointestinal tract, gonads, eye, and muscle may be well visualized. An esophageal probe is especially useful for evaluating internal organs. Ultrasonography is very useful for determining the location, size, and appearance of masses and determining major sources of blood supply to lesions (e.g., neoplasia), even in very small fish (Walsh et al. 1993; Harms et al. 1995). But, compared to plane plain radiography, obtaining high-quality images requires considerable practice and skill.

Highly specialized techniques such as computer assisted tomography (CAT scans), magnetic resonance imaging (MRI), and nuclear scintigraphy can be very informative but are rarely done. A synopsis of methods for performing various imaging techniques is provided in Stetter (2001).

Percutaneous Procedures

Ovarian Biopsy

Eggs from some fish can be sampled by gently inserting a catheter into the oviduct and collecting ova via capillary action (Fig. I-23). This technique is used to sample ova so that their stage of development can be determined, which indicates whether or not the fish is ready to spawn. If a fertile female is gently squeezed in the abdomen, eggs will often be expressed from the gonadal opening.

Fig. I-23. Catheterizing the oviduct of a striped bass to obtain a sample of oocytes from the ovary in order to determine the degree of oocyte maturation. (Photograph courtesy of C. Sullivan.)

Kidney Biopsy

The kidney is the preferred site for isolation of many viral and bacterial diseases in fish (Amos 1985; Thoesen 1994; AFS-FHS 2007). However, if valuable fish are involved (e.g., broodstock), it may not be desirable to sacrifice such fish to determine their health status. An alternative, nonlethal method involves biopsy (Noga et al. 1988b). In teleost fish, the kidney is a long, ribbon-shaped organ that runs retroperitoneally along the length of the peritoneal cavity. Because it is composed of hematopoietic as well as excretory tissues, the kidney does not have the solid structure of normal mammalian renal parenchyma; instead it has the consistency of bone marrow. At its cranial limit, the kidney of most fish curves ventrally and lies just beneath the medial surface of the branchial chamber. This makes it accessible to needle aspiration.

The fish to be biopsied either is anesthetized or is restrained by another person. The gill operculum is lifted, and a 3 cc syringe with a 22 gauge, 1.5 inch needle

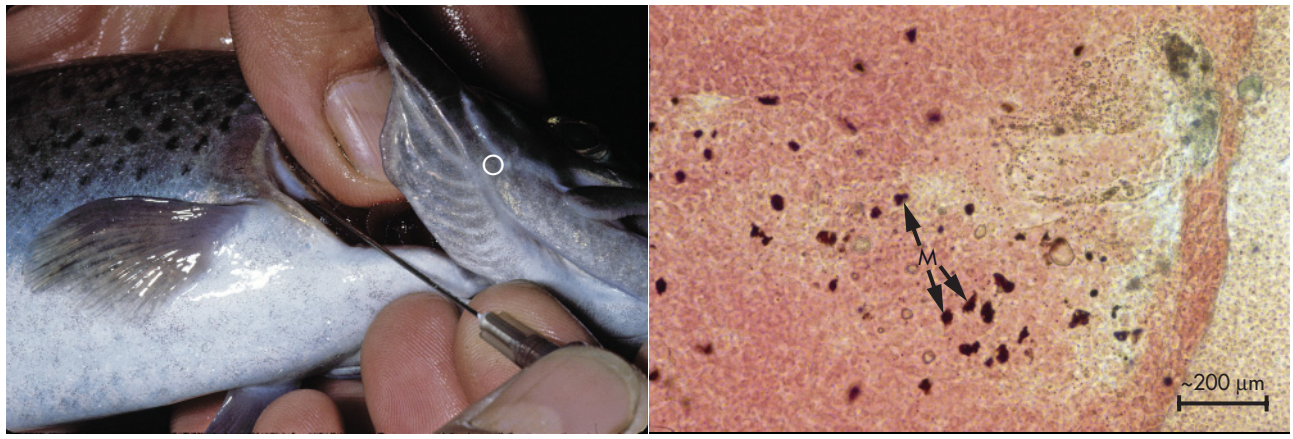


Fig. I-24. Percutaneous kidney biopsy technique. A. Inserting a needle through the medial membrane of the gill chamber and into the kidney. *O* = operculum. B. Confirmation that kidney material has been obtained as indicated by the presence of melanocytes (*M*) in a wet mount of biopsy material. (*A* and *B* photographs from Noga et al. 1988b.)

is directed dorsally and then dorsocaudally into the kidney, just caudal to the last branchial arch (Fig. I-24, A). The syringe is then aspirated until ~0.10 ml of kidney tissue is collected, filling the hub of the syringe. In salmonids, the presence of kidney tissue can be rapidly confirmed by examining a small portion of the aspirate microscopically and confirming that tissue fragments and melanocytes are present (Fig. I-24, B).

This technique is as effective as standard necropsy culture in diagnosing enteric redmouth disease in rainbow trout (Noga et al. 1988b) and would probably be useful for diagnosing other infectious diseases in salmonids. Its usefulness in other fish species remains to be determined and is probably limited to fairly large fish (probably those that are at least 15 cm or 6 inches long). Note that not all fish have melanocytes in the anterior kidney, and thus only tissue fragments may be seen.

Surgical Procedures

Anesthetic Induction and Maintenance

If a procedure is to last 5 minutes or less, anesthesia can be administered "to effect" by leaving fish in an anesthetic bath until adequate sedation is achieved. Such procedures include routine ophthalmic exam, debridement of a skin ulcer, trimming a necrotic fin lesion, excision of embedded parasites (e.g., anchor worms [PROBLEM 14] or digenean metacercaria [PROBLEM 58]), treatment of a corneal ulcer, implantation of a microchip transponder (Harms and Wildgoose, 2001) (see "**Animal Identification**," **p. 77**), or any of the routine clinical procedures listed on **p. 20** ("**Clinical Techniques: Routine Methods**"). However, for longer procedures, such as major surgery, a more precise method of delivering an exact concentration of anesthetic is required. Lewbart and Harms (1999) designed a simple and inexpensive yet highly efficient device for surgical procedures that maintains fish under anesthesia for up to 3 hours (Fig. I-25). While designed for aquarium fish, this setup could easily be scaled up using a larger aquarium/reservoir to accommodate almost any size fish.

Doses for induction and maintenance of anesthesia are given in the "**Pharmacopoeia**." One of the most commonly used anesthetics is tricaine, although eugenol is being used increasingly more frequently. However, there are distinct advantages and disadvantages of various anesthetics and these should be known before using that specific drug. For any procedure, feed should be withheld for 24 hours prior to induction. More details on anesthetic protocols are described in Ross and Ross (2008).

After the procedure, it is best to clean and disinfect (preferably dry out) the surgical platform between uses with a suitable disinfectant such as dilute (1:10) chlorhexidine.

General Guidelines

The following discussion is a general overview of procedures that have proven successful in performing surgery on fish. For more details, see Harms and Lewbart (2000), Wildgoose (2000), Harms and Wildgoose (2001), Harms (2005), and the other papers referencing specific procedures. Also, an extensive review of the basic aspects of fish surgery for individuals lacking prior experience in this area is given in Summerfelt and Smith (1990).

The surgery suite of a typical small animal practice is well suited for fish surgery, and much less elaborate setups are satisfactory as well. While surgical instruments used in small animal practice are feasible for large fish

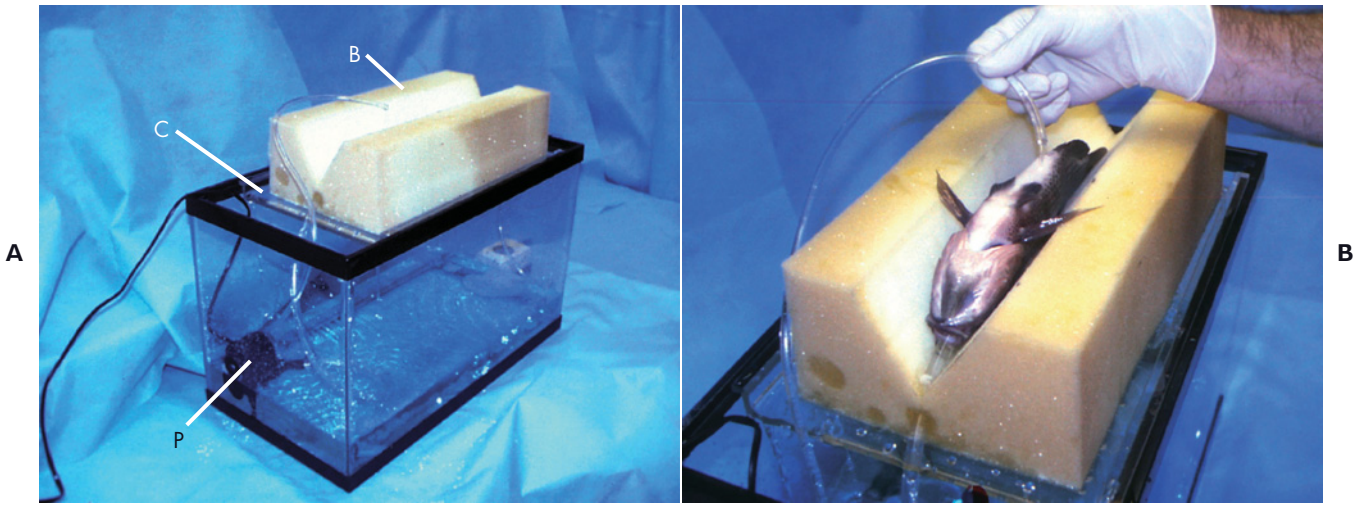


Fig. I-25. The Lewbart-Harms anesthesia device. A. Five gallon (20 liter) glass aquarium having a clear glass or plastic aquarium cover (*C*). If a cover is not available, one can be made by cutting a piece of plexiglass that fits snugly on the inside rim of the aquarium, and also is short enough so that there is space at one end to allow tubing from the water pump (*P*) to exit the aquarium. A surgical foam block (*B*) has been placed on the aquarium cover. A notch was previously carved into the block to allow a fish to rest in dorsal recumbency in the "V" of the block. There is enough water in the aquarium to cover a small submersible water pump. The pump has two tubing outlets: one outlet moves water from the aquarium to the oral cavity of the fish; the other outlet is free to be used to allow constant irrigation of the fish, which keeps the skin and gill tissue moist. B. Fish under anesthesia in the device. (Modified from Lewbart and Harms 1999.)

(e.g., large koi), microsurgical equipment is preferable for most aquarium fish. It is also advisable to use head loupe magnification with center-mounted illumination to see small structures or those deep within the peritoneal cavity.

Prior to performing a procedure, realize that fish anatomy varies greatly and thus a thorough understanding of surgical anatomy, especially internal anatomy, is essential. In this regard, presurgical radiographs and ultrasound to identify the best approach are recommended and will help in defining the surgical field.

Use of preoperative antimicrobial therapy has not been evaluated, but if the fish is to be exposed to a highly contaminated (potentially high bacterial load) or stressful environment, a single dose of enrofloxacin or oxytetracycline (see "**Pharmacopoeia**") may be given immediately prior to surgery.

In terms of preoperative preparation of the surgical field, normal, healthy fish skin has almost no bacteria, much like the cornea of terrestrial animals, due to the presence of potent natural antibiotics and other immune defenses. Thus, a gentle wash with sterile saline or dilute povidone-iodine suffices for preparing the surface for surgery. Harsh antiseptics should be avoided because the living, nonkeratinized skin surface is easily damaged. The skin must be kept moist during the entire procedure; this can be facilitated outside the surgical field by using the Venturi tube on the Lewbart-Harms anesthesia machine, which recirculates the anesthetic solution through the system (Fig. I-25). Details on proper use of anesthetics is given in "**Pharmacopoeia**." Using a clear plastic sterile drape (Steri-Drape, 3M) also retains moisture, does not allow moisture to leak through to the surgical field, and provides a sterile working surface for holding sutures and other materials. During surgery, fish eyes should be shielded from glare as they do not adapt quickly to bright lights of a surgical field.

Approaches for various procedures are described in the sections that follow. Prior to making an incision, it is best to remove the scales along the incision line, although this is not necessary with fine-scaled fish. Similarly, the body wall is usually rigid and retractors are needed for adequate visualization of internal organs. When excising large external masses, skin can be difficult to close because it is immobile compared to that of mammals. Thus, closure in those instances is often better managed via second intention healing. Depending upon skin thickness, a single-layer closure (including both muscle and skin) or a two-layer closure can be used (Harms and Lewbart 2000). Skin is the strength layer. Patterns can be simple interrupted, simple continuous, or continuous Ford interlocking (Harms and Wildgoose 2001).

There have been very few studies examining the suitability of various suture materials for fish tissue. Of the materials examined, including plain gut, chromic gut, silk, monfilament nylon, polyglactin 910, and polyglyconate (Gilliland 1994; Hurty et al. 2002), some differences have been observed in tissue reactivity and time to absorption of the sutures (when using absorbable sutures). However, there are insufficient data to make any clear recommendations for all fish. While none appears to be totally unsatisfactory, some feel that use of a monofilament suture is best since wounds are constantly exposed to bacteria-laden water and multifilament suture could allow wicking of bacteria into wounds (Harms and Wildgoose 2001). Thus, use of polyglyconate, a synthetic, absorbable monofilament suture, has been advocated, since this also induces relatively little inflammatory response (Hurty et al. 2002).

Cyanoacrylate adhesive is not recommended for skin closure. When used alone, it is associated with delayed wound healing and much greater incidence of wound dehiscence (Petering and Johnson 1991); mucus produced by the goblet cells in the skin lifts the glue away from the skin (Harms and Lewbart 2000). The use of surgical staples has had mixed results (Harms and Lewbart 2000).

Postoperatively, there is anecdotal evidence (Harms 2005) that a single application of dilute povidone-iodine solution to the closed incision before returning the fish to the recovery water might reduce the incidence of water mold infections (PROBLEM 34), a very common invader of open wounds in freshwater fish. Antibiotics might be administered if there is bowel penetration or repair of contaminated wounds. Addition of salt (1–3 ppt) to the water of freshwater fish may ease stress from electrolyte loss; this applies to any surgical procedure, not just open wounds prone to direct ion loss, since stress also causes physiological loss of ions via other organs (gill, kidney). Raising the water temperature will increase the rate of healing but should not exceed the optimum physiological temperature range for that species.

While perception of pain in fish is still debated, nocioception (ability to detect an adverse stimulus) has been well documented (see "**Animal Welfare**," **p.** 77). Thus, a single dose of butorphanol just before recovery (Harms et al. 2005) has been shown to reduce the behavioral stress response, but the significance is still uncertain in regard to whether or not this reduces pain in fish.

The anesthetic machine should be left on until the fish has fully recovered. Lengthy exposure to a dissolved anesthetic agent should be avoided because of the redistribution and consequent concentration of the anesthetic in the brain that can lead to overdose (Ross 2001).

External (Skin and Eye) Procedures

The most commonly reported surgeries are removal of external or internal masses of neoplastic or parasitic origin (Harms and Lewbart 2000). Chronic noninfectious masses such as neoplasia can be removed with standard excision techniques (see Fig. I-26) (Probasco et al. 1994). The nonelastic nature of fish skin often requires that the surgical wound heal by second intention; application of silver sulfadiazine creme or antibiotic ointment to the open wound during healing is advisable. Skin masses can be difficult to completely excise and are likely to recur if margins are not thoroughly cleaned (Harms and Wildgoose 2001).

Skin ulcers, such as due to trauma or various infections, can be repaired with standard debridement techniques by removing necrotic debris with dry cotton swabs or gauze. Damaged scales should also be removed, and exposed bone or cartilage must be thoroughly

Fig. I-26. Excision of a fibroma from the skin and muscle of a goldfish. A. Mass prior to excision. B. Healed surgical site. After several months, this mass had not recurred. (Photographs from Probasco et al. 1994).

cleaned and removed if needed. In koi, because their economic value is dictated by the dorsal color pattern, cosmetic restoration should also be kept in mind. The wound is then cleaned with dilute antiseptic (1:40 chlorhexidine or 1:10 povidone iodine). One option is to attempt to close the wound as much as possible to minimize osmotic stress. However, it is usually best to allow healing by secondary intention, since, while scab formation does not occur in fish, re-epithelialization typically occurs very rapidly (Bullock et al. 1978), and keeping the wound open reduces the possibility of abscessation and fistula formation. Because re-epithelialization occurs rapidly, debridement should not be repeated unless necrosis persists. After completing the procedure, a waterproof wound sealant (see "**Pharmacopoeia**") is applied. Depending upon the severity and chronicity of the wound, it might be advisable to administer single or multiple postoperative injections of an antibiotic or administer it in the feed (Harms and Wildgoose 2001).

In the eye, advanced parasitic, infectious, inflammatory, or neoplastic disease may require enucleation. Detailed descriptions have been published (Nadelstein et al. 1997; Harms and Wildgoose 2001). The procedure is fairly simple, but care must be used to avoid damaging cranial nerves V and VII that traverse the retrobulbar space. The fish is placed in lateral recumbency (affected eye up), and then the eye is swabbed with dilute povidone-iodine. Curved tenotomy scissors are then used to bluntly dissect the eye from the circumorbital sulcus. Excess bleeding is controlled via either direct pressure or a drop of 2.5% phenylephrine hydrochloride. The orbit can then be allowed to fill with granulation tissue. Although a method for ocular prothesis implantation has been described, this is not yet reliable (Harms and Lewbart 2000).

Endoscopy

As in other vertebrates, endoscopy can be used in fish for sex identification, as well as visualization and biopsy of viscera (Fig. I-27). The viscera in fish are packed closely together and thus insufflation of the peritoneal cavity with carbon dioxide gas is required. As much gas as possible should be removed after the procedure to avoid buoyancy problems, but any remaining gas will be absorbed. Details on endoscopy procedures are provided

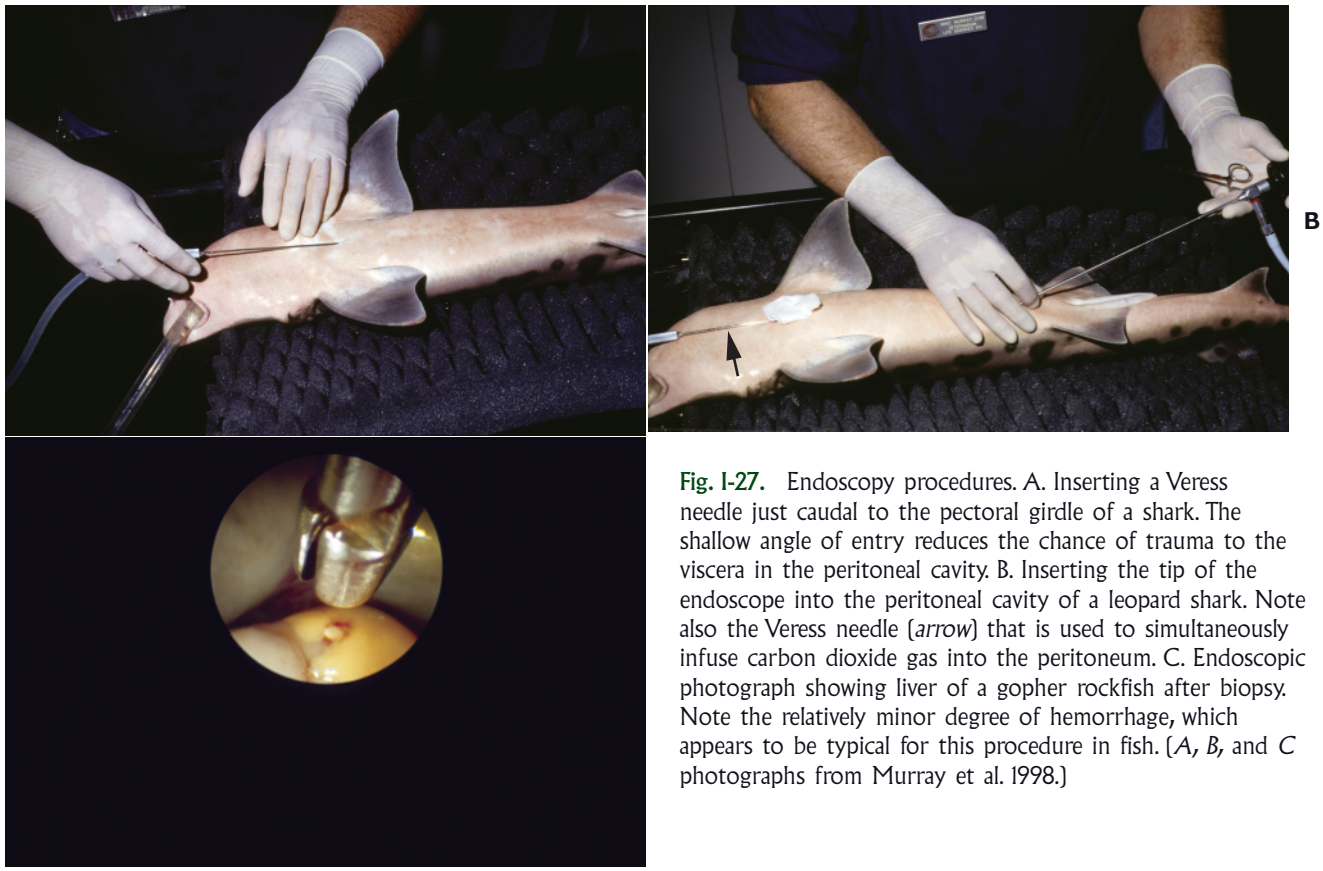


Fig. I-27. Endoscopy procedures. A. Inserting a Veress needle just caudal to the pectoral girdle of a shark. The shallow angle of entry reduces the chance of trauma to the viscera in the peritoneal cavity. B. Inserting the tip of the endoscope into the peritoneal cavity of a leopard shark. Note also the Veress needle (*arrow*) that is used to simultaneously infuse carbon dioxide gas into the peritoneum. C. Endoscopic photograph showing liver of a gopher rockfish after biopsy. Note the relatively minor degree of hemorrhage, which appears to be typical for this procedure in fish. (*A*, *B*, and *C* photographs from Murray et al. 1998.)

in Murray et al. (1998), Hernandez-Divers et al. (2004), and Boone et al. (2008). Endoscopy is of limited use in cyprinids (koi, goldfish) due to the presence of peritoneal tags (Wildgoose 2001).

Abdominal Procedures

Removal of Masses

After placing the fish in dorsal recumbency in a V-shaped trough, the intended incision site is gently swabbed with antiseptic and scales are removed from the area (Fig. I-28). In evolutionarily primitive teleosts (e.g., cyprinids), a ventral midline incision is made from just caudal to the pectoral fins to ~1 cm anterior to the anus. The pelvic girdle is then severed along the ventral midline with a scalpel blade or an osteotome. In more evolutionarily advanced teleosts (e.g., bass), the incision should begin just posterior to the pelvic fins; these fins are more anterior in advanced teleosts and there is usually no need to sever the pelvic girdle.

If needed, access to the surgical site can be enlarged with self-retaining retractors or by making an incision from the ventral midline dorsally. After locating the mass, it is gently freed using blunt and sharp dissection. Bleeding can be controlled with cautery (small vessels) or ligation (large vessels). Prior to closure, the pelvic girdle can be repaired with steel sutures; in small fish, it can simply be incorporated into the suture line. Muscle can be closed in a simple continuous pattern and skin can be closed using a simple interrupted or simple continuous suture.

Kidney Biopsy

For fish whose anatomy does not allow percutaneous biopsy for sampling the kidney, the posterior kidney can be accessed via a surgical approach using a paramedian incision midway between the lateral line and the pelvic fin (Wooster et al. 1993a, 1993b; Fig. I-29). The gonads and mesentery are retracted or partly excised and the swim bladder is gently teased away from the kidney via blunt dissection. The biopsy sample should be taken from only one side to avoid compromising renal function (the kidney is paired but the pairs fuse together in most fish). Hemorrhage is expected but can be reduced by adding a drop of cyanoacrylate tissue adhesive to the scalpel blade just before incising the kidney. Direct pressure or a drop of 2.5% phenylephrine hydrochloride can also be used. In trout, nephrocalcinosis is a common sequela to this type of kidney biopsy but does not appear to cause clinical problems (Wooster et al. 1993a, 1993b).

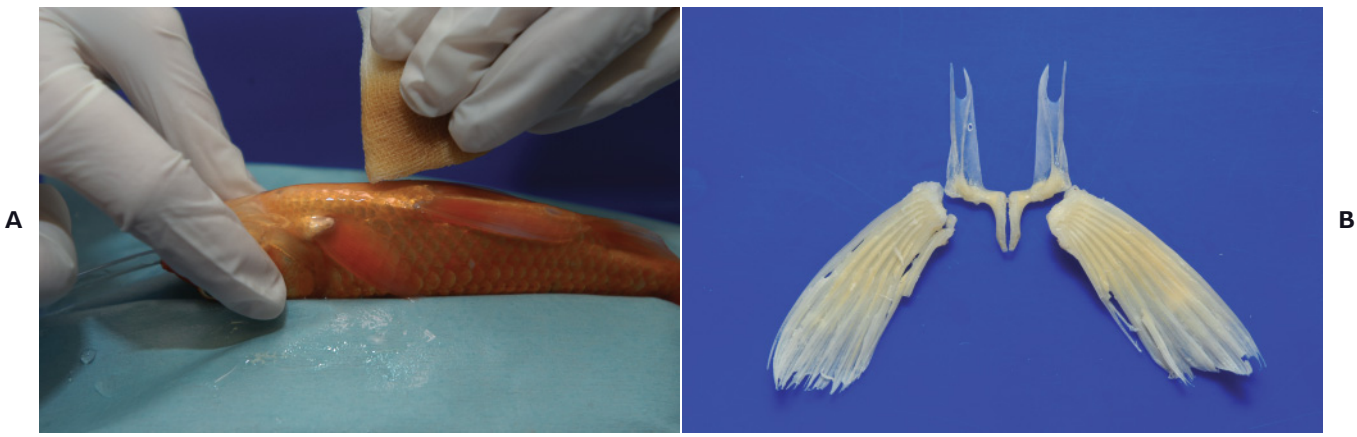


Fig. I-28. Procedures for a midline abdominal surgical approach in a goldfish. A. Excess body mucus is removed and dilute povidone-iodine solution is applied to the surgical site with sterile gauze. Note that the efficacy of using antiseptics on the skin of fish (living tissue) has not been closely examined, so an excessive amount of antisepsis should not be done. On large fish, scales may need to be removed using forceps to allow incision, but this is unnecessary on small fish. B. Bones of the pelvic girdle of a goldfish. This structure is simply embedded in the muscles of the body wall and joined at the midline by a fibrous junction. In older fish, the bones are fused together.

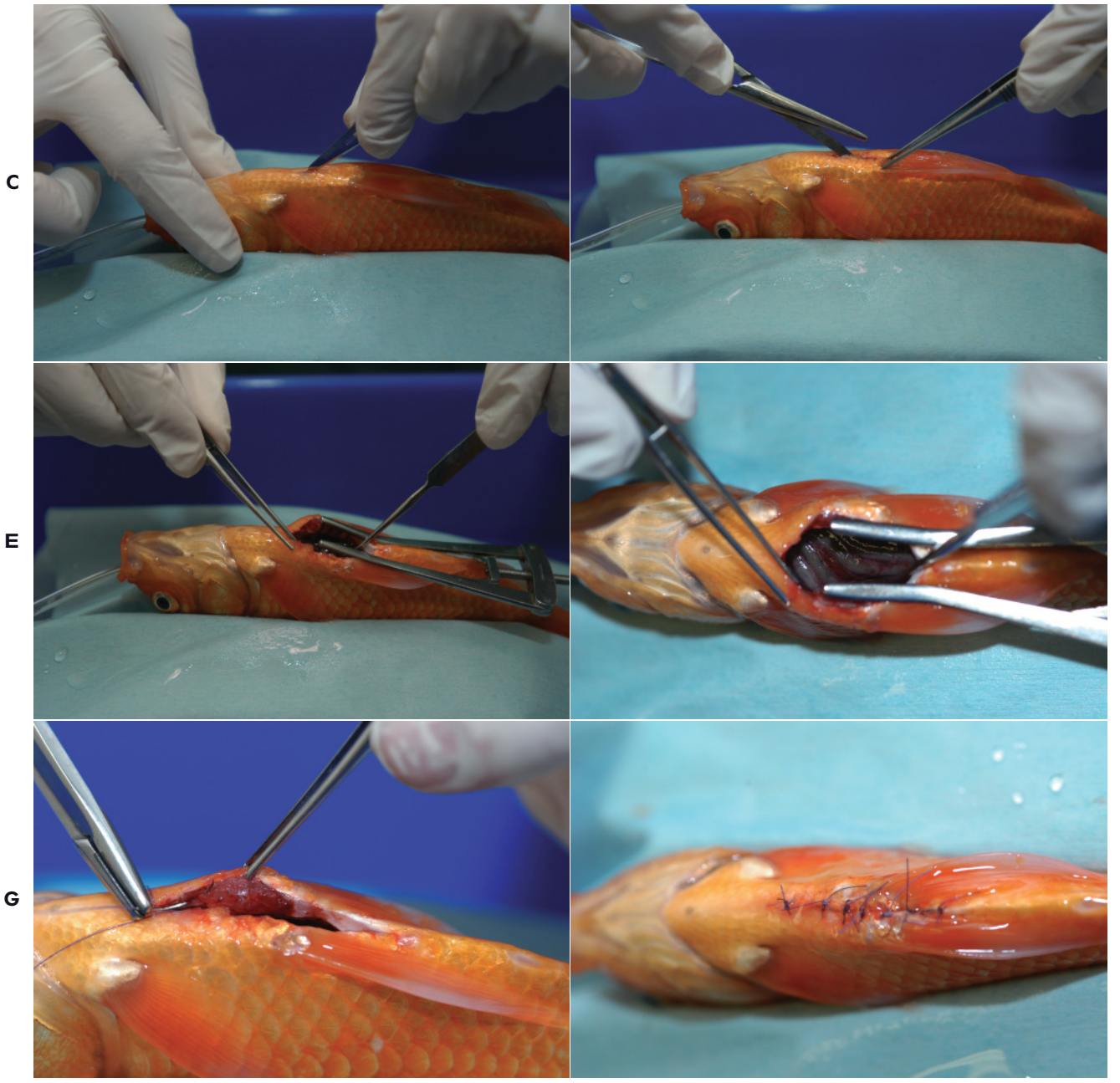


Fig. I-28.—cont'd. C. Using a scalpel, a ventral midline incision is made from just posterior to the pectoral girdle and extending to the pelvic girdle. D. The incision is extended with scissors. A scalpel can be used to cut through the pelvic symphysis in small fish. E. After bisecting the pelvis, the midline incision has been extended toward the vent. Retractors spread the body wall, improving visibility and access to the viscera. A probe and rat-toothed forceps are used to explore the body cavity. F. Intraperitoneal (intracoelomic) view. G. After completing the procedure, the muscle layer is closed with absorbable monofilament suture in a continuous pattern. H. The skin is closed with a nonabsorbable monofilament suture using a simple interrupted pattern. The sutures are usually removed after 1.5–3 weeks.

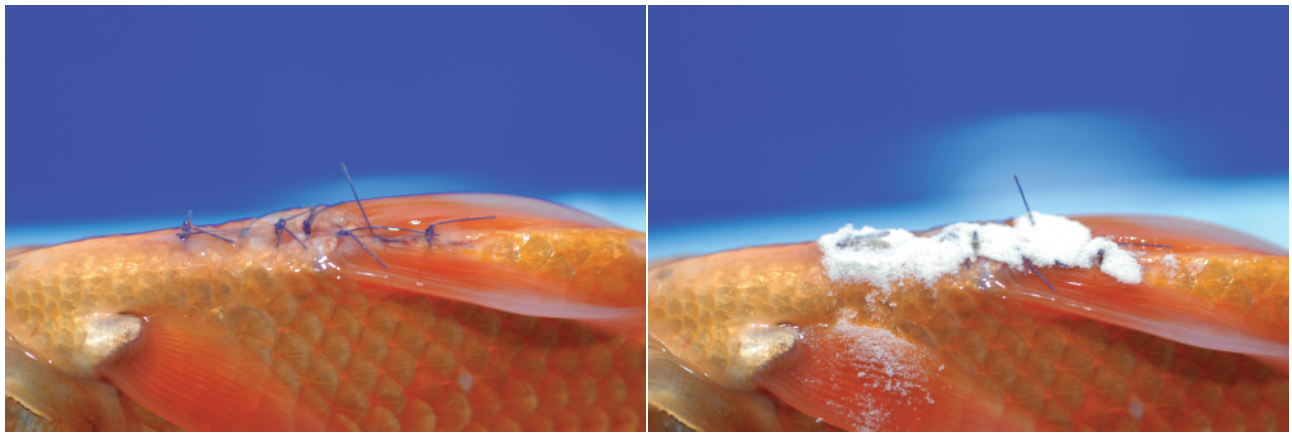


Fig. I-28.—cont'd. I. The suture line the day following surgery, showing epithelialization (cloudy, white area) over the wound. J. Suture line covered with Orahesive® (ConvaTec); this waterproofing powder can be used on fish wounds. (*A* to *J* photographs courtesy of W. Wildgoose.)

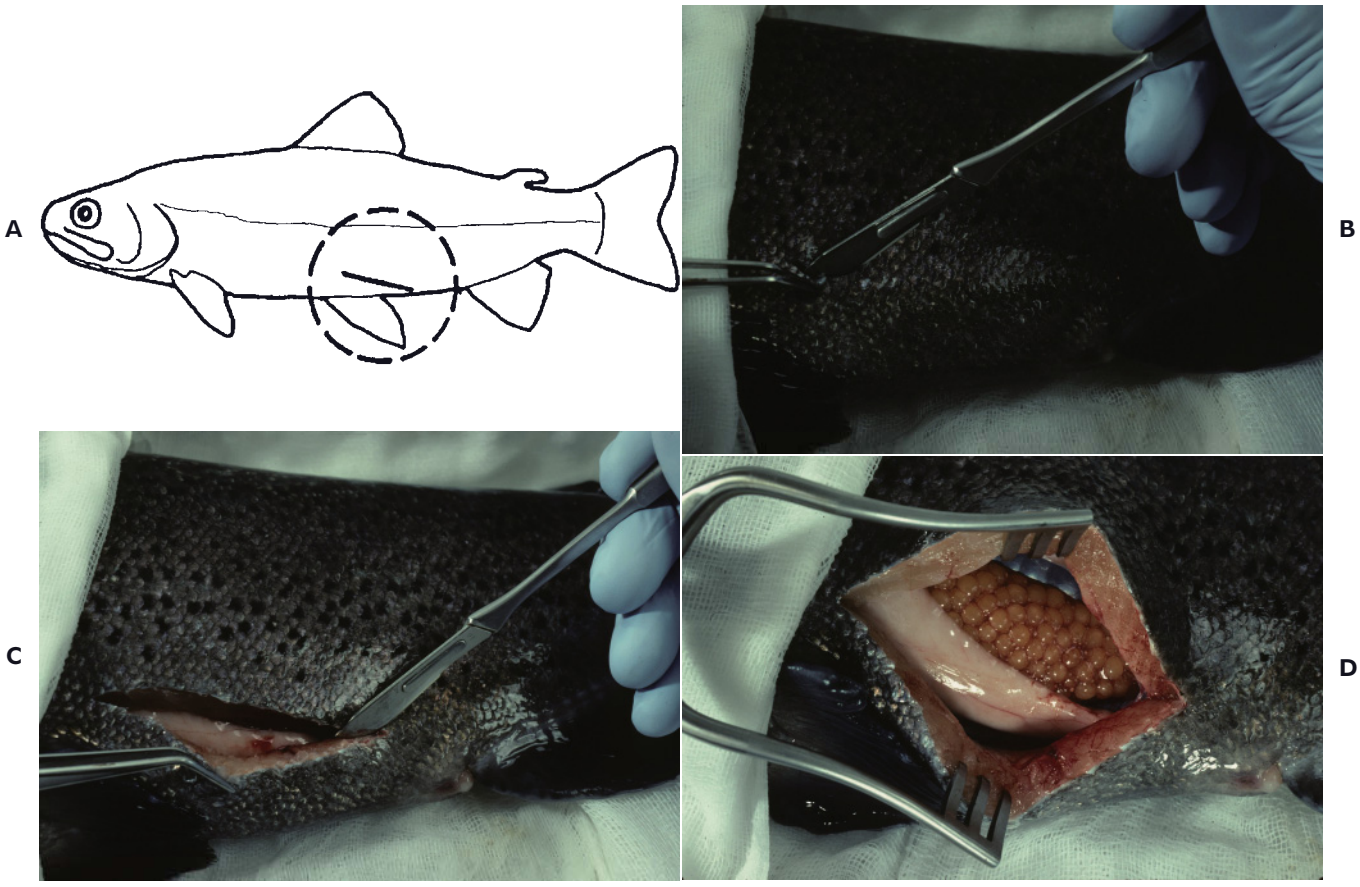


Fig. I-29. Surgical approach to kidney biopsy in trout. A. Site for the surgical incision. B. Location of initial skin incision. C. Full thickness incision through the body wall. D. Retraction of the body wall, exposing the viscera in the peritoneal cavity.

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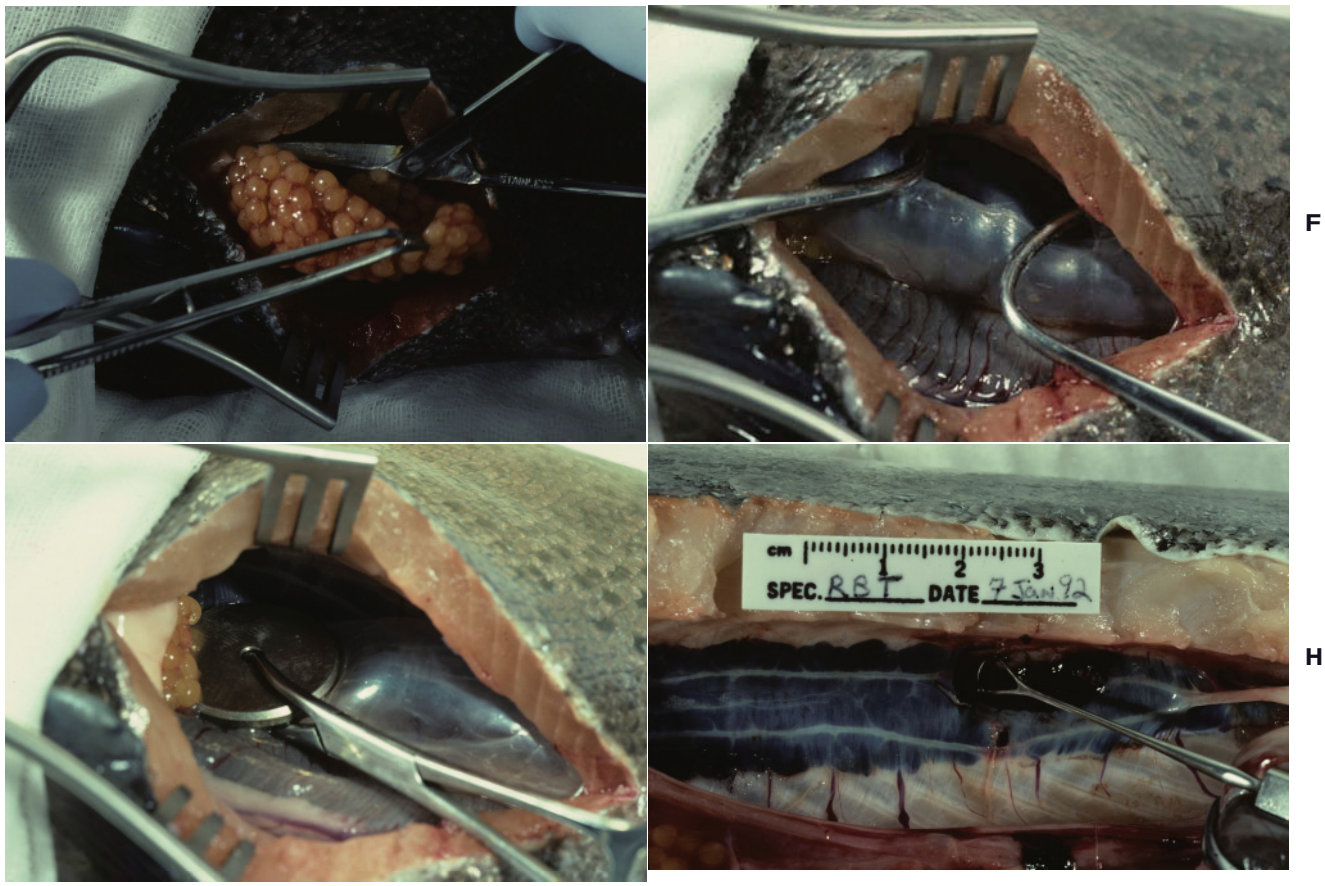


Fig. I-29.—cont'd. E. Excision of the gonads, to allow visualization of the swim bladder. F. Blunt dissection and reflection of the mesenteries away from the swim bladder, exposing the intended site of kidney biopsy. G. Placing downward pressure on the swim bladder with a stainless steel washer to further expose the kidney. H. Using a Lewis lens loop to obtain the kidney sample (body wall and all viscera removed to point out the sampling site). (*A* to *H* photographs from Wooster et al. 1993a.)

CHAPTER 4

Postmortem Techniques

EUTHANASIA

Proper methods for euthanasia are given in "**Pharmacopoeia**."

PRESERVING PARASITES

Live specimens are always preferable for diagnosis, but if assistance is needed for identification and live material cannot be sent to a reference laboratory, samples need to be properly preserved. Table I-4 describes procedures for properly preparing specimens.

Many protozoa can be identified in histological sections, but many can detach from skin or gills with fixation and processing. It is best to fix the gills together, rather than cutting them into individual filaments, especially when loosely attached parasites (e.g., *Chilodonella*, *Trichodina*) may be present. Protozoa can be smeared on a slide, air dried, and stained, the same as for blood smears (see Fig. II-21, E), but this technique is rarely used for identifying protozoa in clinical material. Techniques for preserving metazoan parasites are more commonly used.

CULTURING FOR BACTERIA

It is often desirable to refer fish to a regional reference laboratory if bacterial disease is suspected because the techniques required to properly identify bacterial pathogens of fish are somewhat specialized. Samples should be submitted to a laboratory that is familiar with culturing bacteria from aquatic species because many aquatic pathogens have special requirements. For example, it is best to culture fish isolates at room temperature (22–25°C), not 37°C, as is routinely done in commercial microbiology labs, because some fish pathogens grow poorly or not at all at 37°C. For *Vibrio salmonicida*, *Moritella viscosa*, *Moritella marina*, *Flavobacterium psychrophilum*, and *Renibacterium salmoninarum*, samples should be incubated at 17°C. Samples from marine fish should be cultured on a medium that has a high salt content (e.g., trypticase soy agar with 2% NaCl) or on a nutrient-rich blood agar, such as Columbia agar with 5% defibrinated sheep blood (CBA). CBA or similar nutrient-rich blood agar is a good general-purpose medium for both freshwater and marine bacterial pathogens.

Some bacteria (e.g., flavobacteria, mycobacteria) require other, specialized media, but these media are not routinely used in the clinical workup. *Piscirickettsia* (PROBLEM 56) and *Francisella* (PROBLEM 57) require even more specialized techniques. Anaerobe infections are very uncommon in fish, but if suspected, commercial media that include an anaerobic chamber are available (e.g., OxyPlates™, Oxyrase).

The fact that a general-purpose medium will not be able to isolate all possible pathogens should be borne in mind when interpreting the results of cultures. Selective media can also be used to enhance the isolation of certain pathogens but would not be routinely used in a clinical workup unless prior knowledge of pathogens likely to be encountered warranted it. Not all differential media used for freshwater organisms may be reliable in estuarine environments. For example, Rimmler-Shotts (Shotts and Rimmler 1973), a useful, selective medium for identifying *Aeromonas hydrophila* in freshwater, cannot differentiate between *A. hydrophila* and non-01 vibrios in estuarine waters (Kaper et al. 1981). See Shotts and Teska (1989), Buller (2004), Whitman (2004), and Austin and Austin (2007) for various selective media and culture methods used for bacterial isolation.

Samples may be submitted to a laboratory in one of several ways (Table I-5). Live specimens should be used for culture whenever possible. The only exception is when the only fish displaying clinical signs are dead (i.e., all of the live fish appear healthy). Identification of an obligate pathogen (e.g., *Aeromonas salmonicida*) in a dead fish is a stronger diagnosis than the isolation of an opportunist (e.g., *Aeromonas hydrophila*), especially if large numbers are present.

Whole fish may be frozen and shipped to the laboratory on dry ice. The recoverability of many common bacterial fish pathogens ranges from 20 to 60 days when samples are frozen at –20°C, which is the temperature of a home freezer (Brady and Vinitnantharat 1990). While spleen, liver, and peritoneal fluids are common culture sites, the organ of choice for isolating systemic bacterial pathogens in fish is the kidney, which can be approached dorsally or ventrally (Figs. I-30 and I-31).

Table I-4. Recommended methods of preserving parasites for future identification (modified from Smith and Noga 1993).[1]

Parasite group	Relaxation procedure	"Relaxed" parasite	Fixation	Storage	Final preparation for identification
Monogeneans/Digeneans*	None usually needed for small worms Gently flatten under a coverslip and flood slide with fixative for 5 min	Not contracted Allows some expulsion of eggs from uterus	Hot (55–65°C) AFA or hot NBF	AFA or ETOH	Stained and permanently mounted in mounting medium
Cestodes*	Cold (4–8°C) water or saline for 1–12 hr Gently flatten under a coverslip and flood slide with fixative for 5 min	Not contracted Allows some expulsion of eggs from uterus	Hot AFA or hot NBF or hot ETOH	AFA or ETOH	Stained and permanently mounted in mounting medium
Nematodes	None usually needed for small worms Stretch large worms by holding at both ends with forceps and add fixative for 5 min	Completely uncoiled	Hot AFA or hot ETOH	AFA or ETOH or glycerol:ETOH	Small nematodes can be cleared in glycerol:ETOH and mounted permanently in glycerol jelly. Large nematodes are cleared and temporarily mounted in glycerol:ETOH
Acanthocephalans	Cold (4–8°C) water or saline for 1–12 hr	Proboscis fully extruded	Hot AFA or hot NBF or hot ETOH (puncture cuticle)	AFA or ETOH	Small: stained and mounted. Large: unstained and mounted in glycerol:ETOH
Hirudineans	Tricaine Sodium pentobarbitol	Not contracted	Hot ETOH	ETOH	Small: stained and mounted Large: glycerol:ETOH
Arthropods	Not required	Not required	Cold (4–8°C) ETOH	ETOH	Unstained and cleared in 10% KOH or Hoyer mounting medium
Ciliates, flagellates[1]	N/A	N/A	Air-dry smear	Stain immediately	Stained with Diff-Quik and permanently coverslipped with mounting medium (Permount or equivalent)
Amoebae[1]	N/A	N/A	Air-dry smear	Stain immediately	Stained with Diff-Quik and permanently coverslipped with mounting medium (Permount or equivalent)
Myxozoa	N/A	N/A	Air-dry smear	Stain immediately	Stained with Diff-Quik and permanently coverslipped with mounting medium (Permount or equivalent)
Microsporidians	N/A	N/A	Air-dry smear	Stain immediately	Stained with Diff-Quik or Gram's and permanently coverslipped with mounting medium (Permount or equivalent)

[1]Note that this procedure is less reliable for protozoan identification than routine histopathology but can be useful when submitting specimens to reference laboratories for identification.
Abbreviations: AFA, alcohol-formalin-acetic acid; NBF, 10% neutral buffered formalin; ETOH, 70% ethanol; glycerol:ETOH, glycerol: 70% ethanol.
*Before beginning preservation procedures, encapsulated larvae should be manually dissected out of the capsule or the capsule should be digested with 0.2% pepsin in 0.1M HCl.

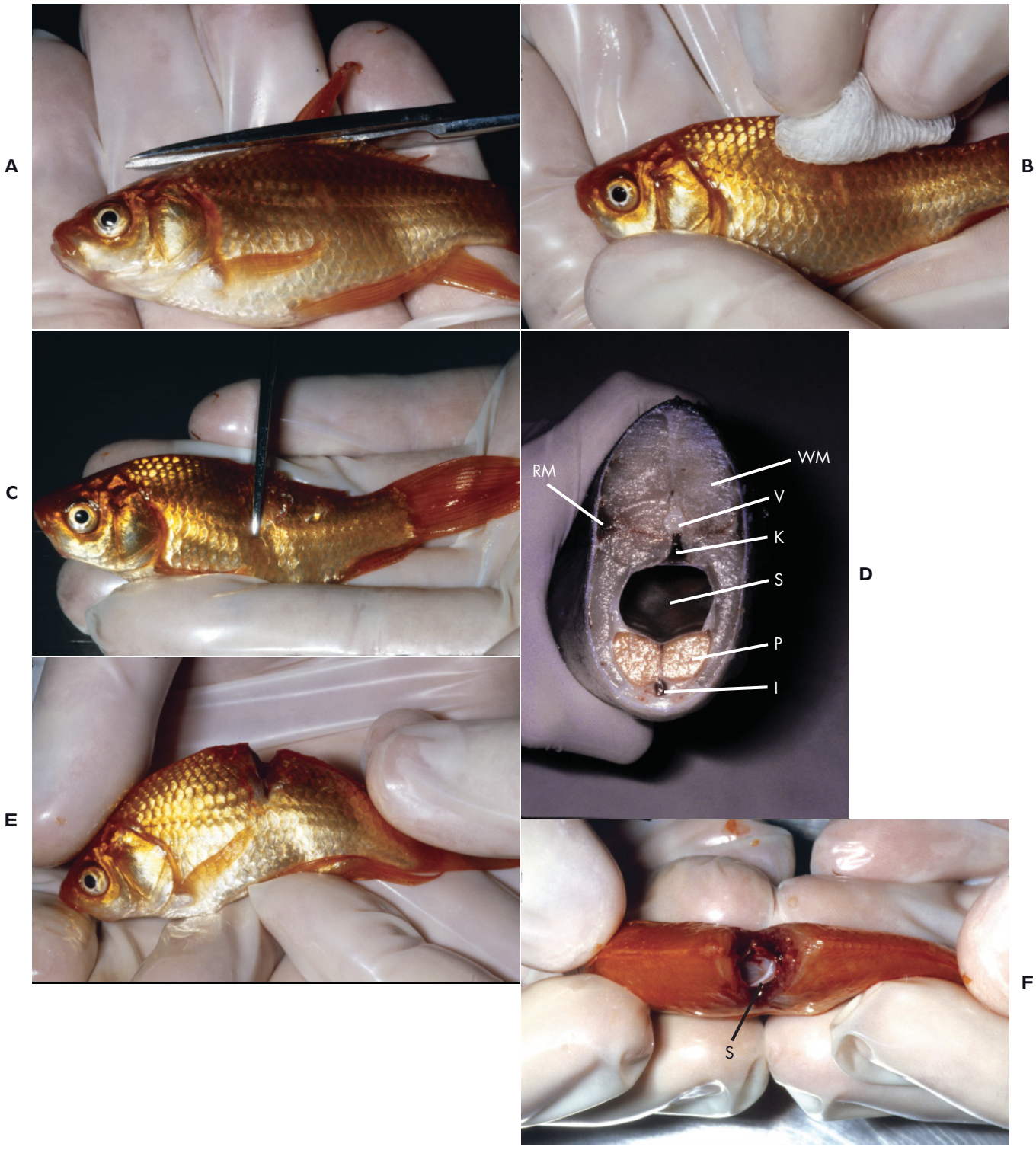


Fig. I-30. Culturing for bacteria after euthanization, using the dorsal approach. A. After anesthetization the dorsal fin is clipped to reduce possible contamination. B. The surface of the back is decontaminated with antiseptic and then dried with a dry, sterile gauze pad. C. The back is cut with sterile scissors. Care is taken not to cut so far as to enter the peritoneal cavity. This step is the most likely time for contamination to occur. D. Whole-body cross-section through a fish. Note that the kidney (*K*) is ventral to the vertebral column (*V*), which must be severed before reaching the kidney. The swim bladder (*S*) is ventral to the kidney. *P* = viscera in the peritoneal cavity, including intestine (*I*). Skeletal muscle includes white muscle (*WM*) and red muscle (*RM*). E. Reflecting the body ventrally (fish in Fig. I-30, *C*) to expose the kidney for culture. F. Entrance into the kidney is indicated by the appearance of a large amount of hemorrhage because of the highly vascular nature of the kidney. The collapsed, white swim bladder (*S*) lies ventral to the kidney; it is not clearly visible on all fish.

Continued.

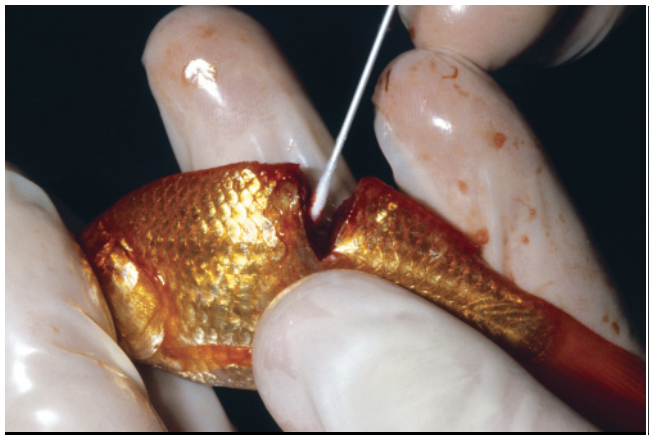
G   H

Fig. I-30.—cont'd. G. Touching a sterile Culturette to the kidney and being careful not to touch other areas, which would cause sample contamination. H. Inoculating a Columbia blood agar plate with the sample, using a Mini-Tip Culturette (Becton-Dickinson) and spreading the inoculum.

Table I-5. Diagnostic usefulness of different tissue preservation techniques for identifying fish pathogens. Note that the ability to recover various pathogens varies greatly; these comparisons are only intended as general guidelines.

Specimen	Protozoan ectoparasites†	Monogenean ectoparasites†	Metazoan parasites (except monogenea)†	Myxozoa and microsporea†	Viral isolation	Bacterial isolation	Gene probe‡	Antibody Probe or histologic value
Live fish	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+++	+ + +
Dead fish[1]	–	–	+ +	+ +	+	–	–	–
Iced fish[2]	+	+ +	+ + +	+ + +	+ +	+	+	+
Frozen fish[3]	+	+	+ +	+ +	+ +	+ +	+++	+
Fixed fish[4]	+ +	+	+	+ +	–	–	+++	+ + +

[1]Dead fish left in water at room temperature for 6–12 hr.
[2]Live fish placed in a plastic bag on wet ice for 6–12 hr.
[3]Live fish placed in a plastic bag frozen at –20°C.
[4]Tissues from a live fish immediately placed in 10% neutral buffered formalin.
+ + + = best; – = virtually useless.
†Comparisons between live, dead, iced, and frozen fish are based upon the ability to identify pathogens in wet mounts; diagnostic usefulness of fixed fish is based upon the ability to identify pathogens in histological sections.
‡For gene probe, rankings of dead, iced, and frozen fish are based upon the ability to detect pathogens using gene amplification (PCR); detection in fixed fish is based upon the ability to identify pathogens via in situ hybridization (ISH). For ISH, tissue should be transferred to 70% ethanol after fixing in formalin for 24 hours.

Culture swabs in transport medium can be shipped immediately to a laboratory. However, the reliability of this method for most fish pathogens has not been determined. Also, since mixed cultures are common in fish lesions, more rapidly growing opportunistic pathogens can overgrow slower-growing pathogens before the sample is plated onto the culture medium.

Culturing Skin Lesions

Skin lesions are common in many bacterial diseases, and some bacterial diseases begin as primary skin infections. It can be difficult to determine the initiating agent because lesions are often overgrown by secondary invaders. It is important to sample early lesions whenever possible to determine the predominant organism, since the latter is often the initiating agent.

To avoid contamination of the sample, it is best to culture skin lesions on fish that have not yet had any other clinical procedures performed. Skin lesions can be cultured with a loop, but it is easier to isolate single colonies when the following procedure is used:

1. Place a sterile, 1 µl volume loop into the leading edge of the skin lesion. It can be useful to aseptically remove some scales from the edge of the lesion to be sure that the leading edge is sampled; however, this is usually not needed.
2. Immediately inoculate the material on the loop into a small, 4 mm^2 area on the periphery of a culture plate.
3. Using a sterile Mini-Tip Culturette (Becton-Dickinson), immediately swab the inoculated area onto half of the plate; then pull the streak across one-quarter of the plate and then across the final quarter of the plate. This procedure almost always results in

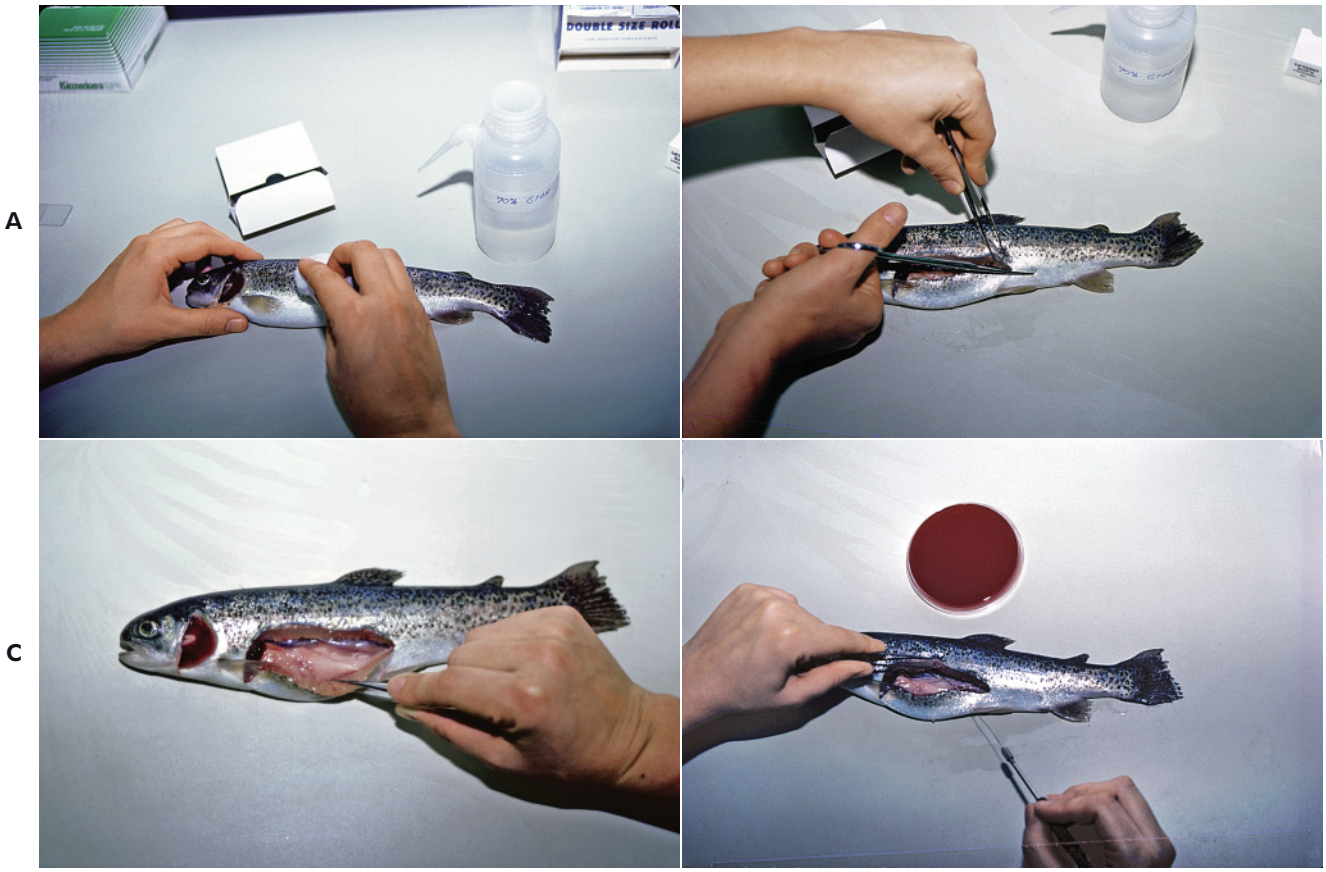


Fig. I-31. Culturing for bacteria after euthanization, using the ventral approach. A. After euthanasia, the flank is swabbed with antiseptic, avoiding the anus and any skin lesions. The area is then dried with a dry sterile gauze pad. B. The body wall is cut with sterile scissors. Care is taken to avoid the anus and to cut close to the body wall to prevent severing the intestine. C. Viscera are aseptically reflected, exposing the swim bladder (also see Fig. I-30, *D*). The swim bladder must be cut or reflected to reach the kidney. D. The kidney is often covered by a tough fibrous capsule, which must be severed to enter the parenchyma.

the isolation of single colonies and also allows you to estimate the number of bacteria present in the lesion (Fig. I-32).

The importance of detecting skin damage early in bacterial infections is exemplified by the studies of Elliott and Shotts (1980), who found that *Aeromonas salmonicida*, the primary bacterial pathogen of ulcer disease of goldfish, could only be isolated from the earliest stages of the disease (i.e., small lesions). Being able to identify and thus culture the earliest lesions, which may not even be visible to the naked eye, improves the ability to identify important pathogens. This might be facilitated by use of the fluorescein test (see **p. 35**).

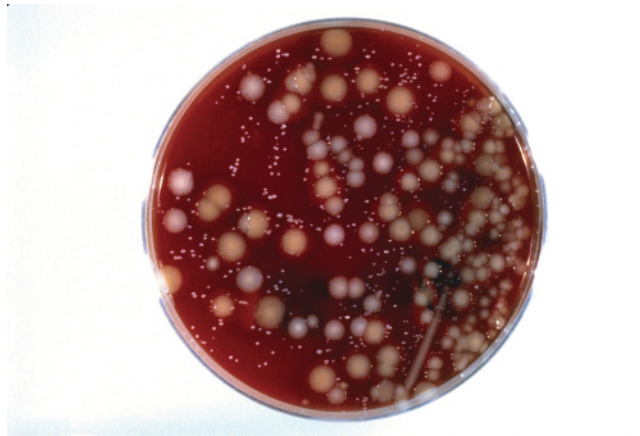


Fig. I-32. A blood agar plate from a skin lesion of a fish having well-isolated bacterial colonies.

Dorsal Approach to Kidney

The fish is euthanized and the dorsal fin is clipped off. The surface of the back is decontaminated either by

swabbing the area with antiseptic (e.g., quaternary ammonium or 70% alcohol) or by searing the skin with a flat, metal object (e.g., knife or spatula) heated in a flame. If an antiseptic is used, the skin should be wiped dry with sterile gauze. Sterile scissors or scalpel is then used to cut into the decontaminated area. The incision should be made just deep enough to cut through the vertebral column. Cutting deeper may enter the peritoneal cavity, possibly rupturing the intestines and contaminating the sample.

The exact incision site varies slightly, depending on the species, but generally, an incision is made just posterior to the dorsal fin. Another useful landmark is to cut at one-quarter of the distance from the anus to the posterior edge of the operculum. Once the incision has been made, the head and tail of the fish should be bent downward (ventrally) to expose the kidney (see Fig. I-30, E). The kidney lies immediately beneath the vertebral column and appears as a dark red, bloody area (see Fig. I-30, F). If the incision is not deep enough, that is, if the incision is only into the epaxial (upper body) muscles, almost no blood will be present, since muscle has much less blood supply than the kidney. Once the kidney is exposed, a sample then can be taken with a sterile loop or a disposable swab (Mini-Tip Culturette, Becton-Dickinson).

Ventral Approach to Kidney

The fish is swabbed with antiseptic, avoiding the anal area, and placed in lateral recumbency. The peritoneal cavity is opened, using aseptic technique, and the body wall is cut away (see Fig. I-31, B). The kidney is reached by gently pushing the viscera in the peritoneal cavity to one side and deflecting the swim bladder away from the vertebral column. This part of the procedure is best done with a sterile, blunt probe. The kidney runs the entire length of the peritoneal cavity, just ventral to the vertebral column. Note that on large fish, you may need to use a scalpel to cut the membrane that separates the kidney from the swim bladder. A loop or swab may be used for culture. This material can then be immediately streaked onto a culture plate or shipped on ice to a diagnostic laboratory if it is placed in a transport medium. Alternatively, a piece of kidney may be removed and placed in a sterile syringe barrel or red-top Vacutainer (Becton-Dickinson) tube; unless it is plated immediately, the specimen should be frozen for shipment to the laboratory.

Culturing Other Viscera

The ventral approach can also be used for sampling other organs, such as the spleen and liver. If the peritoneal cavity has not been entered aseptically, the surface of the organ to be sampled can be seared with a hot scalpel blade and then a loop can be inserted through the seared tissue until unheated tissue is reached; this is only possible with large fish. For smaller fish, whole organs are removed aseptically, and a loop is used to streak the tissue across a plate.

Rapid Screening for Antibiotic Susceptibility

Bacterial infections can spread rapidly through a population, and it is important to treat fish with an appropriate antibiotic as soon as possible, since a matter of a day or two can be crucial. It can thus be useful to rapidly screen for antibiotic susceptibility. This is only a qualitative test at best and does not substitute for a properly performed sensitivity assay. However, it can provide some indication of the best antibiotic to use while the proper test is being performed. A pure culture of the bacterium (i.e., it has been purified by picking a colony from the original plate used for isolation and streaking on another plate) should be used. This simplified test is adapted from Collins (1993):

1. Dampen the tip of a sterile swab with sterile saline. (The condensation water on the lid of a sterile bacterial culture plate can be used if it is not contaminated.)
2. Pick a single bacterial colony with the tip of the swab, and spread it as evenly as possible across the whole surface of the agar. An agar designed to perform sensitivity tests (e.g., Mueller-Hinton agar) is best used, if possible.
3. Use sterile forceps to evenly distribute antibiotic sensitivity disks on the surface of the agar. Be sure the disks are firmly placed on the agar. Disks are available commercially (e.g., Fisher).
4. Replace the lid on the agar plate, and let it stand for a few minutes to ensure that the disks adhere. Then carefully invert the plate and incubate.
5. An inhibition zone of 15–16 mm suggests resistance; sensitive fish pathogens typically have clearing zones of at least 20 mm. These results may vary with the type of disk, antibiotic, agar medium, and thickness of the medium. Testing three to four isolates is advisable.

Because this procedure is not quantitative, it is most useful in ruling out use of an antibiotic (i.e., if there is no detectable inhibition zone) rather than indicating which antibiotic might be best.

Submitting Bacterial Cultures to a Diagnostic Laboratory

When having a laboratory perform antimicrobial susceptibility testing, it would be advisable to have them follow the guidelines developed by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS, www.clsi.

org). The CLSI document M42-P, *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Proposed Guideline*, provides the most up-to-date techniques for disk diffusion susceptibility testing of aquatic species isolates, while *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Proposed Guideline* (M49-P) provides a standardized broth dilution method for determining minimal inhibitory concentrations (MICs) of aquatic bacteria by broth micro- and macrodilution. Using these methods will allow a more accurate comparison of the susceptibility results with those of other laboratories and will thus give a more accurate indication of the isolate's true susceptibility or resistance.

If the laboratory is not highly familiar with fish pathogens, they should also be advised that the great majority of fish pathogens are aerobic and Gram-negative. Note that in some countries (e.g., the United States), many antibiotics are available without prescription in aquarium stores and many are used by wholesalers. In such cases, aquarium fish might have been exposed to many antibiotics prior to submission to the clinician.

For organisms that are difficult to identify or for confirmation of phenotypic characteristics, the ribosomal RNA gene can be sequenced by a commercial lab such as Microbial ID.

SAMPLING FOR WATER MOLDS AND FUNGI

Culture of this group is rarely needed in routine fish disease diagnoses because the most common pathogen isolated, the fungus-like water molds (Oomycetes, see PROBLEMS 34 and 35), can be diagnosed without culture. However, true fungi (see PROBLEM 72) usually require culture for definitive diagnosis. Culture for non-Oomycetes is not usually done unless typical fungus-like organisms are seen either in wet mounts or via histopathology (see PROBLEM 72).

For fungal or water mold culture, plates should be inoculated with a small (approximately 12 mm^3) mass of infected tissue and incubated at room temperature. Once growth of the fungus or water mold is noticeable (usually within several days), it is advisable to transfer the growing edge of the mycelium to a fresh culture plate by aseptically excising a small portion of the agar containing the leading edge of growth. This procedure will help to eliminate any bacterial contaminants that were introduced with the tissue sample.

For non-Oomycetes (true fungi), potato flake agar is a good, commercially available, general-purpose medium for isolating almost all of these pathogens (Sutton et al. 1998). If a non-Oomycete fungal pathogen is suspected, tissue samples should be inoculated onto slants and incubated at room temperature. Be aware that airborne fungal spores can often contaminate cultures; thus, be certain that the type of fungus isolated in culture is morphologically similar to the type of fungus present in the lesions. If the fungus will not grow on the general-purpose medium, other, more specialized media can be tried (see Hatai 1989), or samples can be referred to a specialized laboratory. See PROBLEM 72 for more details about isolation.

Oomycetes are best isolated by using cornmeal agar, YpSs, or another nutrient-poor medium to inhibit growth of contaminating bacteria (Seymour and Fuller 1987). While Oomycetes are usually easily isolated, culturing Oomycetes from bacteria-infected lesions may be difficult because bacteria inhibit Oomycetes, especially slow-growing forms, such as *Aphanomyces* (see PROBLEM 35). In heavily contaminated lesions, adding penicillin (approximately 500 U/ml) and/or streptomycin (approximately 0.2 µg/ml) may improve yields. However, while *Saprolegnia*, the genus most commonly isolated from fish, is usually not significantly inhibited, some Oomycetes (especially *Aphanomyces*) are significantly inhibited by antibiotics (Dykstra et al. 1986).

SAMPLING FOR VIRUSES

Definitive diagnosis of viral infection relies on genetic or immunological identification of the pathogen. Such procedures are best left to competent laboratory personnel who specialize in such techniques. However, reliable use of those techniques depends upon the submission of high-quality samples. Different viruses vary in their abilities to survive preservation procedures; specific recommendations are given for specific viral diseases in the problem list. However, in general, live fish are best submitted when a virus is suspected and the specific agent is uncertain. Otherwise, fish on wet ice or dry ice should be sent immediately by overnight mail. Wet ice is best, but samples should not be stored this way for longer than 48 hours. Fish that cannot be sent immediately should usually be frozen at the lowest temperature possible, although it is best for some viruses to store samples at 4°C if processing will occur in a few days. The types of samples to be collected from various sizes of fish are shown in Box I-3.

EXAMINING TISSUES POSTMORTEM

Circumstances permitting, it is always desirable to do a complete necropsy on selected individuals. Four to six fish showing clinical signs that are typical of the outbreak should be necropsied, if possible. While necropsy may not be possible with highly valuable fish, it is mandatory when a clinician performs an examination of a large fish population that includes expendable fish.

❧ Box I-3 ❧

SAMPLE COLLECTION FOR VIRUS IDENTIFICATION (FROM LAPATRA 2003).

Fish size	Tissues assayed
<4 cm	Entire fish (remove yolk sac)
4–6 cm	Entire viscera (including kidney)
>6 cm	Kidney, spleen, gill filaments
Sexually mature	Ovarian fluid, kidney, spleen, gill filaments

Note: Samples can be pooled, but no more than five fish should be pooled in one sample of tissue or fluid. Pool similar volumes or weights.

Tissues are best stored in a buffer at pH 7.4–7.8 (or within the optimal stability range of the suspected virus). Adding antibiotic is advisable if the tissue is significantly contaminated.

Condition of Tissue

The diagnostic usefulness of the postmortem examination is highly dependent upon the quality of specimens presented (see Table I-5). Whenever possible, live fish should be examined. Owners may present fish that have recently died for diagnosis; however, such fish are often of no diagnostic value. Fish decompose much more rapidly than mammals under similar conditions; this is especially true for small fish. Most ectoparasitic protozoa and Monogenea (see PROBLEMS 17 and 19) die within minutes to hours of host death, depending on temperature and parasite species. Larger parasites, such as copepods (see PROBLEM 14) or branchiurans (see PROBLEM 15), may be detectable for longer periods. Bacterial invasion of both skin and internal organs occurs rapidly after death, making interpretation of culture results difficult. Finally, because fish tissues autolyze rapidly, histological evaluations are compromised.

If submitting live fish is not an option, animals can be put in a plastic bag and placed on wet ice. Again, the diagnostic value of the tissues will deteriorate with time; fish should be examined within several hours of death.

If fish cannot be submitted within several hours, euthanized fish should be frozen immediately. Most ectoparasitic protozoa and Monogenea will usually not be recognizable after freezing, but the macroscopic host response to some protozoa may be visible (e.g., white cysts of *Ichthyophthirius*).

Protozoan ectoparasites and Monogenea usually cannot be identified from wet mounts of chemically preserved (fixed) tissue. Most parasites are recognizable in histological sections, but many ectoparasites detach from the skin and gills during processing, so they may be difficult to find in sections. Granulomas (see Fig. I-45) are easily seen in wet mounts of fixed tissues. Affected tissues can then be histologically processed for a diagnosis. Histology is useful for differentiating many of the diseases affecting internal organs, such as a number of parasites (Bruno et al. 2006).

Necropsy Procedures

Skin and gill examinations should be done as described for biopsy procedures. It is often advisable not to euthanize fish until the skin and gill examinations have been completed because of the aforementioned problems with decomposition. If bacterial cultures are to be taken, these should be done next, as described previously.

After euthanization, place the fish in lateral recumbency, and make a longitudinal incision along the ventral midline from the anal opening to just ventral to the gill chamber. This incision will extend from the posterior peritoneal cavity into the pericardial sac. Make latitudinal incisions at both ends of this previous incision that extend to the dorsal aspect of the body cavity. Reflect the body wall dorsally, exposing the viscera (Fig. I-33).

If fluid is present, make smears as described for blood sampling. Identify and examine the intestines, liver, spleen, gonads, and heart. Reflect the swim bladder ventrally and examine the anterior kidney and posterior kidney (Fig. I-34). The braincase is entered by using a

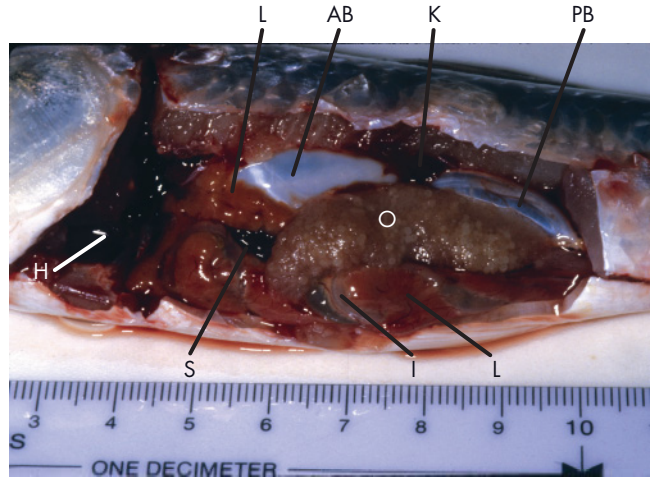


Fig. I-33. Gross anatomy of the viscera of a fish (koi). *H* = heart; *L* = liver, which has several lobes covering the intestine (*I*); *O* = ovary, which is large because this fish was almost ready to spawn; *K* = kidney; *S* = spleen. Note that the swim bladder has anterior (*AB*) and posterior (*PB*) chambers. This is characteristic of cyprinid fish, but other fish have a single chamber. Note that fine connective tissue tags are normally present in healthy koi and should not be mistaken for pathology (adhesions).

pair of sharp scissors to reflect the dorsal cranium anteriorly (Fig. I-35, A and B). After visual inspection, fine scissors and forceps are used to remove the brain in toto (Fig. I-35, C). Direct smears of various tissues can be stained for bacteria, although it is best to stain histological sections appropriately (e.g., Brown and Brenn's Gram stain) so that host response and tissue damage can also be evaluated.

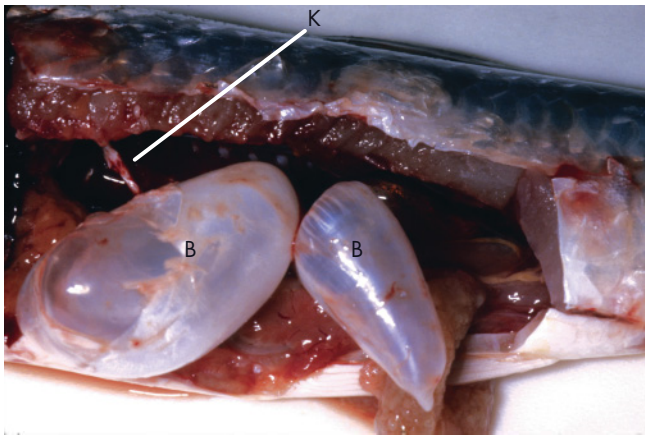


Fig. I-34. Viscera and swim bladder (*B*) in Fig. I-33 have been reflected, revealing the kidney (*K*).

Fixation Procedures for Histology

A 1 cm^3 portion of each lesion and of each organ should be placed in fixative. Even small fish should be dissected to expose internal organs to fixative, although very small fish (<5 mm or 0.2 inch) can usually be fixed in toto without autolysis artifacts. The fixative of choice for routine diagnosis is 10% neutral buffered formalin. Bouin's fluid is considered by some to provide better fixation, but it has several disadvantages (potentially explosive when dry, difficult to remove totally from fixed tissues, and damages fixed tissues if not completely removed) that reduce its attractiveness.

Tissues can be processed routinely using standard histological techniques and embedded in paraffin (Bucke 1989). Note that gills and scaled skin must be decalcified before sectioning. Hematoxylin and eosin and other standard stains can be used on fish tissues. Thus, samples can be submitted to mammalian histopathology laboratories. Atlases of normal fish histology have been published for a number of fish, including channel catfish (Grizzle and Rogers 1976), salmonids (Takashima and

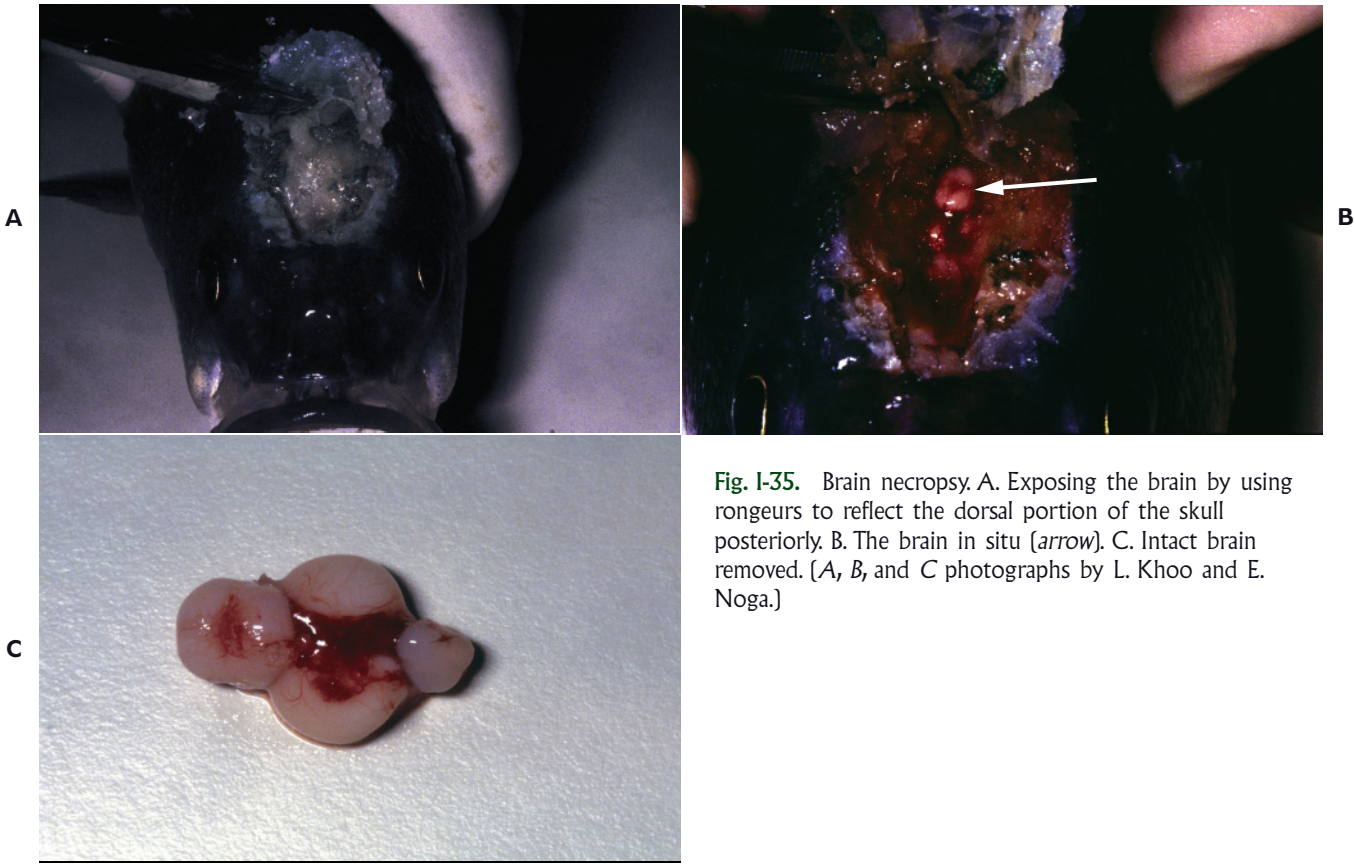


Fig. I-35. Brain necropsy. A. Exposing the brain by using rongeurs to reflect the dorsal portion of the skull posteriorly. B. The brain in situ (*arrow*). C. Intact brain removed. (*A*, *B*, and *C* photographs by L. Khoo and E. Noga.)

Hibiya 1995), tilapia (Morrison et al. 2006), and Atlantic cod (Morrison 1987).

Fixation Procedures for Antibody and Gene Tests

Immune (antibody)-based and gene-based tests are becoming increasingly common for both the rapid identification of a pathogen and for confirmation of the presence of a pathogen (Cunningham 2004; Austin and Austin 2007). This often circumvents the need to isolate/culture the pathogen but rather may allow its detection directly in infected tissue.

Fresh tissues can be used to test for the presence of a pathogen using either an antibody probe or a gene probe. However, fixed tissues are often used since such specialized tests are usually performed by a reference laboratory that might be some distance from the clinician. For antibody tests, tissues are routinely fixed in formalin followed by storage in 70% ethanol. For some tests (e.g., FAT or IFAT), tissues are best tested when frozen. For gene probe tests, tissue samples for PCR may be placed in 70–90% rubbing alcohol (isopropanol). A review of the types of gene probes used to identify fish pathogens is provided in Altinok and Kurt (2003). The effect of various forms of preservation on use of samples in a gene test is summarized in Table I-5. The clinician should consult with the laboratory performing the test to determine the optimal sample preparation.

Wet Mount Procedures

It is often useful to make tissue squashes, especially of kidney, spleen, liver, or any lesions. Small fish can be squashed whole or the entire viscera can be removed and squashed. To make a tissue squash, excise a small (approximately 8 mm^3) piece of tissue and place it on a slide with a drop of water or normal saline. Place the edge of a plastic coverslip near the tissue, and then gently squash it (Fig. I-36). Examine the tissue architecture under low (100×) magnification and look for parasites and granulomas; then crush the tissue into a thin smear and examine it at 100× and high dry (400×) magnification to identify protozoa and bacteria. When examining wet mounts, the condenser should always be adjusted to obtain maximal contrast (see Fig. I-6, H, I).

Structure of Normal Tissues

The viscera of fish are generally similar to those of mammals, but certain peculiarities should be recognized. Small fish, such as most aquarium fish, have little connective tissue stroma, making the viscera flaccid and coincidentally facilitating the preparation of wet mounts. Note that squashes are most easily made from (and thus most useful in) organs of small fish. Organs of large fish (>20–25 cm [>8–10 inches]) have more connective tissue and are harder to squash. Pigmented cells can be a normal finding in virtually all organs and are especially common in hematopoietic tissues. The peritoneum of many fish is lined with melanocytes. Aggregates of pigmented cells, the melanomacrophage centers, are also common (see Fig. I-38).

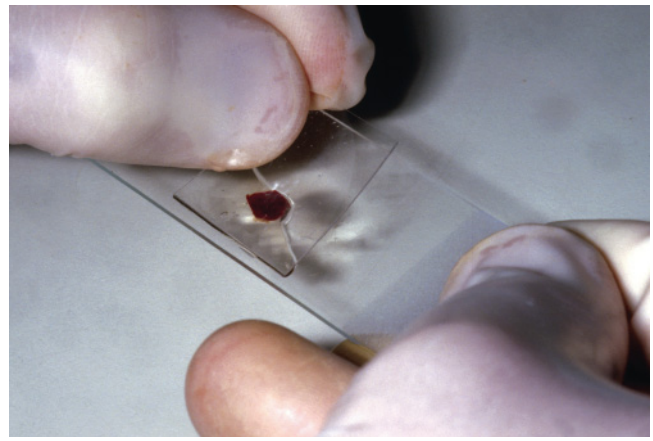


Fig. I-36. Squashing tissue for a wet mount.

Key Features of Internal Organs

Intestine—The intestinal tract is usually the first organ seen when the peritoneal cavity is opened. However, body fat is most commonly deposited in the peritoneal cavity and may obscure the viscera. The intestinal tract is a straight, thin-walled tube. In many aquarium fish the lumen is too small to be easily cut open, but in such fish the intestinal contents can often be seen through the wall (Fig. I-37, A through C). The intestine should be opened after the other viscera have been examined to reduce contamination by bacteria and other organisms. The stomach is larger than the intestines. The presence or absence of food in the intestinal tract is easily assessed. The pancreas, like most glands, is usually not grossly visible (Fig. I-37, D).

Liver—The liver is a brown to red-brown to tan organ in the anterior portion of the peritoneal cavity. Microscopically, normal liver has a homogeneous appearance; an occasional melanomacrophage center may be seen (Fig. I-37).

Gall Bladder—The gall bladder is a large, translucent sac with green or yellowish fluid. It lies close to the liver and is often large (i.e., it is often larger than the spleen), especially if the fish has been anorexic. It may be accidentally ruptured when the peritoneal cavity is opened, tainting the viscera yellow-green.

Spleen—The spleen is a bright red to black organ located in the mesentery. Microscopically, normal spleen

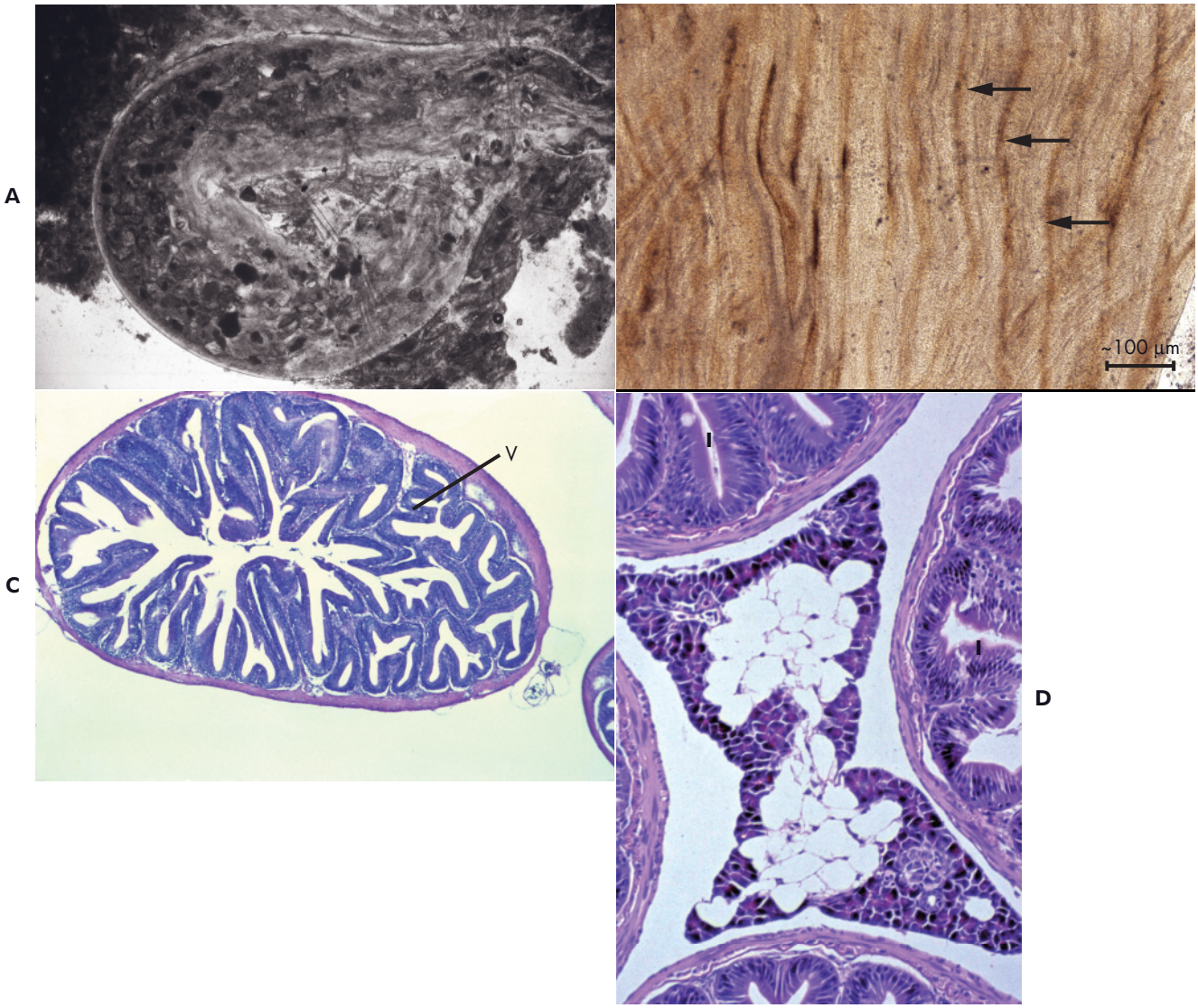


Fig. I-37. A. Wet mount of normal intestine of a small (~2.5 cm) fish. The intestine is thin walled, and the luminal contents are easily seen. B. Wet mount of intestine showing rugae, or folds (*arrows*), which are composed of villi. C. Histological cross-section of normal intestine. *V* = villus. Hematoxylin and eosin. D. Histological section of normal exocrine pancreas with adjacent intestine (I). (*B* photograph by L. Khoo and E. Noga; *D* photograph courtesy of M. McLoughlin.)

has a reticulated appearance because of the network of ellipsoids that are the sites of blood filtration (Fig. I-39).

Gonad—The reproductive organs may be difficult to see in fish that are not sexually mature. In immature fish, the reproductive organs are ribbon-like, grey-white or yellow strips that usually lie just ventral to the swim bladder. In some fish that are ready to spawn, the ovaries may occupy most of the peritoneal cavity and cause gross abdominal distension. Even in immature fish, sex can often be determined by examining a wet mount, which may reveal the presence of sperm (Fig. I-40) in a male or follicles (Fig. I-41, A and B) in a female.

Swim bladder—The swim bladder is a white, shiny organ that lies near the back (dorsum), just ventral to the kidney. Filled with gas, its primary function is to maintain buoyancy.

Kidney—The kidney is a retroperitoneal organ that is functionally (and often morphologically) divided into two segments. The anterior kidney is the primary site of hematopoiesis; it is a dark red to black, soft amorphous tissue that has the consistency of bone marrow

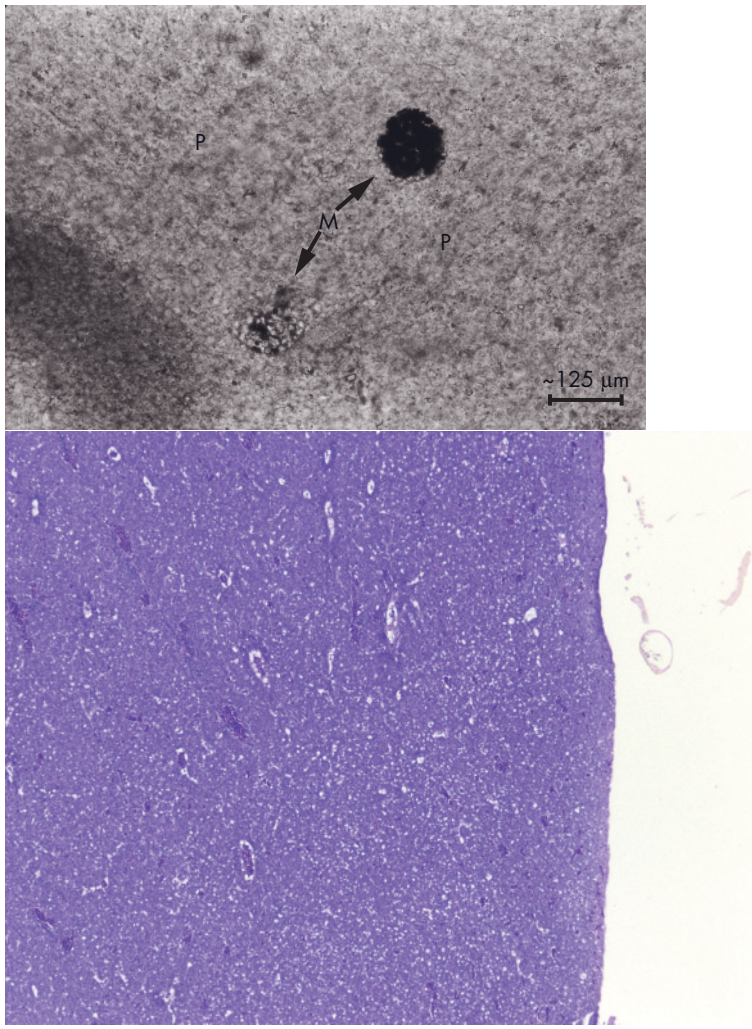


Fig. I-38. A. Wet mount of normal liver. Note homogeneous parenchyma (*P*) and aggregates of pigmented macrophages, the melanomacrophage centers (*M*). B. Histological section of normal liver. Hematoxylin and eosin. (*B* photograph by L. Khoo and E. Noga.)

(Fig. I-42, A and B). The posterior kidney has a similar gross appearance but has renal excretory tissue as well (Fig. I-42, C, D, and E).

Heart/Skeletal Muscle—The heart lies in the pericardial cavity, which is just anterior to the peritoneal cavity in the throat region of the fish. It is a red, highly muscular, two-chambered organ. It empties into the ventral aorta via the white, elastic, bulbus arteriosus. Wet mounts of normal skeletal or cardiac muscle will reveal individual muscle fibers with striations (Fig. I-43).

Brain—The brain is superficially similar to those of mammals, with morphological differentiation of various neural centers. Microscopically, it appears as a grey-white organ that has an amorphous appearance on wet mount.

Glands—Most of the major glandular tissues found in mammals occur in fish; they are only detectable histologically, except for thymus (Fig. I-44). Analogues of the adrenal cortex (interrenal cells) and adrenal medulla (chromaffin cells) are found in the anterior kidney. Pancreatic exocrine and endocrine tissues are usually dispersed throughout the mesentery or may be associated with the liver or spleen. Thyroid tissue is usually dispersed around the ventral aorta but may also be found in the kidney, spleen, or mesentery.

Common Lesions Found in the Viscera

Necropsy can provide information on nutritional status. Aquarium fish are often overfed, resulting in excessive accumulation of fat in the peritoneal cavity. In fish that are fed unbalanced diets, the liver may be pale yellow because of lipidosis. The significance of obesity in pet fish is uncertain, but excessive lipid deposition is commonly associated with clinical disorders in food fish, such as trout (see PROBLEM 89). However, note that normal liver color varies considerably among species; it also varies seasonally, so it is necessary to be aware of the normal physiological color variation for a particular species.

Fluid accumulation in the abdomen ("dropsy"; see Fig. I-3, B, C) is a common clinical presentation. It can

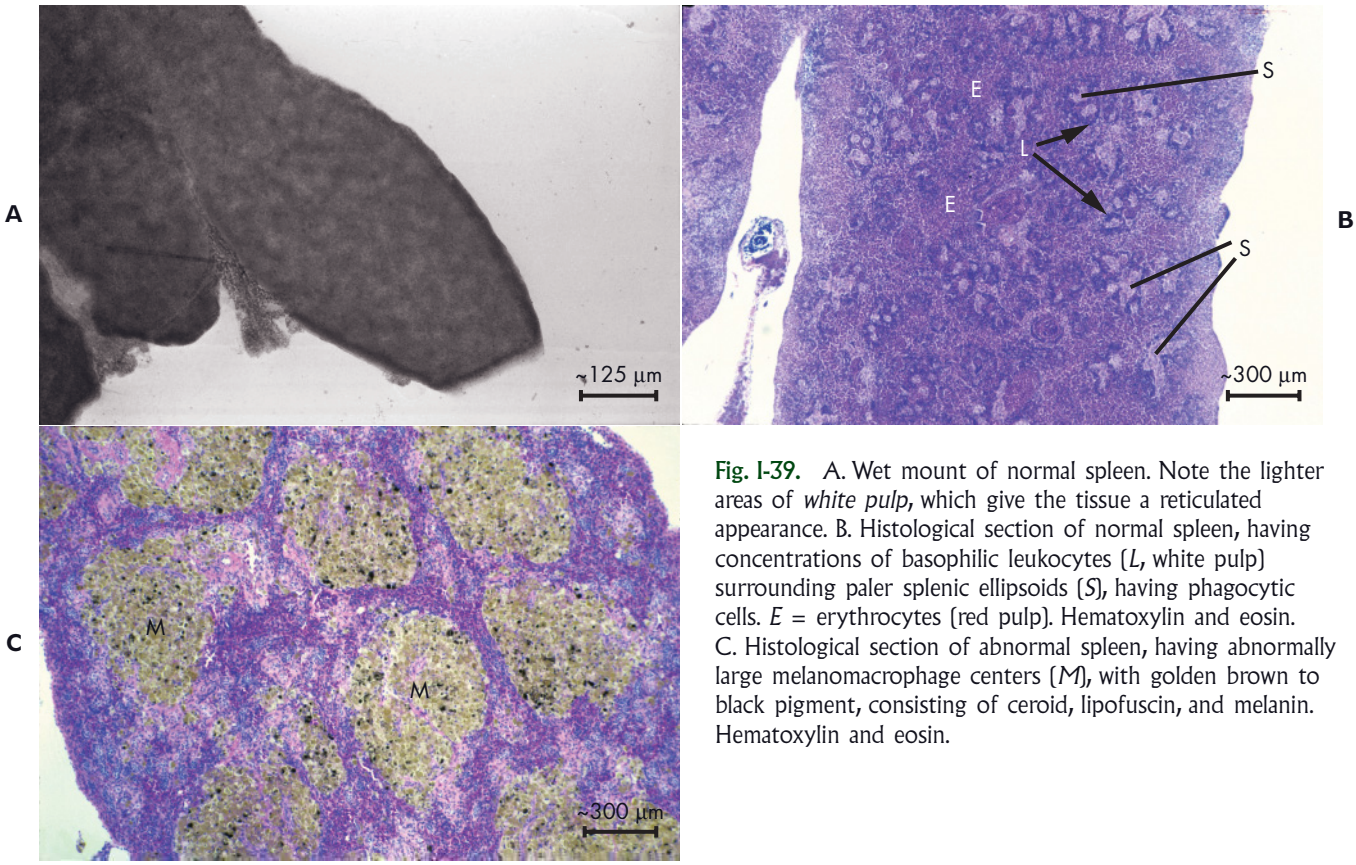


Fig. I-39. A. Wet mount of normal spleen. Note the lighter areas of *white pulp*, which give the tissue a reticulated appearance. B. Histological section of normal spleen, having concentrations of basophilic leukocytes (*L*, white pulp) surrounding paler splenic ellipsoids (*S*), having phagocytic cells. *E* = erythrocytes (red pulp). Hematoxylin and eosin. C. Histological section of abnormal spleen, having abnormally large melanomacrophage centers (*M*), with golden brown to black pigment, consisting of ceroid, lipofuscin, and melanin. Hematoxylin and eosin.

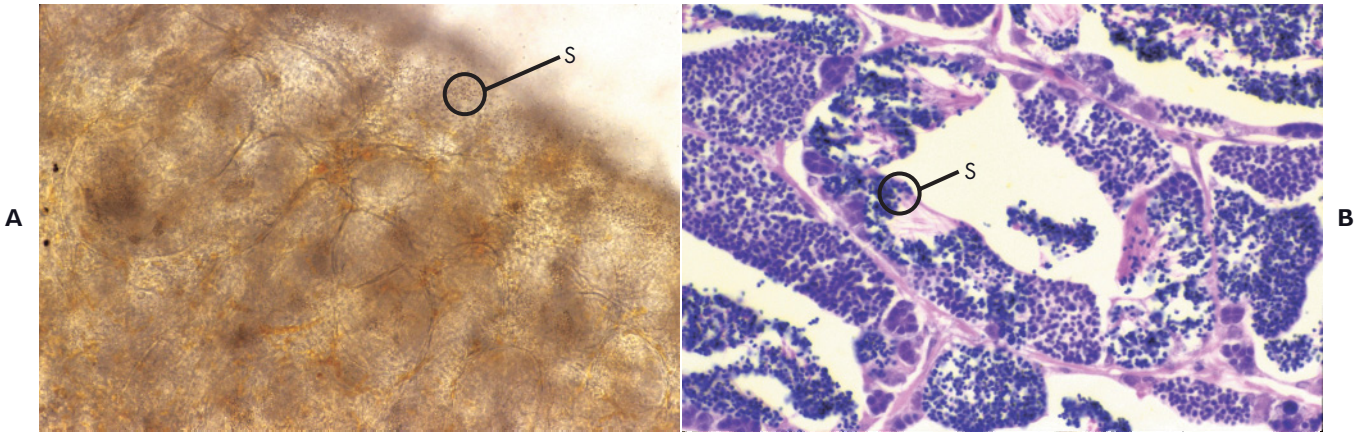


Fig. I-40. A. Wet mount of normal testis. Note individual spermatozoa (*S*) visible on the edge of the cut tissue. B. Histological section of normal testis filled with spermatozoa (*S*). Hematoxylin and eosin. (*A* and *B* photographs by L. Khoo and E. Noga.)

result from infection by viruses, bacteria, or parasites. Examination of abdominal fluid may reveal bacteria or parasites (e.g., diplomonad flagellates). Ascitic fluid may also form from osmoregulatory dysfunction. Hemorrhages in the viscera can be caused by systemic viral or bacterial infections.

Several chronic inflammatory diseases can affect internal organs. Among the most important is mycobacteriosis, which can affect virtually any internal organ. Granulomas produced by this pathogen must be differentiated from neoplasia (see PROBLEM 76), from foreign-body reactions produced against protozoan or

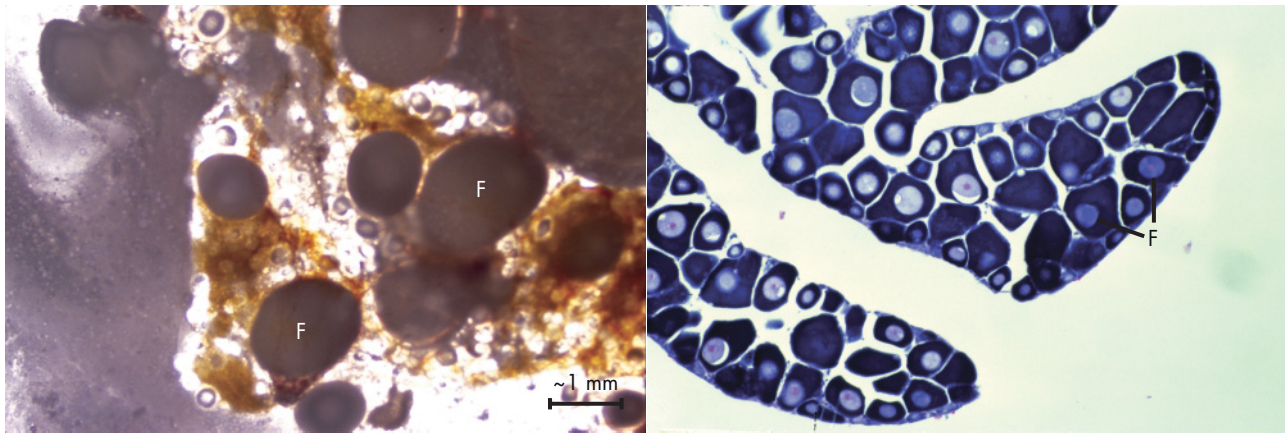


Fig. I-41. A. Wet mount of normal ovary. Compare with testes (Fig. I-40, *A* and *B*). Do not confuse follicles (*F*) with granulomas (see Fig. I-45). B. Histological section of normal ovary. *F* = follicles. Hematoxylin and eosin. (*A* photograph by L. Khoo and E. Noga.)

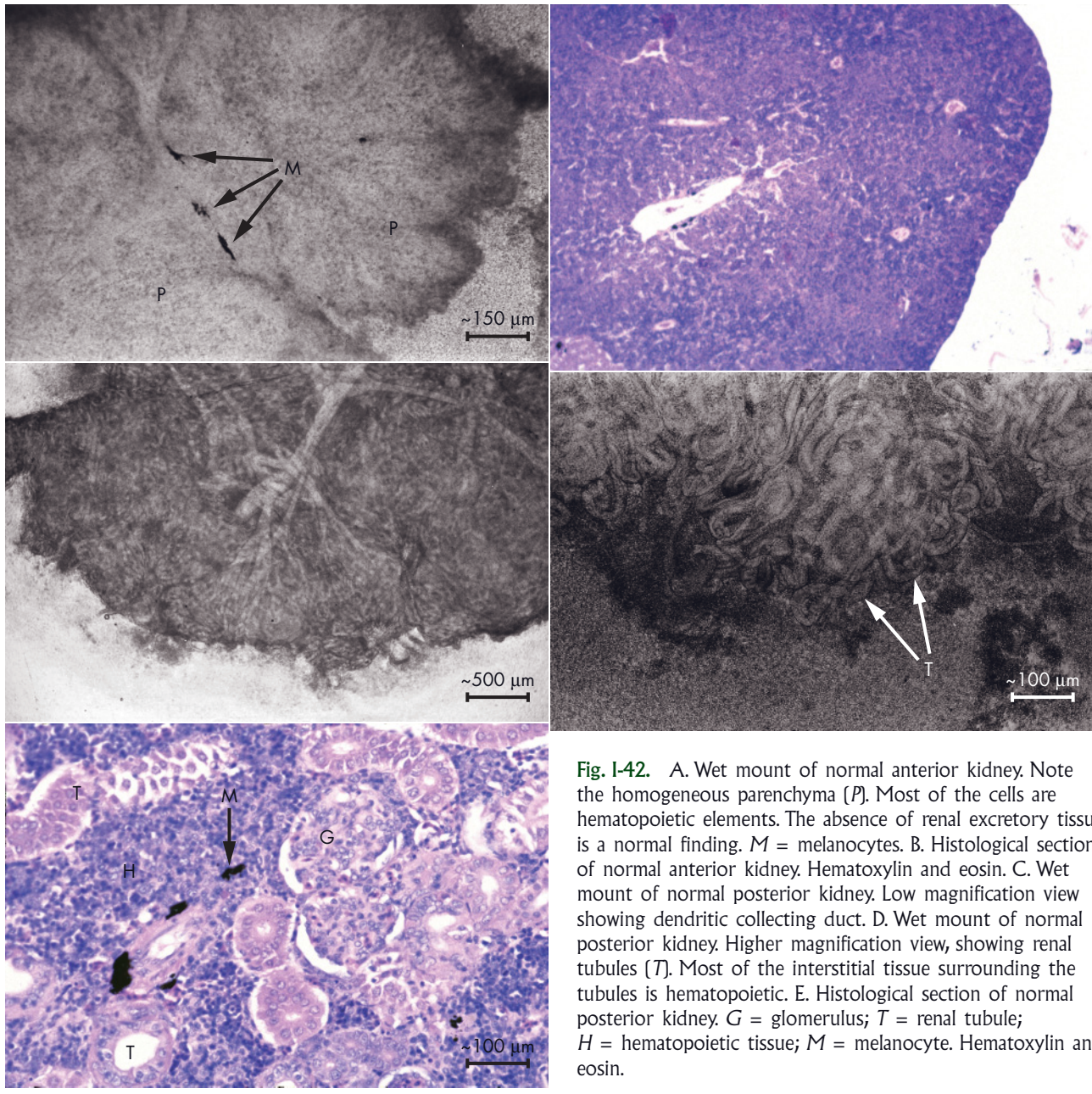


Fig. I-42. A. Wet mount of normal anterior kidney. Note the homogeneous parenchyma (*P*). Most of the cells are hematopoietic elements. The absence of renal excretory tissue is a normal finding. *M* = melanocytes. B. Histological section of normal anterior kidney. Hematoxylin and eosin. C. Wet mount of normal posterior kidney. Low magnification view showing dendritic collecting duct. D. Wet mount of normal posterior kidney. Higher magnification view, showing renal tubules (*T*). Most of the interstitial tissue surrounding the tubules is hematopoietic. E. Histological section of normal posterior kidney. *G* = glomerulus; *T* = renal tubule; *H* = hematopoietic tissue; *M* = melanocyte. Hematoxylin and eosin.

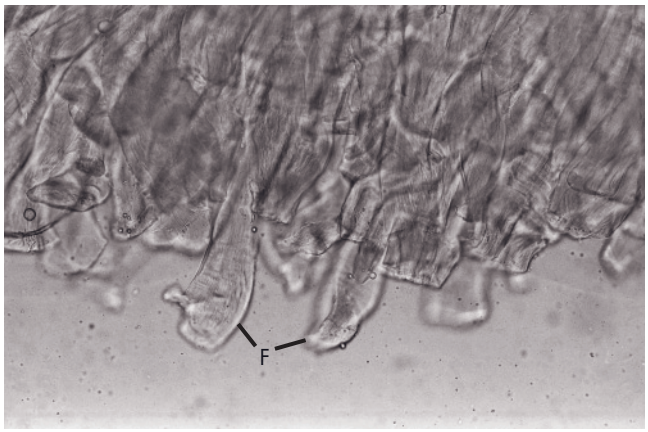


Fig. I-43. Wet mount of normal skeletal (striated) muscle. Note the individual fibers (*F*) with striations.

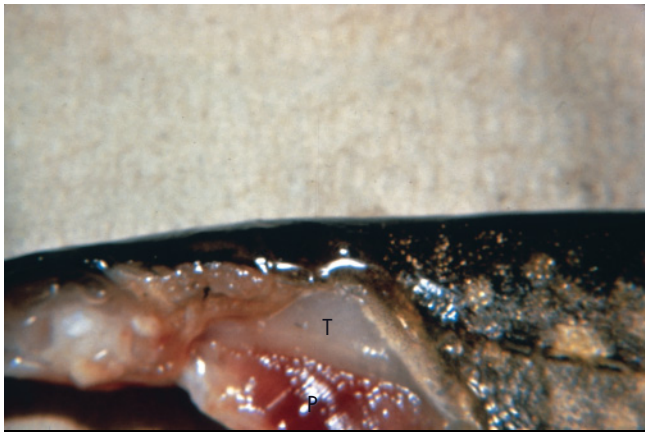


Fig. I-44. Thymus (*T*), located at the dorsomedial aspect of the gill chamber. Head is to the left. *P* = pseudobranch.

metazoan parasites, and from melanomacrophage centers.

Melanomacrophage centers (MMCs) are usually solid foci of cells that have varying amounts of pigment (see Fig. I-38). While these are common in healthy fish, they increase in number with chronic stress (Wolke 1992; Agius and Roberts 2003). Thus, MMCs are indicators of chronic stress; however, it is necessary to know the normal prevalence in a particular fish species to make an accurate diagnosis of chronic stress. Also, a relationship with chronic stress is not always evident (Haaparanta et al. 1996).

In contrast to melanomacrophages, granulomas are usually multilayered structures having a central zone of necrotic debris (Fig. I-45). This necrotic center is the most useful feature for identifying granulomas. It is important to recognize that granulomas may contain pigment, and melanomacrophage centers accumulate in many disease states. Thus, in some cases, histology may

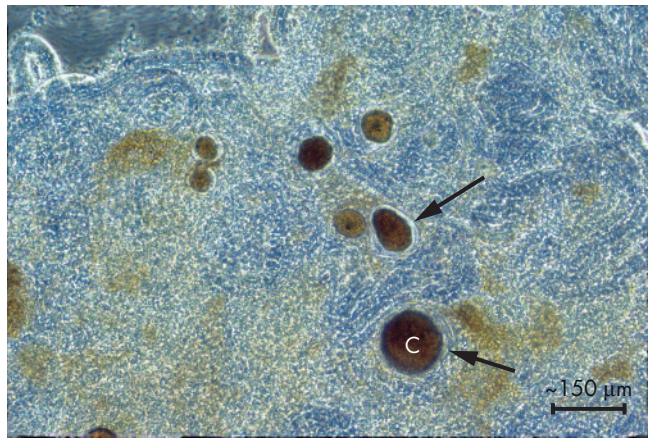


Fig. I-45. Granulomas in a wet mount. Note the dark, necrotic center (*C*) surrounded by lighter, viable, inflammatory cells (*arrows*).

be needed for differentiation, especially if other tests are negative.

Trematodes, nematodes, and cestodes, especially larvae, occur in the mesentery or viscera. Compared with mammals, internal helminths are much less serious problems in fish. However, some internal helminths can cause serious disease.

ZOONOTIC DISEASES AND OTHER HUMAN PATHOGENS

Zoonotic Pathogens

No viruses causing clinical disease in fish are transmissible to humans, although opaleye calicivirus, harbored asymptomatically by the opaleye, has very rarely caused skin lesions in humans (Smith et al. 1998). Relatively few bacterial diseases of fish are transmissible to humans. *Edwardsiella tarda*, some aeromonads (e.g., *Aeromonas hydrophila*), *Photobacterium damselae* subsp. *damselae*, and certain clones of *Streptococcus iniae* (see PROBLEMS 50 and 53) can infect the skin or cause gastroenteritis or systemic infections (Lehane and Rawlin 2000). However, the agent of most concern is *Mycobacterium* (see PROBLEM 55). The so-called "environmental" mycobacteria infect fish; these are the least pathogenic mycobacteria for humans, but some species, in particular *M. marinum* and occasionally *M. fortuitum*, can cause "fish tank granuloma," a chronic infection that is usually limited to the extremities (i.e., fingers and hands). Fortunately, incidences of zoonotic infections with fish pathogens appear to be uncommon events when compared with the relative risk of exposure to these agents. However, appropriate caution is warranted, especially in immunosuppressed individuals (Angulo et al. 1994). Several zoonotic helminths can infect humans (see

PROBLEMS 58, 60, and 61) but can only be contracted after ingestion of infected fish.

Environmental Pathogens

A number of bacteria that are never or rarely pathogenic to fish can infect humans because they can be in the aquatic environment or asymptomatically resident on fish. They are usually transmitted via skin injuries (e.g., due to contact with spines, scales, or teeth) or contamination of existing wounds, but gastrointestinal or systemic infections can also occur. They include a number of vibrios (including *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*), as well as *Photobacterium damselae* subsp. *damselae*. Note that *V. vulnificus* biogroup 1, the main biotype that infects humans, is not a fish pathogen (Lehane and Rawlin 2000). Similarly, the association of *V. cholerae* with fish disease is extremely rare and that of *P. damselae* subsp. *damselae* with fish disease is also relatively rare. Note that *Photobacterium damselae* subsp. *piscicida* (PROBLEM 51) is not a human pathogen. *Erysipelothrix rhusiopathae* has been isolated from the surface of many marine fish (Fidalgo et al. 2000). Any persons that handle fish are at greatest risk. It can cause a localized, reddish-purple lesion on the hands ("fish rose") but can also rarely lead to systemic infection. Home aquaria can be reservoirs of human-pathogenic *Salmonella*, including multidrug-resistant isolates (Levings et al. 2006), but the significance of this finding to salmonellosis in humans is unclear.

All of the bacteria mentioned under zoonotic pathogens may also infect humans via exposure to contaminated water rather than direct contact with fish. Despite the low incidence of infection risk with both zoonotic and environmental pathogens, protective gear (e.g., gloves) should be worn during the clinical workup to prevent human exposure.

CHAPTER 5

Guidelines for Interpreting Clinical Findings

ENVIRONMENT, STRESS, AND FISH DISEASE

The metabolic, biochemical, and physiological processes of fish are basically similar to those of mammals. Fish are susceptible to the same types of pathogens that affect warm-blooded animals, including viruses, bacteria, fungi, and parasites, as well as various noninfectious agents. However, stress appears to play a considerably larger role in causing disease in fish (Collins et al. 1976; Walters and Plumb 1980; Schreck 2000). Stress can be considered as a continuum of insults, varying from mild to severe (Fig. I-46). How much of an impact stress has on a fish depends on the severity and type of stress, its duration, and the physiological state of the fish, among other considerations. Thus, many diseases in fish stem from poor management; this important principle should always be kept in mind when trying to identify the true cause of a fish disease.

Good water quality is the key to successful fish production. Water quality includes all physical, chemical, and biological factors that influence the use of water for fish culture. Any characteristic of water that affects the survival, reproduction, growth, or management of fish is a water-quality variable. An abundant water supply solves many problems associated with intensive fish culture by diluting out accumulated wastes and toxic products, as well as by maintaining optimal water conditions. However, water is a precious and often limiting resource in aquaculture, and thus many methods have been developed to increase the holding capacity of culture systems, since commercial producers are usually trying to push carrying capacity to its limit.

ACCLIMATION

Acclimation is the physiological adaptation of an animal to a new environment. Acclimation is an important concept to understand in fish health because it helps explain why fish may get sick under one set of circumstances but may be perfectly healthy under exactly the same conditions at some other time.

A tank of fish in which the pH has slowly dropped from 7.0 to 5.5 over several months may appear normal; however, if the water is rapidly adjusted back to 7.0, many of the fish may die. Even though pH 5.5 is stressful and not healthy, many fish can tolerate such conditions if they are introduced to the environment slowly. Even though a pH of 7.0 is within the normal range for most freshwater fish, too rapid a return to normal will be dangerous. Thus, the prior environmental history is at least as important as the known tolerated environmental range for that fish.

With the chronic low pH stress described above, where environmental conditions gradually deteriorate, indirect effects of the stress are often seen; these may include failure to reproduce, poor growth, developmental anomalies, or, commonly, the presence of what are referred to as opportunistic infections; that is, diseases that develop when the fish's defenses are not up to par. As you review the problems in the diagnostic guide, you will notice that most of the environmental (water-quality) problems often occur concurrently with opportunistic infections. Most infectious diseases of fish probably take advantage, in one way or another, of compromised defenses; however, some pathogens readily do this. These particular agents are generally considered to have a relatively low pathogenicity for fish and thus can only flourish under immunocompromising conditions. Classical examples of such pathogens include the bacteria *Aeromonas hydrophila* (see PROBLEM 46) and flavobacteria (see PROBLEM 37), water molds (see PROBLEM 34), and the parasites *Trichodina* (see PROBLEM 22) and ectocommensal protozoa (see PROBLEMS 32 and 33). When such pathogens or other opportunists are encountered, look closely for a primary environmental cause.

Inability to acclimate explains why fish often become sick after being handled or transported. The stress created by handling, combined with exposure to new environmental conditions, can cause severe stress against which fish cannot compensate (see PROBLEM 97) (Wendelaar Bonga 1997).

HOW TO USE PART II, THE PROBLEM LIST

The problem list is organized in such a way as to greatly facilitate your ability to diagnose cases. Important: Note that starting on p. 83, **PROBLEMS 1 through 103 are**

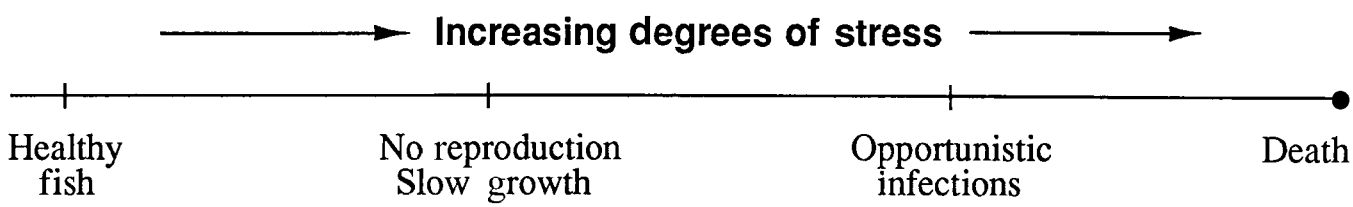


Fig. I-46. Relationship between environmental stress and fish health.

listed in the order that they are encountered in the clinical workup. This listing is summarized in Fig. I-1. Thus, as tests are performed or as tissues are examined, you should record the problems that are identified. This arrangement of problems also allows you to follow along in the diagnostic guide as various clinical techniques are performed.

Thus, problems that are identified from the history are listed first, followed by those that are made from the core water-quality examination, followed by those that are made from the external (skin and gill) examinations, followed by those that are made from the postmortem examination.

It is critical to realize that a fish may have more than one problem, and thus it is important to determine which is the most serious (i.e., which requires treatment first) and which is the primary cause of the disease (see "**Prioritizing Problems**," **p. 68**). In general, water-quality problems can often trigger the development of many infectious diseases, so water quality should often be improved as part of treating an infectious disease. Some infectious diseases are especially notorious as being caused by opportunistic pathogens.

SAMPLE PROBLEM DATA SHEET (KEY TO THE HEADINGS IN PART II)

PROBLEM X

Notifiable to OIE

When a disease is reportable to the Office International des Epizooties (OIE; www.oie.int), it is mentioned here. Diseases notifiable to (listed by) the OIE are in that list because they resist or respond poorly to therapy, have a restricted geographical range, are of high socio-economic importance, and occur in species involved in international trade. Of the listed pathogens, all but two are viruses. Note that some diseases that are not notifiable to the OIE might nonetheless be required to be reported to local or national authorities when they occur.

Prevalence Index

This is a subjective comparison of the prevalence of the stated problem for various fish groups. The absence of a rating means that the disease does not occur in that group. Ratings are as follows: (1) very common; (2) common; (3) uncommon; and (4) rare.

The index is based only on prevalence in cultured fish. Prevalence of a problem in wild fish of the same species may differ considerably from individuals in culture.

The prevalence index is subjective since little published quantitative data exist that document the prevalence of diseases in cultured fish worldwide. Note that certain diseases may be much more or much less common in some geographic areas. Also, when not dealing with the species groups in the prevalence index, the prevalence may not be accurate.

Literally hundreds of fish species are cultured. However, despite this great diversity, many fish species are susceptible to the same diseases. The two most important environmental factors that influence the types of diseases that may affect a particular fish species are salinity and temperature. These two factors play an important role in limiting the distribution of infectious agents and are also an important influence on noninfectious diseases. To help you gain a better understanding of the chance of encountering certain diseases in various fish, prevalence rates are given for the following four ecological categories:

- Warm Freshwater (WF)—These include fish that are submitted from freshwater environments that are warmer than approximately 20°C (68°F). Ranking is based on prevalence of the problem in tropical freshwater aquarium fish and/or prevalence in warm water food fish such as tilapia, ictalurids, and cyprinids, as well as many warm water sport fish, such as centrarchids and striped bass.
- Warm Marine (WM)—These include fish that are submitted from brackish (>~0.5 ppt salinity) or marine environments that are warmer than about 20°C (68°F). Ranking is based on prevalence of the problem in tropical marine aquarium fish and/or prevalence in warm water marine food fish such as groupers, as well as estuarine food fish species, such as mullet, barramundi, red drum, and striped bass.
- Cold Freshwater (CF)—These include fish that are submitted from freshwater environments that are colder than approximately 20°C (68°F). Ranking is based on prevalence of the problem in salmonids

cultured in freshwater and/or prevalence in other cold water fish, such as yellow perch, walleye, and pike.

- Cold Marine (CM)—These include fish that are submitted from brackish or marine environments that are colder than approximately 20°C (68°F). Ranking is based on prevalence of the problem in salmonids cultured in brackish water or seawater and/or prevalence in other cold water marine species, such as flatfish and cod, as well as fish propagated in less cold water (e.g., European seabass, gilthead seabream, summer flounder), and many brackish water species, such as striped bass and sciaenids.

The Prevalence Index is only intended as a general guideline and is mainly intended to allow you to quickly determine if a certain problem may be relevant or not to your case. For example, if the epidemic being examined occurred at 10°C (50°F) in 32 ppt seawater, then one would need to consider only problems that affect cold water marine fish (CM). Problems shown as not affecting that ecological group (e.g., PROBLEM 21) can be ignored. However, in a specific problem, one must carefully note the affected species that are mentioned in the Comments section, since this varies greatly among problems. For example, CCVD (PROBLEM 78) has a Prevalence Index of WF-2 since it is common in channel catfish. However, it does not affect other warm water fish species. Thus, the numerical score of the Prevalence Index relates only to the species mentioned in the Comments.

Note also that many species overlap into more than one category. For example, channel catfish are normally cultured in climates where water temperatures may range from 10°C (50°F) to 30°C (86°F). The pathogens and other problems that this species encounters depend on the ecological conditions prevailing at that time. Thus, salinity and temperature should be used as the primary guides for assessing probable prevalence.

Method of Diagnosis

This gives the data or procedure needed for the diagnosis. In some cases a definitive diagnosis cannot be obtained under typical clinical conditions. In any case the method of diagnosis that provides the most reliable result under typical clinical conditions is listed first, followed by other, usually less definitive, methods.

History

This is self-explanatory. Note that all of the features listed rarely will be present in any single case. None may be present.

Physical Examination

This is self-explanatory. Note that all of the clinical signs rarely will listed be present in any single case. None may be present.

Treatment

Different treatments are listed numerically (1, 2, 3, etc.). Some treatments require multiple steps; these are indicated as alphabetical subheadings of that treatment (e.g., 1a, 1b, 1c, etc.). Detailed treatment procedures are given in "**Pharmacopoeia**." See "**General Concepts in Therapy**," **p. 347**, and "**Pharmacopoeia**" for legal considerations in treating fish.

CLINICAL DECISION MAKING: HAVE THE MAJOR PROBLEMS BEEN IDENTIFIED?

When core water-quality parameters have been measured and fish have been examined for infectious agents and lesions, using biopsy, culture, and necropsy exam (and possibly a more specialized clinical technique), a decision must be made about which problems are most important and whether those identified are sufficient to explain the morbidity and mortality patterns and the clinical signs. For example, a heavy monogenean infestation on the gills, combined with clinical hypoxia, low (~1% per week) mortality, and moderately elevated unionized ammonia (0.03 mg/l UIA) would be consistent with a diagnosis of monogenean-induced mortality. The monogenean infestation could be explained by the sublethal, but stressful, ammonia concentration.

Conversely, a mild trichodinosis infestation on the gills, absence of any other infectious agents in the clinical workup, and normal core water-quality readings would not be sufficient to explain a high, acute (~5% per day) mortality rate in a population. If no other problems are identified in the clinical workup (i.e., if all these problems are ruled out as the major cause of the fish mortalities), look to a rule-out diagnosis(es) to explain the mortalities (see Fig. I-1).

It is important to realize that rule-out diagnoses are considered after the clinically identifiable problems (i.e., see PROBLEMS 1 through 76) have been eliminated from consideration as the major cause of the disease. Rule-out diagnoses can sometimes be presumptively identified from the history (e.g., use of outdated feed in combination with clinical signs of nutritional deficiency are strongly suggestive of a nutritional imbalance; see PROBLEM 89). However, definitive confirmation of a rule-out diagnosis requires specialized tests that are not routinely performed in most clinics (e.g., chemical analysis of feed composition, viral isolation). Thus definitive confirmation of rule-out diagnoses requires referral to a specialized laboratory (see "**When to Refer Cases**" below). Note that it may be impossible or economically unfeasible to obtain a definitive diagnosis. Instead, it may be better to correct the presumed problem (e.g., replace old feed with fresh feed if nutritional imbalance is suspected) and monitor for a favorable clinical response,

rather than try to identify a specific problem (e.g., test for vitamin C deficiency in suspect feed).

PRIORITIZING PROBLEMS

More than one problem is usually identified in a clinical workup. Thus, the clinician must prioritize the problems using the following criteria:

1. Which problem is primarily responsible for the morbidity and/or mortality?
2. Which problem is most life-threatening?
3. Which problem is safest to treat first?
4. Which problem is of most concern to the animals' welfare?

All four questions are closely interrelated; consider which problem is best addressed first. For example, gill parasites are generally considered more dangerous than skin parasites, so it would be advisable to treat gill pathogens first, especially if the fish had to be moved or otherwise stressed to treat the skin disease. In another case, if a bacterial infection and a skin parasite were both present but the fish is not eating, it would be best to treat for the skin parasite, which may stimulate the fish to begin eating, even though the bacterial infection may be more life-threatening. An antibiotic-medicated feed might then be used to effectively treat the bacterial infection. Details on treatments are given in "**Pharmacopoeia**."

TREATMENT PLANS

The clinician should provide the following two types of plans to the client:

1. Short-term plan—This plan should include the means to control the immediate problem that is usually the cause of the presenting complaint; this typically involves various types of medications to control infectious disease and large-scale water treatments to reduce environmental stress and toxins.
2. Long-term plan—This plan is often the most important and involves recommendations for improving management that will prevent the recurrence of similar problems in the future.

More details about treatment plans are given in **chapter 6**.

WHEN TO REFER CASES

Most fish disease cases can be diagnosed by the clinician using the relatively simple techniques described in this book. However, some cases may require additional tests that are not routinely performed in most clinics (Anderson and Barney 1991). Many rule-out diagnoses (see PROBLEMS 77 through 99) can be definitively diagnosed by using specialized tests. Specific details for proper referral of such cases to appropriate referral laboratories are described under individual problems. Clinicians usually have access to at least one government agency that can provide assistance in fish disease diagnosis. These are often affiliated with universities (veterinary colleges, fisheries departments) or state/provincial agricultural or fisheries agencies. Some national and international reference laboratories also perform fish disease diagnosis. Also a number of private laboratories provide fish disease diagnostic services.

A searchable database of diagnostic laboratories and clinicians in aquatic medicine worldwide is available at www.AquaVets.com. This database is relatively new and does not include all laboratories and clinicians but should become more comprehensive with time. Other sources of disease diagnostic expertise are members of the European Association of Fish Pathologists (www.eafp.org), Fish Health Section of the Asian Fisheries Society (http://afs-fhs.seafdec.org.ph), Fish Veterinary Society (http://www.fishvetsociety.org.uk), Japanese Society of Fish Pathology (www.fish-pathology.com), Fish Health Section of the American Fisheries Society (www.fisheries.org/units/fhs), International Association for Aquatic Animal Medicine (www.iaaam.org), and American Association of Zoo Veterinarians (www.aazv.org).

A listing of laboratories that are certified as the reference laboratory for each OIE-notifiable disease is available at http://www.oie.int/eng/OIE/organisation/en_LR.htm?e1d8. Before collecting or sending any samples from fish with a suspected exotic disease (i.e., a foreign animal disease, not endemic to the area), contact the proper authorities (local and/or national) so that the appropriate procedures for handling samples can be verified. Such samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.

CHAPTER 6

Health Management

The long-term goal for all aquaculture operations should be the elimination of all disease. For a long list of both technical and economic reasons, elimination of all disease is not possible at this time. Nonetheless, the clinician should strive to manage the health of the population so that the incidences of specific diseases are minimized to as great an extent as possible. In this regard, *proactive* health management strategies (as compared to *reactive* disease treatments) are playing a greater role in aquaculture and will be increasingly more important in the future. A striking example of the power of this strategy is how the Norwegian aquaculture industry was able to dramatically reduce drug use through the introduction of effective vaccines, selection of more optimal farm sites, and improvement of farm biosecurity. As a result, the amount of antibacterial agents used in Norwegian finfish aquaculture was reduced by 98% from 1987 to 2003 (Norwegian National Institute of Nutrition and Seafood Research, http://www.nifes.no).

A successful aquaculture operation must also pay close attention to other matters that are not directly related to fish health because public concerns have a significant influence on how and where fish are cultured. Thus, the clinician must be aware of and assist the producer in minimizing environmental impacts of the culture operation and, when dealing with food fish, ensuring that the product is safe and wholesome for human consumption. Finally, the clinician must ensure that all animals are treated humanely.

Note that the specific strategies to be employed must be closely tailored to the particular farm, including its physical makeup, fish species, and present and future pathogen risks.

BIOSECURITY

General Guidelines

Continued global exchange of organisms and climate change are predicted to lead to an increase and spread of new pathogens in humans and wildlife (Tompkins and Wilson 1998). In fact, this has already had a major impact on both cultured and wild aquatic animal populations. Many (if not most) of the diseases that are among the most serious problems in cultured fish are caused by exotic pathogens; that is, they were inadvertently introduced into a region via infected fish from another geographic area. At the same time, worldwide demand for high-quality aquaculture products makes disease control increasingly important. Thus, an effective biosecurity program is vital to maintaining healthy animals and to reducing the risk of acquiring disease in a facility.

Biosecurity refers to the implementation of methods to prevent (or manage if already present) the transmission of infectious diseases in a culture operation. Effective biosecurity can also exclude or reduce the spread of pathogens that might be endemic to a farm's geographic region but have not yet contaminated that particular farm. Biosecurity is also important in preventing the escape of pathogens from a farm, if it is contaminated with a certain pathogen, so that it does not affect wild populations or adjacent farms.

The general features of an effective biosecurity program apply to all culture systems, whether they are land-based (e.g., ponds), water-based (e.g., cages), flow-through (e.g., raceways), or recirculating (e.g., aquaria) systems and include two major components: external barriers and internal barriers. Two key methods used to maintain biosecurity are pathogen inactivation strategies and inhibiting fish-to-fish transmission. The relative importance of each component of a biosecurity program will vary with the aquaculture operation (i.e., farm size, fish species, pathogens of concern, sources of the pathogens, etc.), but the general principles apply in all cases. See Arthur et al. (2008) for general guidelines on biosecurity design and see specific references for more detailed guidelines on particular fish groups, including aquarium fish (Lewbart and Harms 1992), laboratory fish (Kent et al. 2009), and salmonids (Scarfe et al. 2005).

External Barriers

External barriers prevent the spread of pathogens *onto and off of* a farm and include:

- Using a specific-pathogen-free (SPF) water source when possible (e.g., land-based farms; see definition of SPF on **p. 71**)

- Never introducing fish from other farms or at least never introducing fish from farms having older or less healthy fish
- Restricting the movement of fish between farm sites of the same operator
- If new fish must be introduced, using SPF fish or at least those with a known history of health. The history of the fish will also dictate the quarantine procedures needed (see **p. 71**)
- Strict sanitary measures for all persons (including farm workers and visitors) entering the farm (see "**Pathogen Inactivation Strategies**" below)
- Restricting access to the farm site (e.g., fencing the site, locking all doors, restricting visitors, etc.)
- A pest management control program
- A feed hygiene program

Internal Barriers

Internal barriers prevent the spread of pathogens *within* a farm and include:

- Partitioning the farm into isolation units
- Physically separating each unit and keeping all units isolated from each other
- Having specific sanitation and personnel hygiene protocols (e.g., cleaning, disinfection, antisepsis, pest control) for each unit (see "**Pathogen Inactivation Strategies**")
- Having specific sanitation protocols for movement of fish or materials between units (e.g., never allowing any transfers from unit X to unit Y)

Pathogen Inactivation Strategies

Pathogens can be spread either by the fish (which may shed the pathogen) or as fomites (objects other than the fish that are contaminated with the pathogen). Pathogens on fish (including eggs) are reduced or eliminated with antiseptics, while fomites are inactivated with disinfectants. The proper use of antiseptics and disinfectants is described in "**Pharmacopoeia**."

Disinfection

Disinfection is a crucial component of a successful biosecurity strategy. It must be practiced throughout the production cycle to eliminate pathogens on rearing units, equipment, water, and even certain feeds such as live brine shrimp, tubifex worms, or diets containing fish products. Fomites include any item that could possibly come in contact with water (and thus the fish), including nets, aeration equipment (e.g., airstones, paddlewheels, pumps, etc.), filters, filter media, plumbing (pipes, etc.), water-handling containers (buckets, plastic bags, live hauling units, etc.), clothes, shoes, and hands. The rearing water itself can be considered a single, large fomite. Fomites can even circumvent well-designed isolation systems via aerosolization; bacteria, parasites, and most probably viruses can be introduced in this manner (Bishop et al. 2003; Roberts-Thomson et al. 2006), necessitating that an effective containment strategy be in place (e.g., tight-fitting covers on aquaria, minimizing aerosolization from aerators, etc.). A hygiene program should also be established for all farm personnel (disinfectant foot dips, hand hygiene, protective clothing that is frequently sanitized).

When designing a disinfection strategy, it is useful to identify "critical control points" (i.e., the points where there is the highest risk of contamination). These are the points where disinfectant use can yield maximum benefit. Keeping areas clean and clear of debris is also important because organic matter reduces the effectiveness of disinfectants. For intensive, closed culture systems, in-line disinfection, such as ultraviolet light or ozone, is highly recommended when multiple culture units (e.g., several aquaria) are connected together, such as by a single filtration system.

Antisepsis

Treatment of fish and eggs with antiseptics is an important component of disease management. On fish, antiseptics are only effective against some skin or gill pathogens, having no effect on internal or gut pathogens. And while antiseptics are very effective in reducing the load of certain pathogens (especially parasites), there is little scientific evidence proving that any antiseptic can totally eliminate a pathogen from a fish or a population. However, treatment of the egg stage with certain antiseptics is well known to totally prevent the vertical transmission of certain microbes, if they are carried only on the surface of (and not within) the egg.

Prophylactic treatments (e.g., salt, formalin, etc.) are often advocated prior to the introduction of fish into a new environment. However, the efficacy of this procedure depends upon the specific circumstances. For example, if a fish population has a significant number of ectoparasites, it is justified to treat with an appropriate parasiticide prior to introduction in order to reduce the parasite load on the stressed fish. This is especially true for wild-caught fish, which often carry significant parasite burdens. On the contrary, if a population is clinically healthy and has no significant pathogen burden, the added stress of a drug treatment might not be advisable and might increase the susceptibility to opportunistic pathogens that are commonly resident in the environment. Thus, the proper prophylactic procedures must be tailored to each particular situation.

Fallowing

Fallowing, the removal of all fish from a culture site to cause the elimination (natural death) of a pathogen from the site, can be a very effective strategy in many culture systems (aquariums to ponds to cages), so long as the microbe is an obligate fish pathogen (i.e., it requires a

fish host to survive). It cannot be used for pathogens that can live on sites other than the fish host, if those will continue to be available while the fish are removed. For example, water molds (PROBLEM 34) are saprophytes and do not require a living host. It is also not effective if significant living reservoirs besides the host fish will retain viable pathogens during fallowing (e.g., aquatic worm hosts for myxozoans, PROBLEM 63). Fallowing's success requires knowing the amount of time that a pathogen can survive in that particular environment without a fish host. This will vary with the pathogen and also with the environment (e.g., pathogens often linger longer at low temperature). In some cases, the length of time required for fallowing to eliminate a particular pathogen may be impractical. Fallowing details for specific pathogens are provided in various PROBLEMS.

Inhibiting Fish-to-Fish Transmission

Geographic Isolation of Farms

Physical separation is a highly useful barrier to disease transmission. Pathogens can be transmitted via water source (e.g., from upstream, from adjacent sea cages), aerosol, fish farm escapees, or pests (insects, birds, mammals). The farther or more difficult it is for such disease carriers to travel from an infected farm or site, the less likely that it will occur.

Limiting Human Access to Farms

The visitation of unauthorized personnel (i.e., nonemployees) to a farm should be highly discouraged, especially individuals that have had prior contact with other farm operations that might harbor unwanted pathogens. Entry of unauthorized individuals also provides the opportunity for sabotage or intentional contamination of a facility. While such instances are probably rare, the potential for mischief should not be ignored (R.A. Bullis, personal communication). There should also be strict guidelines on when and how farm personnel should be allowed to visit other farm operations. This should be avoided unless absolutely necessary.

Separation of Age Classes

Many fish diseases have a pronounced difference in pathogenicity with age, with younger fish often being much more susceptible. At the same time, older fish are often asymptomatic carriers and thus can transmit an infection to susceptible fish without appearing sick. Thus, keeping age classes segregated (e.g., separate fry, fingerling, growout, broodstock facilities) is very important.

Disease-Resistant Strains

While a considerable amount of classical genetic selection has been tried to produce fish strains that are resistant to a certain disease (e.g., BKD [PROBLEM 54]), furunculosis [PROBLEM 47]) and some strains of fish are more resistant to certain diseases, relatively little practical progress has been made in this arena. With the significant advances in quantitative molecular genetics currently being applied to fish, as well as a greater understanding of fish immunology, much better results are expected in the future. Farmers already take advantage of the natural resistance of certain fish species to particular diseases (e.g., greater natural resistance of rainbow trout to furunculosis compared to brown trout). Hybrids can also be superior in terms of resistance to stress and disease (e.g., hybrid striped bass).

Specific-Pathogen-Free Stocks

Specific-pathogen-free refers to the absence of a certain pathogen (i.e., a specific bacterium, a specific virus, a specific parasite, etc.) in a fish population. It is often mistakenly referred to as "disease-free," which implies the absence of all pathogens; no such fish stocks exist. Development of SPF stocks has advanced the most in salmonids, where commercial suppliers provide fish that are certified free of certain pathogens (especially some viruses). Specific-pathogen-free zebrafish have also been recently developed as a laboratory animal model. Examples of the unfortunate, inadvertent introduction of serious pathogens into new environments are too numerous to mention (see PROBLEMS 59 and 83 as illustrations). Obviously, excluding a pathogen is by far the best option in disease management. Thus, it is highly advantageous for a farmer to use SPF stocks when available. Also, local regulations might mandate that only fish free of certain diseases be allowed on a site (see "**Regulatory Issues: Reportable Diseases and Certification of Stocks**," **p. 73**). When SPF stocks are not commercially available, the next best option is to allow only fish on the farm that have undergone a very thorough health exam and quarantine (which should already be part of any biosecurity plan). However, there are limitations to this strategy, especially if there is a significant reservoir of the pathogen in the water source used to raise the fish (e.g., natural stream, sea cage). Thus, it is most easily implemented in closed systems or those with a pathogen-free water source (e.g., well water).

Quarantine

Quarantine, the isolation of a new population of fish prior to their placement within the established population, is intended to prevent the introduction of a specific disease into an aquatic system from which eradication would be difficult or impossible, as well as to protect the resident fish population. Quarantine also allows the acclimation of new stock to the new environment in a relatively controlled setting that allows close observation.

One of the most critical times during quarantine is the initial introduction of the fish into the quarantine system. Water quality (temperature, pH, salinity, etc.) should be

within a certain maximum range of the shipment water (e.g., see PROBLEMS 2, 7, and 10 for details). Exposure to highly different water quality or placing in small holding units when the fish were previously held in large systems can be severely stressful (see PROBLEM 97).

Quarantine procedures and systems range from simple to complex. They are highly desirable for even the hobbyist with a single aquarium and absolutely essential for any significant culture systems (e.g., commercial facilities). All materiel (holding systems, nets, etc.) used for quarantine should ideally only be used in the quarantine system. After use, everything should be treated with a high-level disinfectant or at least one that is known to inactivate all major pathogens of concern to that particular operation (see "**Pharmacopoeia**").

The most certain means of quarantine is physical isolation of the new stock as far away as feasible from the established population. When not possible, water disinfection such as via ultraviolet light, ozone, or ultrafiltration (see "**Pharmacopoeia**") can be used but is more prone to a failure during quarantine. Nonetheless, inlet water disinfection should always be applied in flow-through quarantine systems that use potentially contaminated sources (e.g., surface water).

Quarantine holding systems should be as simple as possible to allow ease of cleaning, but for some species (especially aquarium fish) it is best to provide some shelter (e.g., plastic pipe or other inert structure) to reduce stress. A minimum number of fish is often needed to reduce stress and aggression and encourage feeding. Feeding during quarantine is necessary, but fish placed in a new environment will often be anorectic, even for as long as 1 week or more. This behavioral anorexia must be differentiated from that caused by disease.

Some advocate keeping the temperature at the upper end of the fish species' optimum range in order to speed up parasite life cycles (e.g., ich). However, high temperature is also more stressful for fish (see PROBLEM 2), although this may also facilitate the fish "breaking" with a subclinical infection, allowing its detection (see PROBLEM 47, *Aeromonas salmonicida* infection). The advantage of using prophylactic drug treatments during quarantine is not clear-cut because objective, scientific data demonstrating its efficacy is lacking. However, clinical experience of many practitioners suggests that it is warranted in many cases, such as for many wild-caught fish that might have a significant parasite burden. Most commonly used are broad-spectrum ectoparasiticidal treatments, such as formalin, copper, or salt/freshwater exposure, which might also have some limited effects against skin or gill bacterial pathogens. However, many treatments are quite stressful and whether this should be done is best evaluated on a case-by-case basis that is influenced by the history of problems with a particular fish stock. One of the most innocuous treatments for freshwater fish in closed culture systems is use of a low concentration of salt as a prolonged immersion, which also has a positive physiological influence (see "**Pharmacopoeia**"). Maintaining marine fish at the low end of their salinity tolerance might also be advantageous but is less proven. Antibiotic prophylaxis is rarely advisable.

Health examinations are typically performed no sooner than 4–7 days after commencement of quarantine (unless fish appear sick) to allow acclimation to the new system before handling (Lewbart and Harms 1992). Screening procedures should be tailored to the disease predilections of the fish stocks as well as the history of problems that may be present in the introduced stock. If a true statistical sampling of the population is desired, a specific number of fish are sampled that depends upon the total population size and desired probability of pathogen detection if present. Details on sampling methodologies are provided in the *FHS Blue Book* (AFS-FHS 2007) and des Clers (1994).

If disease occurs during quarantine, it should be treated promptly and then testing done again to confirm that the population has fully recovered. Then a decision must be made as to whether and how to introduce the fish into the established stock. For many diseases, the fish may remain carriers long after they have clinically recovered, and thus detection of an infection that one wishes to remain excluded would probably warrant banning the stock from the facility.

The time needed for fish to remain in quarantine depends upon the specific pathogen(s), its clinical course, and its life cycle (i.e., how long before clinical signs appear; if cultured, how long before the pathogen will grow and can be identified; if a parasite, how long its life cycle is). Cold water fish species are often quarantined longer than tropical species because some diseases may take longer to become apparent. Personal experience with particular diseases also influences the quarantine strategy. Times range from as little as 1 week to 30 days (American Association of Zoological Parks and Aquaria 1991) or more. When screening for slow-growing pathogens such as mycobacteria (PROBLEM 55), quarantine might require up to 90 days (Astrofsky et al. 2000). Quarantine procedures must be tailored to each particular situation (Whitaker 1999). It is important to keep good quarantine records that include water quality, if/when clinical signs appear, and movements in or out of the system.

It is important to realize that the relative success of any quarantine procedure is highly dependent upon the ability to detect the presence of the pathogen of interest. In many cases, the tests for detecting the target pathogen have low sensitivity and thus are unlikely to be even close to 100% effective in assuring that a pathogen is not introduced. However, this varies greatly with the patho-

gen; and in some cases, a diagnostic test is available that greatly increases the likelihood that a certain pathogen can be excluded from a farm or operation (e.g., see PROBLEM 86).

Another important consideration is that the time periods that are typically used for quarantine (e.g., 7 days, 90 days, etc.) are quite arbitrary, and published scientific data to validate the periods used are scarce. To effectively ensure that some pathogens are not introduced, a very long quarantine period should be used.

Ideally, the quarantined population should be permanently segregated (never to be introduced onto the farm) and only the progeny of the quarantined population should eventually be introduced, after several rounds of disease screening of both the parental and offspring populations, all showing absence of the pathogen(s). Where this strategy has been implemented, it has at times resulted in major increases in productivity.

It is also very important to realize that quarantine does not ensure that a pathogen will not be introduced, but only increases the likelihood that it can be detected before it can do harm. In summary, the longer the quarantine and the more generations having documented lack of detection of a pathogen, the greater the likelihood of excluding that pathogen.

Regulatory Issues: Reportable Diseases and Certification of Stocks

Numerous local, state, and national regulations dictate that governmental authorities must be notified about the status of certain animal diseases. These regulations mainly pertain to traditional agricultural species but regulations increasingly involve aquatic animals. The clinician should be familiar with local and nationwide regulations, since failure to comply with these regulations can lead to punitive sanctions and might place that affected aquaculture species at risk.

Health codes vary significantly from one country to another. In most countries, movements within the country or foreign imports are licensed and should be accompanied by a health certificate from a local authority (usually the agricultural or fisheries ministry) of the exporting locality or country. Procedures governing the movement of fish stocks are available on many governmental websites (e.g., European Union [http://ec.europa.eu/food/animal/liveanimals/aquaculture]; Canada [http://www.pac.dfo-mpo.gc.ca/sci/aqua/pages/fhprot_e.htm]; Japan [http://www.maff.go.jp/e/index.html]; Australia [http://www.daff.gov.au/aqis]). In the United States, the secretary of agriculture is authorized to protect and control the health of aquatic animals. A list, country by country, of agencies that deal with animal health and food safety is given at http://www.fda.gov/oia/agencies.htm. The *FHS Blue Book* (AFS-FHS 2007) describes generally accepted methods for diagnosing and certifying the disease-free status of many important fish diseases in the United States.

At the international level, the Office International des Epizooties (OIE) is charged with collecting and disseminating information on animal diseases to its approximately 150 member countries, as well as promoting standards for health regulations applied to international trade. This includes defining the minimum health standards required of international trading partners to avoid the risk of spreading aquatic animal diseases. The OIE develops scientifically based risk assessment criteria that are reflected in the *International Aquatic Animal Health Code* (OIE 2008), which provides international guidelines and recommendations designed to help countries survey, prevent, and control infectious aquatic animal diseases. Diseases of concern that are listed in the code are addressed in the *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2006), which provides methods for a uniform approach to the diagnosis of these diseases, so that the requirements for health certification for international trade in aquatic animals and aquatic animal products can be met. A listing of laboratories that are certified as the reference laboratory for each OIE-notifiable disease is also available (OIE 2006). Various international regions also have governmental bodies that monitor diseases of particular concern to that region, such as NACA (Network of Aquaculture Centres in the Asia-Pacific) (http://www.enaca.org) and the European Commission in the European Union (http://ec.europa.eu/food/animal/liveanimals/aquaculture/index_en.htm).

In order to move fish or fish products to a particular location, it is often necessary to certify that the stock is free of certain diseases. Simon and Schill (1984), Amos (1985), des Clers (1994), Thoesen (1994), OIE (2006), and AFS-FHS (2007) provide detailed methods for certifying fish to be free of specific diseases and methods for ongoing surveillance to maintain pathogen-free status.

In addition to health issues, certification may also involve assuring that an aquaculture facility does not have a negative impact on animal welfare, food safety, or the environment (see Boyd et al. 2005 and discussion below for details).

HEALTH PROMOTION AND MAINTENANCE

Vaccines

A successful health management strategy is predicated upon the identification of the controllable risk factors in disease prevention, rather than spending large efforts in attempting to eliminate a pathogen (Mitchell 1996). Vaccination has become an integral component of health management in many segments of the aquaculture indus-

try and continues to grow in importance as drug use becomes ever more scrutinized for many reasons. The salmonid industry has lead the way in this effort and most commercial products are designed for use in these species. However, new vaccines for many other species continue to be released, and even products intended for one species can sometimes be used to protect against the same disease in other species (e.g., vibriosis).

The rationale for vaccination of fish is similar to that in other animals, in that an effective vaccine reduces mortality, reduces the need for drug use (especially antibiotics), enhances the response to medication (even if an epidemic still occurs), improves growth rate/feed conversion, and improves general health throughout the production cycle. These positive effects lead to a reduced overall cost for the producer.

However, there are some special factors to be considered when vaccinating fish. These include:

1. A large number of vaccinees—The large number of individuals makes it expensive and limits the use of injectable preparations to very valuable individuals (broodstock, expensive ornamentals, high-value food fish, etc.). This often precludes individually handling fish. It also requires vaccination at an age when immunocompetence may not be fully developed. For example, salmonids less than 1 gram have a weak immune response and short duration of immunity. Smaller fish are also more fragile. It also requires the use of vaccines that delicately balance low pathogenicity with high potency (provide adequate protection while avoiding a vaccine "break").
2. High disease susceptibility at a young age—Many microbes are most virulent in very young fish (e.g., IPNV, IHNV), possibly before the fish's immune system is fully developed. Thus, time of vaccination must be closely correlated with immune status, which varies among fish species.
3. Environmental conditions—Especially important is temperature. Pathogen and host immune response both have temperature optima, and these two optima are not necessarily the same. For example, IHN causes the most severe mortalities in chinook salmon at about 10°C (50°F), but the temperature optimum for the immune response of chinook salmon is considerably higher than 10°C. There is a more rapid immune response with increasing temperature, but some portions of immune response are less dependent upon temperature.
4. Health status—The manipulations involved in vaccination (handling, crowding, temperature change, etc.) are stressful, providing an opportunity for latent or opportunistic pathogens (bacteria, water molds, parasites, etc.) to cause disease.

In addition, vaccines vary greatly in efficacy. Experimental data does not always mirror results in the commercial operations and the clinician must closely monitor efficacy to determine if the product is providing the desired result.

Table I-8. Comparison of the advantages and disadvantages of the three major routes used to vaccinate fish.

	Water-borne	Injection	Oral
Ease of administration	Moderate	Low	High
Cost	Moderate	High	Moderate
Amount of stress	Moderate	High	Low
Potential injury to fish	Moderate[1]	High[2]	Low
Survival	Moderate	Low	High
Dosage control	Moderate	High	Low[3]
Labor	Moderate	High	Low
Duration of protection	3–12 months	12–24 months	2–4 months
Effectiveness	Moderate	High	Low

[1]Damage due to handling (mainly skin and eye damage).
[2]Can cause adhesions that can affect fillet quality (IM injection) or affect digestion (IP injection). The smallest fish might have the most severe side effects. Side effects are relatively mild 1–3 months after injection, become more severe 3–12 months after injection, and then begin to resolve after 12 months (Intervet 2003). Other side effects include temporarily decreased feeding, inadvertent puncture of the intestine, and creation of a wound that could become infected.
[3]Varies with food intake.

There are three routes used to vaccinate fish (water-borne, injection, and oral), each having advantages and disadvantages (see Table I-8). Bathing fish in a vaccine solution (water-borne vaccination) is one of the most common methods used to vaccinate them. Protection typically lasts 3–12 months, which is not long enough for the production cycle of some fish species. If fish will not be moved after stocking into a production unit, immersion can only be used at stocking. Immersion is not cost-effective for large fish (Vinitnantharat et al. 1999). Injection is also highly common but is typically used for more valuable fish (e.g., salmonids); commercial operations can typically inject up to 2,000 fish per hour using repeating injection guns. It is impractical to inject fish smaller than 5 grams. While oral vaccines have several attractive features, they are least used because they are not highly protective. They are not cost-effective for larger fish. Which method is best in a particular situation depends upon the actual and perceived risk (history of outbreaks and disease persistence), fish age, farmer's risk aversion, and return on investment (Mitchell 1996). On larger farms, it is useful to perform a cost-benefit analysis before deciding whether, when, and how to vaccinate. Lillehaug (1989) provides details on performing such a study.

Instructions for proper administration of specific vaccines are provided with the literature accompanying each product. Vaccination should be scheduled so it is done during other routine procedures (e.g., grading) to minimize labor and fish stress. Fish should be fasted prior to

vaccination. Fasting is often for 24 hours but might be less with small fish or at high temperature (Evelyn 1997). Only healthy fish should be vaccinated, and vaccination should be done at least 21 days prior to any likelihood of exposure to the infectious agent in the vaccine. Fish should usually not be vaccinated within 21 days of slaughter or release.

An increasing number of commercial vaccines are available for important diseases of fish (see "**Vaccines**," **p. 419**, for a list of suppliers). Bacterins (killed bacteria) predominate, but modified live vaccines for bacterial and viral diseases as well as killed viral vaccines are available. Subunit vaccines and DNA vaccines have only been used experimentally (Thompson and Adams 2004; LaPatra et al. 2004).

In some cases, a commercially licensed vaccine is not available for an important disease, and in such cases, autogenous vaccines might be used. An autogenous vaccine is a vaccine derived from a specific pathogen that is isolated as the cause of disease in an individual fish, lot of fish, facility, or geographic region. Autogenous vaccines are custom-made vaccines prepared from a pathogen isolated during a specific epidemic, rather than commercial vaccines that are prepared from standardized cultures (Haskell et al. 2004). A few companies can provide autogenous vaccines for certain diseases.

Regulations governing the use of autogenous vaccines vary among countries. In the United States, autogenous vaccines must be prepared from cultures of microorganisms that have been inactivated and are nontoxic. The product must be prepared for use only by or under the direction of a veterinarian and under a veterinarian-client-patient relationship, except that such products may be prepared for use under the direction of a person of appropriate expertise in specialized situations such as aquaculture, if approved by the USDA. Autogenous vaccines must be produced using seed organisms isolated from sick or dead animals in the population of origin; however, the USDA (APHIS) may authorize preparation of an autogenous vaccine for use in populations adjacent to the population of origin or in populations that are not adjacent to the population of origin. For example, the USDA has stated that the appearance (isolation) of infectious salmon anemia virus (PROBLEM 82) in U.S. waters would be considered adequate justification for authorizing the use of an autogenous vaccine in adjacent and nonadjacent commercial U.S. salmon production facilities.

In the United States, prior to initiating work with a pathogen, an applicant must designate the facilities to be used, specify the precautions that will be taken to prevent contamination of licensed products, and must submit this information to the Center for Veterinary Biologics. Distribution in each state is limited to authorized recipients designated by the USDA (APHIS Veterinary Services) and the proper state officials, under such additional conditions as these authorities may require (USDA 1999).

Nonspecific Immunostimulants

Nonspecific immunity (i.e., comprised of defenses that combat a broad array of pathogens at one time rather than a single microbe) seems to be especially important in fish. Many chemical and cellular host defenses in fish are nonspecific. A number of drugs and microbial products (e.g., levamisole, lipopolysaccharide, peptidoglycan, killed bacteria) can experimentally "turn on" nonspecific immunity and thus act as an immunostimulant. Most commercial immunostimulants are derived from the cell walls of fungi, the most well known of which are the β-glucans. Commercial products are used either alone to "boost" fish immunity or in combination with vaccines to enhance protection. For commercially available products, see "**Immunostimulants**," **p. 403**.

A number of controlled experimental laboratory studies have shown that immunostimulants can help protect against infectious disease, especially bacterial pathogens. However, not all lab studies have demonstrated protection and there is a lack of controlled field trials that have demonstrated efficacy. While immunostimulants can probably protect under some circumstances and hold great promise, there is still much information needed on optimally using these products in the real world.

Probiotics/Competitive Exclusion

Fish are normally exposed to a wide array of microbes; not all are pathogens but rather many are innocuous components of the host's normal flora. Probiotics seek to exploit this by enhancing the "good" microbial flora with the intention of "competitively excluding" the "bad" (fish-pathogenic) populations (Gómez et al. 2007). The goal is usually to prevent colonization of the gut, but there is also interest in microbes that can protect at the external body surfaces (skin, gill).

Experimental studies have shown that administering some live bacterial ("probiotic") strains to fish can protect against pathogenic bacteria (Balcázar et al. 2006). But again, data are lacking from controlled field trials. Thus, while probiotics can probably protect under some circumstances, much information is still needed to optimally use these products. Some bacteria considered to be probiotics may actually be acting as immunostimulants (Birkbeck 2004).

Biological Control

Biological control is the use of an organism to specifically prey upon, parasitize, or otherwise reduce the levels of

an undesirable organism (usually a pest). Very important to a successful biological control is that it is specific (i.e., only kills or preys upon the pest and does not harm "nontarget" organisms). Biological control is somewhat similar to the use of probiotics but includes virtually any organism (microbe to mammal) that can kill a pest or pathogen; also, biological control does not target microbes but rather multicellular organisms such as parasites.

The most well-known example of biological control in fish is its use in controlling ectoparasites. Some fish and invertebrates feed on the parasites of other fish ("clients") in a process called "cleaning." Cleaning associations involve cleaner organisms that remove ectoparasites and other material, such as mucus and skin, from the body surface of apparently cooperating individuals. The best well-documented fish that display this behavior are the wrasses (labrids). For most species, cleaning is done as a juvenile or facultatively, but Indo-Pacific tropical wrasses belonging to the genus *Labroides* feed almost exclusively by cleaning (Côté 2000). The blue-lined cleaner wrasse has been shown experimentally to be a very important predator of ectoparasites, such as monogeneans. They can eat over 1,000 worms per day, significantly reducing parasite burden (Becker and Grutter 2004). Caribbean cleanerfish include neon gobies, other members of the genera *Elacatinus* and *Gobiosoma,* and Spanish hogfish. Many other small tropical marine fish, including juvenile butterflyfish and angelfish, can also be cleaners (Zann 1988). Neon gobies have been used to control ectoparasite infestations in tropical marine fish broodstock (Zimmerman et al. 2001). In temperate seas, other wrasses are commonly used to control sea lice on marine salmon (see PROBLEM 14).

Tropical cleaner shrimp belonging to genera such as *Periclimenes* and *Urocaridella* also feed on many ectoparasites, including isopods, copepods, and monogenean worms (Becker and Grutter 2004). A number of other shrimps, including coral banded shrimps (*Stenopus* spp.) and cleaner shrimps (*Lysmata* spp.) also display similar behavior in the wild (Zann 1988) (Fig. I-47).

Biological control has considerable potential in aquaculture if appropriate control species can be identified and obtained in sufficient numbers.

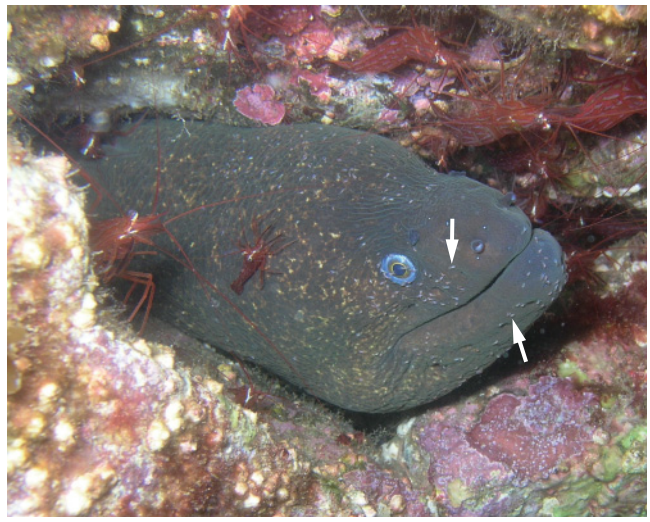


Fig. I-47. Moray eel infested with ectoparasitic copepods (*arrows*) that has entered a cleaner shrimp cleaning station. Note the red shrimp on the moray eel's skin. (Photograph courtesy of S. Salger.)

Fig. I-48. Typical presentation of an acute, high mortality event ("fish kill"). (Photograph courtesy of H. Möller.)

Health Monitoring

Day-to-Day Operations

Aquaculture operations should have a predetermined, routine monitoring schedule that includes water-quality testing, inspecting fish for signs of disease (both the cultured fish and, when appropriate, feral fish in adjacent waters such as near net-pens), and randomly sampling moribund and/or asymptomatic fish for routine diagnostic procedures using standard methods done either on the site or sent if needed to a certified laboratory. Dead or moribund animals must be promptly disposed of in an appropriate site and should not be released into natural waters. Fish kills (Fig. I-48) may require more involved efforts (see "**Mortality Management**," **p. 78**). Needless to say, operations that are unkempt or dirty are

more difficult to disinfect and also are likely to reflect the degree of care that personnel have taken in following biosecurity guidelines.

In some operations, especially those concerned about possible escape of certain pathogens off the farm, it is advisable to institute a sentinel animal program by placing fish in a location where they will be exposed to most or all of the effluent water from the culture systems. Fish are then sampled from this sentinel population at regular intervals (e.g., every few months), and a standard clinical workup is performed to look for pathogens. Operations should always strive to reduce stress; mitigation strategies should be focused on the high disease risk periods (e.g., handling, high temperature, etc.).

Animal Identification

Identification of individual animals is becoming routine in many areas of agriculture and laboratory animal research and is standard practice for many pet animals (e.g., dogs). Government bodies are moving toward this being mandatory for farm animals to allow complete traceability of an animal from birth to store shelf. One of the most useful methods for animal identification is implantation of a microchip transponder. A microchip transponder registers a unique alphanumeric code when activated by a suitable scanner that uniquely identifies that individual. Some scanners have limited detection range in tissue, so guidelines have been developed by the British Veterinary Zoological Society (http://www.bvzs.org) suggesting that implantation of all fish should be deep in the midline, anterior to the dorsal fin. Other sites of implantation have included the left side at the base of the dorsal fin in fish over 30 cm in length and in the peritoneal cavity in smaller fish (Harms and Wildgoose 2001). These transponders in fish are often referred to as PIT (passive integrated transponder) tags (Biomark). Fish can also be tagged using various plastic tags (Floy Tag), similar to ear tags in cattle. Only marking methods that cause minimal damage to the fish should be used (ECPAKFP 2006).

ANIMAL WELFARE

The major, central, unresolved issue in fish welfare is whether fish can feel pain. Fish have a complex nervous system and the presence of nociceptors, receptors that detect noxious stimuli. Also, the behavioral response of at least some fish to analgesics is similar to that of rodents (Sneddon 2003). This suggests that fish have the sense organs and the sensory processing systems to perceive harmful stimuli and that their central nervous system can probably experience at least some of the adverse states that are associated with pain (Adams et al. 2002). But how these responses are processed (i.e., do fish truly feel pain?) is uncertain (Rose 2002).

Many guidelines on humane treatment of fish relate to those held in research facilities (see Erickson 2003 for a comprehensive list of references), but recent articles have started to address fish welfare in a broader context (e.g., Adams et al. 2002), although there are still few criteria proposed that comprehensively address fish welfare in a clinical setting. However, comprehensive synopses of welfare in husbandry systems for Atlantic salmon, trout, common carp, European sea bass and gilthead sea bream have recently been published (EFSA, 2008a–2008d). As has been proposed for other animals, guidelines for humane treatment of fish can be divided into five "freedoms" that encompass the major areas affecting welfare (Adams et al. 2002; ECPAKFP 2006):

1. Freedom from hunger and thirst—For fish, this means providing a nutritious and palatable feed that maintains full health and vigor.
2. Freedom from environmental challenge—For fish, this would include appropriate water flow, nontraumatic substrate, proper lighting, and lack of disturbances (sounds, vibrations, etc.). It would also involve provision of a water supply with fully supportive constituents (oxygen, temperature, pH, etc.) and nonstressful levels of toxins (ammonia, nitrite, etc.).
3. Freedom from injury and disease—For fish, this includes treating disease promptly and appropriately, performing procedures (handling, medical therapies) in a nonstressful manner, and, when needed, properly using sedatives and anesthetics for mitigating stress and alleviating pain.
4. Freedom to express normal behavior—For fish, this would include proper population density and holding units with an appropriate size, shape, and substrate.
5. Freedom from fear and distress—For fish, this means avoiding all conditions that cause mental or physical suffering, including aggression, cannibalism, trauma, and inappropriate handling or display.

Implicit in these guidelines is that conditions should be tailored to the particular species, life stage, and environmental setting. As can be seen above, fish welfare closely parallels the avoidance or remediation of deleterious conditions outlined in the PROBLEM LIST (part II), and thus adequate fish welfare is quite simply reflected in providing an environment for optimal functioning of the individual and population. Specific details of the issues relating to these guidelines are provided for specific PROBLEMS. Responsible choice of fish for an aquarium has been discussed on **p. 5**.

To facilitate the day-to-day evaluation of fish welfare, several simple indicators have been proposed (Adams et al. 2002). Note that these indicators are also part of a routine diagnostic workup (**p. 17**) but also should be part of the routine, daily assessment of a fish population:

- Color
- Ventilation rate

- Swimming pattern and other behaviors
- Food intake
- Growth rate
- Condition
- Presence of morphological abnormalities
- Injury
- Disease
- Reproductive performance

Even in mammals, assessment of welfare is a challenging, highly contested issue; and considerable work is needed to have a clearer understanding of how it is best evaluated. For the most recent, in-depth discussion of fish welfare issues, see Branson (2007).

FOOD SAFETY

Antibiotic-Resistant Pathogens

The possible development and transfer of antibiotic-resistant pathogens from farm animals to humans has been a hotly debated topic in recent years. Zoonotic pathogens are much less prevalent in fish than in mammalian food animals, and there are no studies that strongly support the transfer of antibiotic-resistant pathogens from fish to humans. Nonetheless, the clinician must keep in mind that repeated antibiotic treatment of bacteria leads to resistance and the use of antibiotics should be minimized (see "**Antibiotics**," **p. 377**, for more information).

Chemical Contaminants

Drug Residues

The rapid growth of aquaculture has resulted in a concomitant increase in drug use to combat infectious diseases. As aquaculture has become an increasingly significant part of the world's food supply, concerns about drug contamination have increased (Costello et al. 2001). This has been complicated by the large variations in drug use (both legal and illegal) among different countries. Testing of fish products for drug contamination is being done by local authorities and importing countries with increasing frequency, especially for substances banned from food animal use in many countries (e.g., chloramphenicol, nitrofurans, dimetridazole, malachite green). The discovery of illegal residues can have highly serious ramifications, not only for the clinician and the farm where the contaminants originated but also for the nation if the product is exported. Thus it is imperative that clinicians adhere to legal guidelines regarding the use of drugs, including required withdrawal times for legal drugs (Lupin et al. 2003). Details for specific drugs are described in "**Pharmacopoeia**." GESAMP (1997) also provides information on the potential impacts on human health of drugs commonly used in coastal aquaculture. There are commercial kits available to test for the presence of antibiotic residues from Neogen Corporation and Charm Sciences.

Environmental Toxins

There have been recent concerns about the possible contamination of cultured fish stocks by environmental toxins, including PCBs (polychlorinated biphenyls; Hites et al. 2004; Mozaffarian and Rimm 2006). However, the data supporting this claim has been contested (Gochfeld and Burger 2005). For example, samples examined in another study found that contaminants (PCBs and mercury) in all salmon, wild or farmed, were at levels well below consumption thresholds considered safe (Ikonomou et al. 2007). Nonetheless, this controversy points out that as aquaculture continues to expand globally, such issues will need to be addressed by the industry.

ENVIRONMENTAL SAFETY

Mortality Management

Fish euthanized at the clinic should be disposed of using standard biohazard guidelines for infectious waste. All fomites should be disinfected using a high-level disinfectant.

On farms, there are three main environmental concerns with dead fish, especially after a large kill. First, carcasses and associated pathogens might be released from holding systems (ponds, cages, etc.) into public waters. Second, decomposition of dead fish can cause effluent water quality to decline. Third, odors might be a nuisance where farms are near homes. In many intensive culture systems, it is not unusual to see occasional dead fish especially during certain times of the year; these should be removed as part of normal biosecurity measures. A few dead fish are not a major environmental concern; but, after a large kill, fish should be promptly removed and either placed in a permitted landfill or appropriately prepared (incinerated, composted, rendered, or ground up) so they can be applied to land as fertilizer. In ponds, riser pipes should be equipped with trash racks to prevent floating dead fish from entering discharge pipes (Anonymous 2002).

Insurance is available in some countries to indemnify some fish farming enterprises against certain disease losses (van Anrooy et al. 2006; Sempier et al. 2007) and if a claim is to be made, certain procedures for documenting the losses may need to be followed.

Drugs in the Environment

Aquaculture practices have the potential to negatively impact the environment (Cabello 2006), especially in

semi-open and open systems where drugs are not easily contained. Some drugs may persist for very long times. For example, the antibiotics oxytetracycline, oxolinic acid, and flumequine are detectable in sediments at least 6 months after being used for treatment in sea cages (Weston 1996); and under some circumstances, potentiated sulfas and quinolones can persist for over 1 year (Bakal and Stoskopf 2001; Zuccato et al. 2004). Persistent antibiotics may inhibit microbial activity in the sediment, reducing the rate of aerobic organic matter decomposition (Hansen et al. 1992b). Persistent antibiotics can also induce selection for antibiotic-resistant bacteria, but whether this leads to development of antibiotic-resistant human pathogens is unknown (Zuccato et al. 2004). While not directly related to aquaculture, there has been concern about the presence of some pharmaceuticals in quite high concentrations in rivers and even drinking water (Zuccato et al. 2004).

Drug-contaminated particulates (food, feces, etc.) originating from fish medicated in sea cages can result in the drug appearing in both fish and filter-feeding mollusks near the cages. For example, oxolinic acid or oxytetracycline residues can persist in fish or shellfish around a salmon cage for 2 weeks after drug treatment has ended (Capone et al. 1996). This is especially a concern when commercial or sport fishermen frequent cage sites due to the especially high numbers of wild fish attracted to such sites. Drugs can also kill or injure nontarget aquatic species; pesticides (e.g., organophosphates used as ectoparasiticides), disinfectants, and anti-fouling agents are most problematic.

Drugs in water can be degraded via either abiotic means (photoxidation or hydrolysis) or biotic means (aerobic or anerobic microbes). The most persistent chemicals tend to be those resistant to photodegradation and hydrolysis (Boxall et al. 2004). Details about the fate and effects of specific drugs used in aquaculture are discussed in "**Pharmacopoeia**." GESAMP (1997) also provides details on the potential environmental impacts of drugs commonly used in coastal aquaculture.

Exotic Pathogens and Exotic Hosts

For the sake of this discussion, "exotic pathogen" is defined as any pathogen that presently does not exist in a particular geographic area. This area could be a country, a state/province, or a much smaller area, including a single farm. The point of this definition is to emphasize that all efforts should be made to keep such pathogens excluded from the region. The introduction of an exotic pathogen represents one of the greatest threats to the health and economic viability of aquatic animal populations. As will be evident going through the problem list, many, if not most, of the most important diseases affecting cultured fish have been inadvertently spread to nonnative localities because of the lack of proper biosecurity measures. A number of diseases affecting wild fish populations have also been spread in this manner. Thus, it is critical to ensure that appropriate measures always be taken to prevent further introductions. In addition, the suspicion that an exotic agent might have been introduced should promptly be reported to appropriate authorities (Davenport et al. 2003).

Another possible concern in terms of novel host-pathogen relationships is that endemic pathogens might be much more pathogenic to exotic fish that are imported into a region for culture. An exotic fish species may be inherently more susceptible to an endemic pathogen that it has not previously encountered in nature; subsequently, the exotic host may act as a major reservoir for that pathogen, resulting in the amplification of that pathogen's abundance in the environment, leading to epidemic disease in native populations. This has been suspected to be one possible reason for the epidemic prevalence of mycobacteriosis in native Red Sea fish species after the introduction of the European sea bass for aquaculture (A. Colorni, personal communication).

PART II

PROBLEM LIST

CHAPTER 7

PROBLEMS 1 through 10

Diagnoses made with commercially available water-quality test kits or equipment that should be present in the clinician's clinic

1. Environmental hypoxia
2. Temperature stress
3. Temperature stratification
4. Ammonia poisoning
5. Nitrite poisoning
6. Nitrate poisoning
7. Too low (too acidic) pH
8. Too high (too alkaline) pH
9. Improper hardness
10. Improper salinity

PROBLEM 1
Environmental Hypoxia

Prevalence Index

WF - 1, WM - 1, CF - 1, CM - 1

Method of Diagnosis

1. Measurement of oxygen concentration
2. History

History

General: Overcrowding; low water flow in raceway; algae crash in pond; several overcast days over pond

Acute environmental hypoxia: Acute shutdown of aeration caused by power failure; acute mortality of all but air-breathing fish; fish piping for air; gathering at water inflow; depression; death with opercula flared and mouth agape; acute stress response; large fish die (small fish may survive)

Chronic environmental hypoxia: Chronic stress response

Physical Examination

See "**History**"

Treatment

1. Acute hypoxia
   a. Restore oxygen levels immediately
   b. Monitor ammonia (see PROBLEM 4) and nitrite (see PROBLEM 5) daily for 1 week to be sure that biological filtration is functioning properly (aquaria only)
2. Chronic hypoxia
   a. Increase aeration
   b. Reduce feeding
   c. Reduce fish density

COMMENTS

Definition of Environmental Hypoxia

Environmental hypoxia means that a low concentration of dissolved oxygen (DO) exists in the water. Oxygen is the most important water-quality factor for proper fish health, but it is poorly soluble in water. For example, the maximum amount of oxygen that can dissolve in freshwater at 28°C (82°F) is 7.84 mg/l (Fig. II-1, C; Table II-1; Murray and Riley 1969). This compares with over 150 mg O_2 per liter of air at sea level; there may be less oxygen if the culture system is crowded or has inadequate aeration. Temperature and, to a much lesser extent, salinity have a significant influence on oxygen solubility. Thus, the higher the temperature and higher the salinity, the lower the total amount of oxygen that will dissolve in the water.

Sources and Users of Oxygen

Oxygen can enter water from photosynthesis or by diffusion of atmospheric oxygen. In a pond without mechanical aerators, photosynthesis is the most important source of oxygen (and is also the cause of many diurnal changes in pond water quality; see Fig. II-1, D; Noga and Francis-Floyd 1991). A certain amount of algae in a pond is desirable, because it increases oxygen production and thus allows a greater number of fish to be stocked. High fish stocking densities also result in large algae populations because of the plant nutrients that are released from fish excrement.

The effect of algal metabolism on oxygen levels is dramatically illustrated by the marked diurnal variation in oxygen concentration in a pond with a large algae population (see Fig. II-1, D). Oxygen concentration is highest near sunset because net oxygen production

A

B

C

Correction factor for oxygen saturation at various altitudes

Altitude Feet	Altitude Meters	Pressure mm	Factor
0	0	760	1.00
330	100	750	1.01
655	200	741	1.03
980	300	732	1.04
1310	400	723	1.05
1640	500	714	1.06
1970	600	705	1.08
2300	700	696	1.09
2630	800	687	1.11
2950	900	679	1.12
3280	1000	671	1.13
3610	1100	663	1.15
3940	1200	655	1.16
4270	1300	647	1.17
4600	1400	639	1.19
4930	1500	631	1.20
5250	1600	623	1.22
5580	1700	615	1.24
5910	1800	608	1.25
6240	1900	601	1.26
6560	2000	594	1.28
6900	2100	587	1.30
7220	2200	580	1.31
7550	2300	573	1.33
7880	2400	566	1.34
8200	2500	560	1.36

Fig. II-I. A. Fish piping for air near the surface of the water because of low dissolved oxygen (DO). The air-water interface has the highest concentration of oxygen. B. Fish that died because of acute environmental hypoxia. C. Rawson's oxygen saturation values at various temperatures and altitudes.

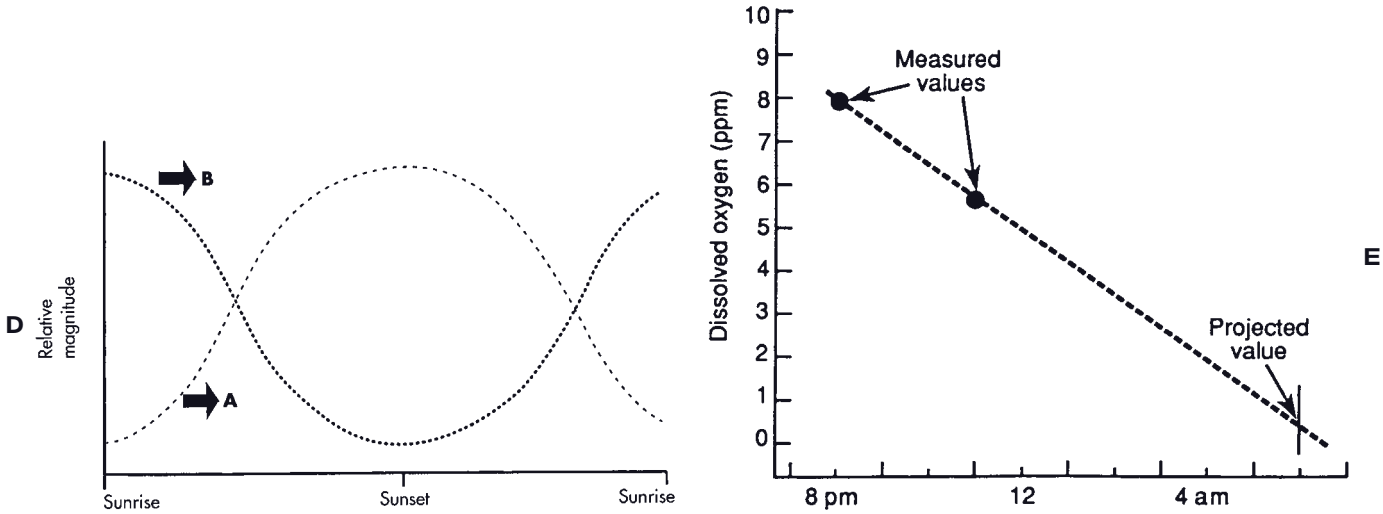


Fig. II-I.—cont'd. D. Diurnal fluctuations in water-quality variables in a pond. Dissolved oxygen, temperature, pH, and percentage of unionized ammonia usually increase during the day (*curve A*); dissolved carbon dioxide levels usually increase overnight (*curve B*). E. Extrapolation method for estimating nighttime decline in dissolved oxygen in a pond. (*A* photograph courtesy of T. Wenzel; *B* photograph from H. Möller; *C* from Piper et al. 1982; *D* from Noga and Francis-Floyd 1991; *E* from Boyd et al. 1978.)

Table II-I. Dissolved oxygen (mg $O_2 l^{-1}$) at saturation in freshwater, brackish water, and seawater at different temperatures. Calculated from data in Murray and Riley (1969) from the table in Spotte (1979a). Note that chlorinity is a close approximation of salinity.

	Chlorinity (‰)										
(°C)	0	2	4	6	8	10	12	14	16	18	20
1	14.24	13.87	13.54	13.22	12.91	12.59	12.29	11.99	11.70	11.42	11.15
2	13.84	13.50	13.18	12.88	12.56	12.56	11.98	11.69	11.40	11.13	10.86
3	13.45	13.14	12.84	12.55	12.25	11.96	11.68	11.39	11.12	10.85	10.50
4	13.09	12.79	12.51	12.22	11.93	11.65	11.38	11.10	10.83	10.59	10.34
5	12.75	12.45	12.18	11.91	11.63	11.36	11.09	10.83	10.57	10.33	10.10
6	12.44	12.15	11.86	11.60	11.33	11.07	10.82	10.56	10.32	10.09	9.86
7	12.13	11.85	11.58	11.32	11.06	10.82	10.56	10.32	10.07	9.84	9.63
8	11.85	11.56	11.29	11.05	10.80	10.56	10.32	10.07	9.84	9.61	9.40
9	11.56	11.29	11.02	10.77	10.54	10.30	10.07	9.84	9.61	9.40	9.20
10	11.29	11.03	10.77	10.53	10.30	10.07	9.84	9.61	9.40	9.20	9.00
11	11.05	10.77	10.53	10.29	10.07	9.84	9.63	9.41	9.20	9.00	8.80
12	10.80	10.53	10.29	10.06	9.84	9.63	9.41	9.21	9.00	8.80	8.61
13	10.56	10.30	10.07	9.84	9.63	9.41	9.21	9.01	8.81	8.61	8.42
14	10.33	10.07	9.86	9.63	9.41	9.21	9.01	8.81	8.62	8.44	8.25
15	10.10	9.86	9.64	9.43	9.23	9.03	8.83	8.64	8.44	8.27	8.09
16	9.89	9.66	9.44	9.24	9.03	8.84	8.64	8.47	8.28	8.11	7.94
17	9.67	9.46	9.26	9.05	8.85	8.65	8.47	8.30	8.11	7.94	7.78
18	9.47	9.27	9.07	8.87	8.67	8.48	8.31	8.14	7.97	7.79	7.64
19	9.28	9.08	8.88	8.68	8.50	8.31	8.15	7.98	7.08	7.65	7.49
20	9.11	8.90	8.70	8.51	8.32	8.15	7.99	7.84	7.66	7.51	7.36
21	8.93	8.72	8.54	8.35	8.17	7.99	7.84	7.69	7.52	7.38	7.23
22	8.75	8.55	8.38	8.19	8.02	7.85	7.69	7.54	7.39	7.25	7.11
23	8.60	8.40	8.22	8.04	7.87	7.71	7.55	7.41	7.26	7.12	6.99
24	8.44	8.25	8.07	7.89	7.72	7.56	7.42	7.28	7.13	6.99	6.86
25	8.27	8.09	7.92	7.75	7.58	7.44	7.29	7.15	7.01	6.88	6.75
26	8.12	7.94	7.78	7.62	7.45	7.31	7.16	7.03	6.89	6.76	6.63
27	7.98	7.79	7.64	7.49	7.32	7.18	7.04	6.91	6.78	6.65	6.52
28	7.84	7.65	7.51	7.36	7.19	7.06	6.92	6.79	6.66	6.53	6.40
29	7.69	7.52	7.38	7.23	7.08	6.95	6.82	6.68	6.55	6.42	6.29
30	7.56	7.39	7.25	7.12	6.96	6.83	6.70	6.58	6.45	6.32	6.19

occurs during the day. At night, oxygen levels decline because of the cessation of photosynthesis. Because plant and animal respiration (including microbial degradation of organic matter) occurs continuously, a net loss of oxygen occurs at night. Thus, oxygen levels are at their lowest level just before sunrise.

In aquaria, raceways, and other high-density culture systems, algal photosynthesis is not sufficient to support the high fish biomass. Thus, oxygen levels must be supplemented by either constant mechanical aeration (aquaria) or by constantly running oxygenated water through the system (raceways). Raceways most commonly use surface water from a natural stream or lake; the latter are usually nearly saturated with oxygen.

Causes of Environmental Hypoxia

PONDS

Low oxygen is common in ponds, especially in summer, when warm temperatures both decrease oxygen's solubility (Table II-1; Fig. II-1, C) and increase the pond organisms' metabolism and subsequent oxygen demand. An intimate relationship exists between oxygen levels and pond metabolism. While it is desirable to have algae in a pond to increase oxygen production, too much algae can cause wide fluctuations in DO, because algae are both the major producers and consumers of oxygen in most ponds. Consequently, the large nocturnal oxygen demand can cause a low DO by sunrise.

Other circumstances can also lead to environmental hypoxia in ponds. Cloudy weather decreases photosynthesis and thus reduces net oxygen production (Tucker 1985). Overcast weather may cause severe hypoxia by steadily decreasing oxygen production from lower light intensity. A crash or massive death of algae, a common but usually unpredictable event, can cause severe oxygen depletion (Boyd 1979). Decreased oxygen production is exacerbated by the great oxygen demand of the decaying algae. Many chemicals that are commonly used to treat fish diseases (e.g., copper sulfate, potassium permanganate, or formalin) are algicidal (Schnick et al. 1989). These agents must be used with extreme caution in ponds having large algae populations.

If ice forms on a pond in winter, the oxygen can become progressively depleted, leading to anoxia (Barica and Mathias 1979). This most commonly occurs in late winter and early spring in ponds that have a permanent winter ice cover. The ice and snow prevent oxygen diffusion into the pond and block photosynthesis, while respiration of pond organisms continues, albeit at a low metabolic rate. Many factors determine whether a kill will occur, including how long the ice cover persists and the amount of decaying matter in the pond. Shallow ponds are more susceptible because of the smaller total amount of oxygen. Other metabolic processes, such as increased CO_2 (see PROBLEM 90) and hydrogen sulfide (see PROBLEM 91), probably contribute to mortalities.

This type of winter kill should not be confused with winter kill due to water mold infection (see PROBLEM 34).

AQUARIA AND OTHER HIGH-DENSITY SYSTEMS

The most obvious cause of environmental hypoxia in high-density, closed-culture systems is due to failure of aerators, which leads to an acutely low oxygen level. This is a common sequela to an electrical power failure and can cause acute mortality in a home aquarium or other system. Because the oxygen may be off for only a short time and since the power often returns to normal after the fish have died without the aquarist observing the power failure, such events must be diagnosed from the history. Survival of only air-breathing fish (e.g., anabantids, clariid catfish) is one clue that acute hypoxia may be responsible. Heavily planted aquaria could become hypoxic at night because of the plants' respiration. However, this is rare, since mechanical aeration prevents this problem.

While surface water from streams or lakes is usually well oxygenated, ground (well or spring) water is typically low in dissolved oxygen and high in other gases; both conditions can cause hypoxia (see PROBLEMS 11 and 90). Nets used to confine fish in cages or pens can become fouled, impeding water flow and reducing oxygen supply.

Clinical Signs of Environmental Hypoxia

Acute environmental hypoxia is defined as a rapid (within minutes to hours) drop in DO to lethal or near-lethal levels. It is often accompanied by acute and frequently catastrophic mortalities. Common behavioral signs include lethargy (Scott and Rogers 1980) and the congregating of fish near the air-water interface (piping; Fig. II-1, A), where oxygen levels are highest (Francis-Floyd 1988). Hypoxic fish are often anorectic. A classical sign of asphyxiation is an agonal response, with the mouth open and the opercula flared (Fig. II-1, B), although this is not pathognomonic for environmental hypoxia.

Chronic environmental hypoxia is defined as a long-term (days or longer) suboptimal dissolved oxygen level in a culture system. Chronic hypoxia does not kill fish outright but causes considerable stress. At least 5 mg/l of dissolved oxygen is needed for optimal growth and reproduction of most fish (Tucker 1985). Below this level, food consumption decreases and becomes less efficient (Hollerman and Boyd 1980) and growth slows (Andrews et al. 1973). A DO of less than 2 mg/l is very stressful and may predispose fish to opportunistic infections (Scott and Rogers 1980). If DO remains below 1 mg/l for any period of time, most fish die (Tucker 1985). Many warm water fish can survive for long periods in 2 to 3 mg/l

oxygen. Amazingly, goldfish can live for 20 hours in *anoxic* water at 20°C (68°F; Nakamura 1995). At the other extreme, many cold water species (e.g., salmonids) only tolerate 4–5 mg/l oxygen for long periods.

Channel catfish that recover from acute environmental hypoxia may develop deep, necrotic ulcers (see PROBLEM 102, Red Fillet Syndrome; Plumb 1984).

Diagnosis of Environmental Hypoxia

Definitive diagnosis of low DO can only be done by measuring the DO in water at the site or by immediately preserving the water sample. Once the sample is removed from the aquarium or pond, its oxygen concentration changes immediately because it is mixed with air. Oxygen measurements made on unpreserved water samples submitted to the clinic are not valid. Thus, a diagnosis of environmental hypoxia is based upon the history, unless an electronic meter is used to measure oxygen on site or unless the sample is immediately preserved, using a commercial test kit.

In flow-through systems, DO is highest at the inflow and lowest at the outflow. In ponds, clinical signs are most commonly evident in early morning; hypoxic conditions often dissipate rapidly after sunrise, masking the event. The largest fish are usually most susceptible to oxygen depletion. In aquaria, survival of only air-breathing fish is strongly suggestive of acute environmental hypoxia. Although not air breathers, goldfish can also withstand low DO for a long time.

Environmental hypoxia must be differentiated from other causes of hypoxia, including nitrite toxicity (see PROBLEM 5) and gill parasitosis.

Treatment of Environmental Hypoxia

Acute environmental hypoxia is an emergency situation, and immediate steps must be taken to provide fish with oxygenated water. The catastrophic nature of acute environmental hypoxia dictates that all possible measures be taken to prevent the development of this situation. For large culture systems holding high fish densities, this means having adequate emergency aerators and power sources to handle hypoxic events. Chronic environmental hypoxia is less of an emergency but is still a serious problem that should be addressed expeditiously. Reducing feeding will reduce fish and algal oxygen consumption (Andrews and Matsuda 1975).

PONDS

Supplemental aeration should begin if DO drops below 3–4 mg/l (Tucker 1985) in channel catfish ponds and 4–5 mg/l in salmonid culture. Aeration equipment includes both pneumatic (i.e., air pumps) and mechanical (i.e., paddlewheels) devices. Water may also be transferred from an adjacent pond to the hypoxic pond. Oxygenated well water may also be used. Aeration usually does not increase the DO throughout the entire pond but provides local areas of oxygen-rich water (Tucker 1985), where fish remain until the dissolved oxygen level in the entire pond returns to acceptable levels. Thus, circulation is just as important as aeration because circulation increases the volume of oxygenated water in the pond. Dissolved oxygen concentrations may vary significantly at different ends of a pond because of differences in algal densities, wind direction, and related factors. Aerators should be placed where the DO is highest and thus where fish are congregating. While tractors may be used to operate aerators, electrical power is much more convenient and economical. Principles of aeration and management of dissolved oxygen problems are reviewed by Tucker (1985).

If pond fish are raised at high densities (e.g., channel catfish at greater than 1,900 kg/ha = 2,000 lb/ac), keep constant vigilance for environmental hypoxia. An oxygen meter is essential for a commercial aquaculture enterprise. Measuring DO in a pond both at dusk and 2 or 3 hours later allows the clinician to draw a straight line that can reliably predict the DO concentration at dawn (Fig. II-1, E).

Algae concentrations should be routinely monitored by the farmer. Algal density can be estimated by placing a Secchi disk (Tucker 1985) or some other object (e.g., yardstick) into the water to measure turbidity. In general, pond water visibility should be no less than 0.5 m from the water's surface (Noga and Francis-Floyd 1991). Be aware that turbidity may also result from suspended clay or other particles.

AQUARIA

Chronic environmental hypoxia is rarely a problem in aquaria because of the considerable amount of mechanical aeration. However, acute environmental hypoxia does occur because of electrical or mechanical failure of aeration equipment. Some evidence exists that adding hydrogen peroxide can provide a short-term increase in DO concentration (Maranthe et al. 1975). Ammonia and nitrite levels should be closely monitored in aquaria that have experienced an acute drop in DO because the bacteria that remove these toxins require oxygen and thus may be harmed (see PROBLEMS 4 and 5).

FLOW-THROUGH SYSTEMS

Water used for culture should be at close to 100% saturation. When well water is used for flow-through culture, it must usually be aerated or at least allowed to equilibrate with the atmosphere before fish are exposed to it (see PROBLEMS 11 and 90). In flow-through systems that are without supplemental aeration, stocking density is usually limited by oxygen concentration, especially at >10°C (>50°F) (Piper et al. 1982). In trout culture, low oxygen is usually a problem in summer when low water flow, high metabolism, high organic decay, and large amounts of algae occur. The maximum stocking density

recommended for salmonids in raceways can be calculated using the following Flow Index:

$$F = \frac{W}{L \times I}$$

F = Flow Index
W = the permissible weight (pounds) of fish at a given inflow
I = gallons per minute for a given fish size
L = inches

Flow Index will vary with different hatcheries, depending upon water saturation and water chemistry. Piper et al. (1982) discuss how to calculate the Flow Index for various species. Other factors will also influence stocking density (see PROBLEMS 4 and 98). For salmonids, oxygen levels should ideally not be less than 6 mg/l or 80% saturation and should never drop below 5 mg/l at the end of the raceway.

CAGES AND NET-PENS

Cage and net-pen systems rely on tidal currents or other water movement to supply oxygen. Fouling organisms must be removed from netting regularly to prevent blockage of water flow.

PROBLEM 2
Temperature Stress

Prevalence Index

Hypothermia: WF - 2, WM - 2, CF - 4, CM - 4
Hyperthermia: WF - 4, WM - 4, CF - 2, CM - 2

Method of Diagnosis

1. Measurement of abnormal temperature
2. History

History

General: Acute to chronic stress response

Hypothermia: Temperature at or near lower lethal limit of that particular species; shutdown of aquarium heater because of power failure or broken thermostat; thermometer not working properly; heater wattage too small for aquarium; aquarium next to window or draft; shimmies; lethargy; mortality of all but the most cold-tolerant aquarium fish (e.g., goldfish and koi); water mold infection

Hyperthermia: Temperature near the upper lethal limit of that particular species; dyspnea; heater thermostat improperly set; heater not adequately submerged or thermostat broken; heater wattage too large for aquarium; aquarium next to heat source or window; summer

Physical Examination

See "**History**"

Treatment

1. Restore proper temperature within appropriate period of time (as soon as physiologically tolerable)
2. Move fish to environment with proper temperature

COMMENTS

Effects of Temperature on Fish Physiology

Fish are poikilothermic; therefore temperature dramatically affects their metabolism, including immunity (Finn and Nielsen 1971; Avtalion et al. 1973). Decrease in water temperature suppresses the immune response (Clem et al. 1984). Perturbations in immune function may partly explain why many pond fish diseases are most common in the spring and fall (MacMillan 1985), when temperature fluctuation is greatest.

Definition of Temperature Stress

There are standard environmental temperature (SET) ranges for individual fish species that define the temperatures for optimal growth. However, absolute temperature ranges for health or survival do not exist because temperature tolerance depends on several factors, including the temperature to which the individual has been acclimated, salinity (for estuarine species), life stage, and reproductive status. The speed of temperature change is also important (see "**Acclimation**," **p. 65**). Thus, it is difficult to generalize about temperature tolerance because it is influenced by so many factors. However, it is important to be aware of the general temperature ranges (Table II-2, A) for the species being examined and the conditions that may influence it.

Temperate species, such as channel catfish, striped bass, and largemouth bass, often tolerate a wider temperature range than tropical fish or cold water species (e.g., salmonids). All fish are susceptible to rapid temperature changes.

Table II-2, A. Optimal and tolerable temperature ranges in °C (°F) for representative fish. Note that these are general guidelines that vary considerably, depending on species, prior acclimation, and the prevailing environmental conditions.

Group	Optimal	Upper tolerance	Lower tolerance
Freshwater tropicals	22–27 (72–81)	30–40 (86–104)	8–18 (46–64)
Marine tropicals	22–27 (72–81)	30–40 (86–104)	8–18 (46–64)
Goldfish; koi	15–22 (59–72)	30 (86)	2–4 (36–39)
Sunfish	26–30 (79–86)		
American eel		30 (86)	
Striped bass juvenile	18–28 (64–82)		
Striped bass adult	18–25 (64–77)		
Channel catfish	28–30 (82–86)	35 (95)	0–2 (32–36)
Red drum	22–25 (72–77)		
Atlantic salmon	17 (63)	19 (66)	
Rainbow trout	15 (59)	19 (66)	
Brook trout	15 (59)	18 (64)	
Pacific salmon	12 (54)	18 (64)	

Table II-2, B. Required wattage of electric heaters (from Sterba 1983). For example, if 50 liters of water must be raised 10°C, a heater of at least 77 watts is required.

Tank volume (liters)	Tank volume (gallons)	How high temperature must be raised in °C (°F) (temperature differential)		
		5 (9)	10 (18)	15 (27)
		Wattage Required		
10	2.6	11	22	33
50	13	39	77	115
100	26	50	100	150

In general, most fish seem to tolerate a rapid drop in temperature better than an equivalent rise in temperature. This is probably due to the physiological changes that occur with increasing temperatures: metabolic rate (and thus oxygen consumption) increases with temperature. However, oxygen is less soluble at higher temperatures (see Table II-1). Thus, hypoxia may exacerbate hyperthermia. Also, stress hormone release increases with temperature. Immune function may also take time to equilibrate to the higher temperature, while pathogens can adjust much more quickly; this may explain why many bacterial and parasitic diseases are more common in spring (Meyer 1978).

Diagnosis

As with dissolved oxygen, water temperature can only be accurately measured at the site, so a diagnosis of temperature stress at the clinic is based on the history. A history of temperature stress will vary, depending on the variables discussed above—how low or high the temperature becomes and how quickly it takes to arrive at the stressful temperature. For example, many tropical aquarium fish can withstand a relatively low temperature as long as the change occurs slowly; this might occur during fall in an unheated aquarium. Conversely, if the heater stopped working in an aquarium in the middle of winter, dropping the temperature 10°C in 1 day, it might cause many fish to die immediately.

It is important to realize that in ponds, water temperatures may normally fluctuate as much as 10°C daily without any apparent harm to the fish (Boyd 1990). This emphasizes the importance that acclimation plays in determining the effect of a temperature change.

HYPOTHERMIA

Because fish are cold-blooded animals, their activity depends on temperature. Thus, at low temperatures, fish become inactive and depressed. Fish exposed to suboptimal temperatures are especially susceptible to water mold infections (see PROBLEM 34).

Fish that are stocked outside their normal geographic range may succumb to hypothermia. For example, tilapia, a hardy cichlid, is often stocked in summer in subtropical or temperate areas of the United States, but tilapia usually die when temperatures reach less than approximately 12°C. Low pond temperatures have been associated with an idiopathic syndrome also known as winter kill (see PROBLEM 34). Do not confuse this problem with winter kill that is caused by oxygen depletion (see PROBLEM 1).

HYPERTHERMIA

Hyperthermia can be a serious problem in salmonids, when temperatures in some culture systems may approach their upper lethal limit, such as during summer with trout cultured in the southern Appalachian region of the United States; this often increases susceptibility to opportunistic infections.

Treatment and Prevention

Temperature control is feasible and routine in small, closed systems (e.g., aquaria). In general, marine reef fish are more sensitive to temperature stress than freshwater fish and marine reef tanks should have a heater of sufficient wattage to keep the aquarium at 24°C (75°F). About 3–10 watts/gallon is usually satisfactory (Table II-2, B). The heater should not be more powerful than needed to maintain the temperature, because the higher the wattage, the faster the temperature will rise when the heater is turned on. If the tank has only fish, occasional excursions to the low 80s°F are tolerated. However, invertebrates are less tolerant of hyperthermia, and if the temperature does not remain below 27°C (80°F), a chiller should be used (Shute and Tullock 1995).

In ponds or other culture systems with large volumes of water, temperature stress is usually economically unfeasible to control. Some tropical fish farmers use plastic sheeting to insulate ponds during cold snaps, but this is not practical for food fish ponds. In flow-through systems, temperature control is only feasible when either recycling most of the water or when egg incubation systems that use very little water are employed.

When fish are removed from water (e.g., netted into hauling tank, etc.), avoid hyperthermia by doing it at the cooler time of the day and avoiding direct sunlight exposure. In winter, cold air and wind chill can cause temperature shock (Jensen 1990).

It is difficult to give exact recommendations for allowable temperature change because it varies with species, environment, and prior acclimation conditions. For example, fish acclimated to a higher temperature often can withstand hyperthermia better than the same species maintained at a lower temperature. When acclimating fish to a certain temperature, a rule of thumb is that water temperature should not be changed more than about 1°C (or 1°F) per hour. While some fish may be stressed by this change, many others tolerate even more rapid changes. For example, when transporting warm water food fish (e.g., channel catfish), it is advisable to lower the temperature to reduce stress. Prior to hauling, the

temperature can be reduced up to 5°C (10°F) every 20 minutes, unless the fish are very small (Jensen 1990). Fish that are normally exposed to wide temperature fluctuations (e.g., in ponds) are probably more tolerant of rapid temperature change than fish that are kept under more stable conditions (e.g., thermostatically controlled temperature in an aquarium).

HYPOTHERMIA

Tropical aquarium fish that are shipped to temperate regions in winter may be exposed to large temperature drops. If the fish are exhibiting clinical signs of hypothermia and the temperature is well outside their normal range, it should be returned to at least near their normal temperature range as quickly as possible. One way this can be done is by filling plastic aquarium bags with warm water and floating them in the shipment water.

Note that temperate species (e.g., channel catfish, salmonids) are often deliberately cooled quickly before shipping to reduce transport stress (Piper et al. 1982).

HYPERTHERMIA

As it is with hypothermia, the ability of a fish to tolerate hyperthermia depends not only on how high the temperature becomes but also on how quickly it rises. Slow increases in temperature are tolerated much better. When acclimating aquarium-kept fish to a high temperature (such as for breeding), it is best not to raise the temperature more than approximately 3°C (or 3°F) per day. In some cases, such as when transporting fish, the temperature may be unavoidably raised above this maximum. When temperature increases, oxygen should be as close to saturation as possible because low oxygen inhibits the ability of fish to acclimate to temperature change (Weatherly 1970). During transport, it is advisable to lower the temperature to the low end of the physiological range for that species (see "**Hypothermia**," **above**).

When the temperature is near a species' upper lethal limit, it is often wise to reduce or stop feeding, since the amount of oxygen needed for both homeostasis and digestion of food may exceed the amount of oxygen that can be extracted from the water (Stevenson 1987).

PROBLEM 3
Temperature Stratification

Prevalence Index

WF - 1, CF - 1

Method of Diagnosis

Detection of a significant thermocline

History

Deep pond (>1.5 m [5 feet]); spring/summer/fall; eutrophic pond

Physical Examination

Varies with sequela

Treatment

1. Provide emergency aeration
2. Prevent future stratification events

COMMENTS

Definition

Temperature stratification is not a problem in itself, but instead it causes changes in pond water quality, which can be lethal. Temperature stratification refers to the development of two distinct temperature zones in a pond (Fig. II-3); it occurs when the surface water of a pond warms up, while the bottom water remains cooler. Temperature stratification is a common problem in farm and watershed-type ponds that are often over 1.5 m deep, but it is rarely a problem in commercial channel catfish ponds, which are usually less than 1.5 m (5 feet) deep.

Causes

Stratification is most likely to develop during hot, calm summer days when little water mixing occurs by wind action. As the temperature difference between the surface water (epilimnion) and bottom water (hypolimnion) increases, the pond stratifies into two layers of water that are separated by the metalimnion, or thermocline, where water temperature changes rapidly from the warm surface temperatures to the cool bottom temperatures. Warm water is lighter, and thus the thermocline acts as a physical barrier between the epilimnion and hypolimnion, and a considerable amount of energy is required to mix, or "turn over," the pond. The DO in the hypolimnion is rapidly depleted by pond metabolism and an oxygen demand builds up as anaerobic reactions are not sufficient to form final degradation products of pond metabolites. Toxic substances, such as hydrogen sulfide and methane, may accumulate under these reducing conditions.

Consequences

The longer the stratified state persists, the greater the danger of a lethal oxygen depletion and toxin release when the pond finally mixes. Inclement weather (heavy winds or cold rain), harvesting (seining), or aeration can mix a stratified pond. In addition, a stratified pond will eventually turn over in fall, when surface water temperatures cool. Stratification can be prevented by having the farmer run weekly oxygen profiles on each pond in at least two places (Noga and Francis-Floyd 1991). The DO and temperature are measured at 0.3 m (1 foot) intervals from surface to bottom. If stratification is present, both temperature and DO will rapidly change at the thermocline, and there may be little oxygen below that depth. Any evidence of stratification should be corrected immediately by aeration. Early detection is essential to preventing a catastrophe.

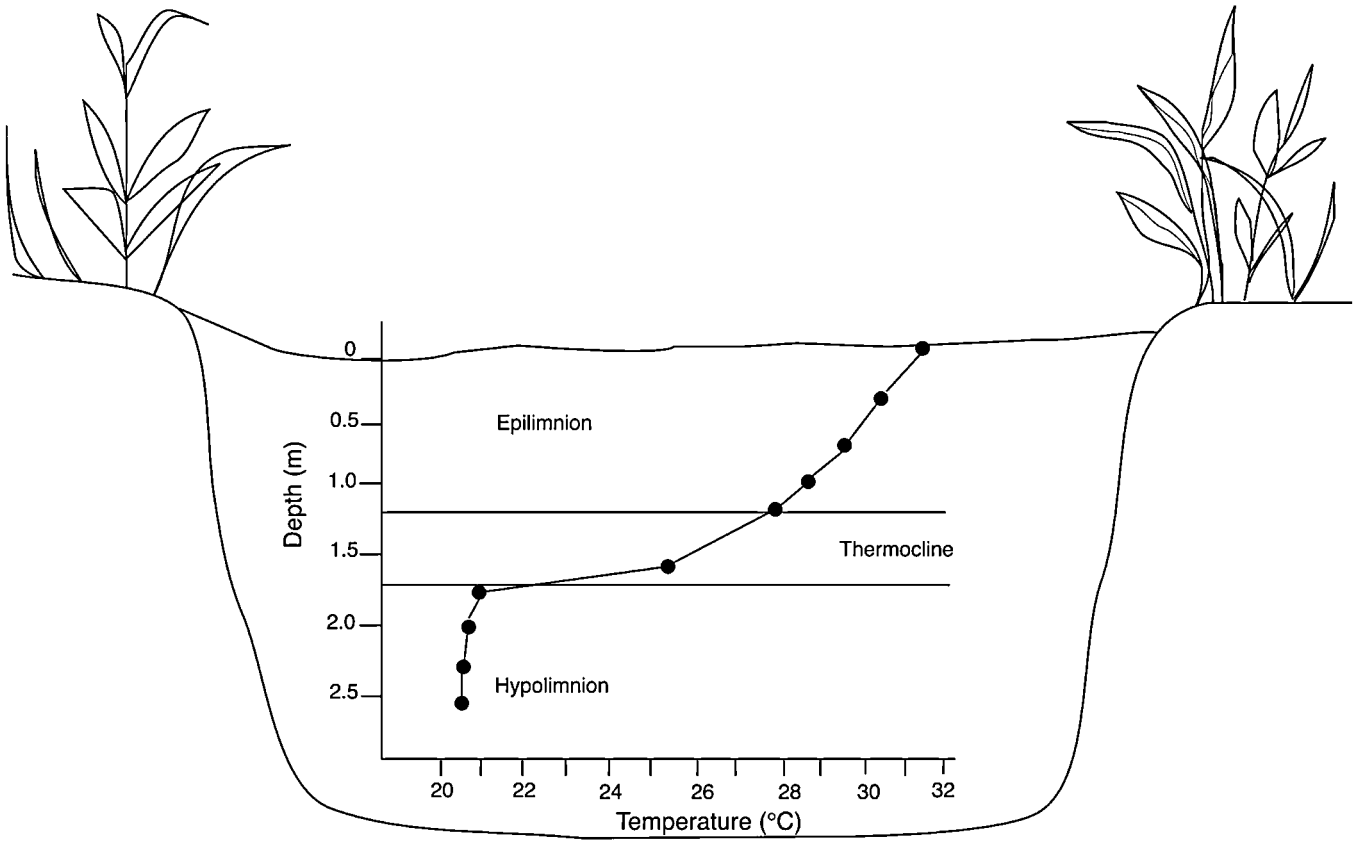


Fig. II-3. Typical temperature zones in a stratified pond.

PROBLEM 4
Ammonia Poisoning

Prevalence Index

WF - 1, WM - 1, CF - 1, CM - 1

Method of Diagnosis

Chemical measurement of high unionized ammonia
Lethal poisoning: >~1.00 mg UIA/l
Sublethal poisoning: >~0.05 mg UIA/l

History

Overcrowding; recent medication or other chemicals added; newly established aquarium; aquarium gravel recently washed or other filters recently cleaned; failure of biological filters; recent algal crash in pond; reduced water flow in raceway; hyperexcitability, possibly other neurological signs if acute (UIA >0.20 mg/l); acute to chronic stress response

Physical Examination

See "**History**"

Treatment

AQUARIA

1. 25–50% water change (daily to weekly, depending on ammonia concentration)
2. Add zeolite
3. Add buffer to reduce pH (freshwater only)
4. Add nitrifying bacteria
5. Add biological filtration
6. Decrease density
7. Temporarily reduce or stop feeding

Monitor closely for possible nitrite increase

PONDS, FLOW-THROUGH SYSTEMS

1. Stop or reduce feeding
2. Decrease density
3. Add water or increase water flow

COMMENTS

Ammonia poisoning is one of the most common water quality problems diagnosed in aquaculture (Meade 1985). Ammonia is the primary nitrogenous waste product of fish and also originates from the decay of complex nitrogenous compounds (e.g., protein). Ammonia can cause acute mortality, but most often it presents as a sublethal stress.

Aquaria

In an aquarium, ammonia accumulation is due to an inadequate number of bacteria that oxidize ammonia into nitrite (Fig. II-4, A). *Nitrosomonas europaea* is the predominant ammonia oxidizer in both freshwater and

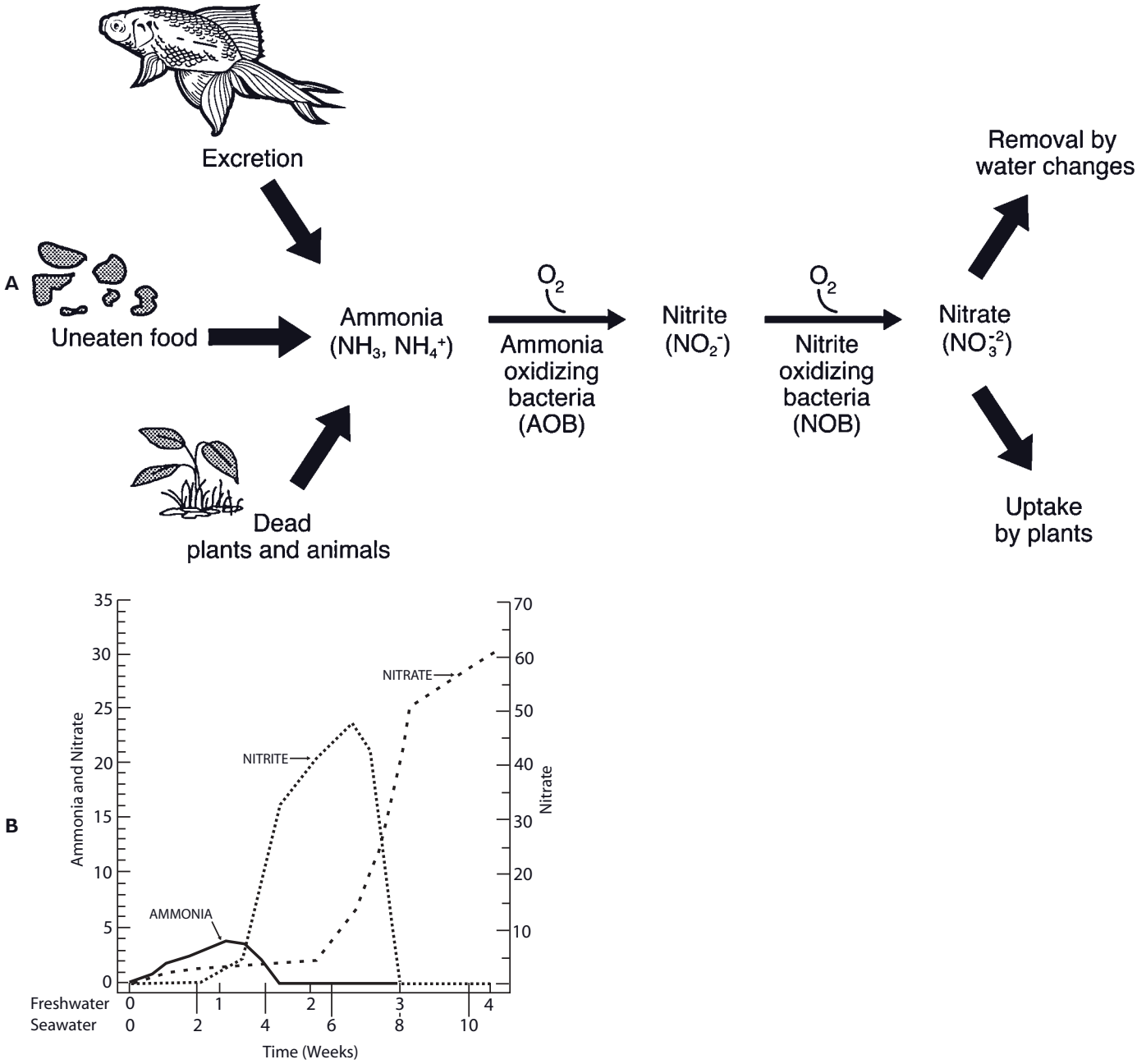


Fig. II-4. A. Major sources of nitrogen input and removal in a culture system. B. Typical ammonia and nitrite concentrations present during new tank syndrome if fish are added on day 0. Time required to establish an active biological filter and reduce ammonia and nitrite to nontoxic levels are ~3 weeks in freshwater aquaria (Carmignani and Bennett 1977) and ~8 weeks in marine aquaria at ~20–22°C (~68–72°F; Bower and Turner 1981). Time required to establish the filter increases considerably at lower temperatures and may vary considerably depending upon other environmental conditions.

marine environments, but other *Nitrosomonas* species dominate under certain conditions. *Nitrosococcus* and *Nitrosospira* species are also well-known ammonia oxidizers (Hovanec and DeLong 1996; Hovanec 1998; Burrell et al. 2001).

In a new aquarium setup, these ammonia-oxidizing bacteria (AOB) are scarce. So, when fish are added to the new tank, the ammonia rapidly rises (Fig. II-4, B), killing the fish; this is often referred to as new tank syndrome.

Table II-4, A. Fraction of the total ammonia nitrogen that is present as unionized ammonia at various temperature-pH combinations (modified from Emerson et al. 1975). Reprinted with permission. Values for °F are rounded off to the closest integer.

Temp		pH								
(°C)	(°F)	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	32	.0001	.0003	.0008	.0026	.0082	.0255	.0764	.207	.453
1	34	.0001	.0003	.0009	.0028	.0089	.0277	.0825	.221	.473
2	36	.0001	.0003	.0010	.0031	.0097	.0300	.0890	.236	.494
3	37	.0001	.0003	.0011	.0034	.0105	.0325	.0960	.251	.515
4	39	.0001	.0004	.0012	.0036	.0114	.0352	.103	.267	.535
5	41	.0001	.0004	.0013	.0040	.0123	.0380	.111	.283	.556
6	43	.0001	.0004	.0014	.0043	.0134	.0411	.119	.300	.576
7	45	.0001	.0005	.0015	.0046	.0145	.0444	.128	.317	.595
8	46	.0002	.0005	.0016	.0050	.0157	.0479	.137	.335	.614
9	48	.0002	.0005	.0017	.0054	.0169	.0516	.147	.353	.633
10	50	.0002	.0006	.0019	.0059	.0183	.0556	.157	.371	.651
11	52	.0002	.0006	.0020	.0063	.0197	.0599	.168	.389	.668
12	54	.0002	.0007	.0022	.0068	.0213	.0644	.179	.408	.685
13	55	.0002	.0007	.0024	.0074	.0230	.0692	.190	.426	.702
14	57	.0003	.0008	.0025	.0080	.0248	.0743	.202	.445	.717
15	59	.0003	.0009	.0027	.0086	.0267	.0797	.215	.464	.733
16	61	.0003	.0009	.0029	.0093	.0287	.0854	.228	.483	.747
17	63	.0003	.0010	.0032	.0100	.0308	.0914	.241	.502	.761
18	64	.0003	.0011	.0034	.0107	.0331	.0978	.255	.520	.774
19	66	.0004	.0012	.0037	.0115	.0356	.105	.270	.539	.787
20	68	.0004	.0013	.0040	.0124	.0382	.112	.284	.557	.799
21	70	.0004	.0014	.0043	.0133	.0410	.119	.299	.575	.810
22	72	.0005	.0015	.0046	.0143	.0439	.127	.315	.592	.821
23	73	.0005	.0016	.0049	.0154	.0470	.135	.330	.609	.832
24	75	.0005	.0017	.0053	.0165	.0503	.144	.346	.626	.841
25	77	.0006	.0018	.0057	.0177	.0538	.153	.363	.643	.851
26	79	.0006	.0019	.0061	.0189	.0575	.162	.379	.659	.859
27	81	.0007	.0021	.0065	.0203	.0615	.172	.396	.674	.868
28	82	.0007	.0022	.0070	.0217	.0656	.182	.412	.689	.875
29	84	.0008	.0024	.0075	.0232	.0700	.192	.429	.704	.883
30	86	.0008	.0025	.0080	.0248	.0746	.203	.446	.718	.890

Table II-4, B. Effect of various drugs on ammonia detoxification when used as prolonged immersions at recommended therapeutic levels.

Agent	Effect	Reference
Chloramphenicol	FW: None	Collins et al. (1976b)
	SW: Slight increase, accompanied by clouding of the water	Bower and Turner (1982b)
Copper sulfate	FW: None	Collins et al. (1975)
	SW: Slight to moderate increase	Bower and Turner (1982b); Kabasawa and Yamada (1972)
Erythromycin	FW: Substantial increase	Collins et al. (1976b)
Formalin	FW: None	Collins et al. (1975)
		Heinen et al. (1995)
Gentamicin sulfate	SW: None	Bower and Turner (1982b)
Malachite green	FW: None	Collins et al. (1975)
Methylene blue	FW: Substantial increase	Collins et al. (1975)
	SW: Slight to moderate increase	Bower and Turner (1982b)
Metronidazole	FW: None	Halling-Sørensen (2001)
Neomycin sulfate	SW: Slight to moderate increase	Bower and Turner (1982b)
Nifurpirinol	FW: None;	Collins et al. (1976b)
	SW: None	Bower and Turner (1982b)
Oxolinic acid	FW: None	Skjølstrup et al. (2000)
Oxytetracycline	FW: None	Collins et al. (1976b)
Potassium permanganate	FW: None	Collins et al. (1975)
Quinacrine hydrochloride	SW: None	Bower and Turner (1982b)
Sulfadiazine	FW: None	Halling-Sørensen (2001)
Sulfamerazine	SW: None	Collins et al. (1976b)
Chloroquine diphosphate	SW: None	C.E. Bower (unpublished data)

FW = freshwater; SW = seawater.

Ammonia poisoning can also occur in long-established aquaria. If fish are added to a tank that has many fish already present or if fish are overfed, causing an accumulation of decaying food in the tank, ammonia can rise. The total amount of ammonia that can be converted to nitrite depends entirely on the amount of biological filtration in the tank. Biological filtration (more appropriately termed microbiological filtration, since it refers to the filtration of water over microbes) occurs when the aquarium water passes over a surface coated with AOB. Thus, biological filtration (and ammonia removal) is greatest where there is a high water flow over a large surface area. This occurs in the aquarium's filters (undergravel, box, power filters). If the biological filtration capacity is too low to remove all the ammonia produced by the fish, ammonia will rise. If filters are cleaned too vigorously (e.g., gravel stirred excessively), it will cause an ammonia spike, since the bacteria are easily dislodged from the substrate and are susceptible to changes in environmental conditions.

Ponds

Ammonia is usually not a problem unless supplemental aeration is used, preventing environmental hypoxia and thus allowing higher fish densities (Boyd 1990). As in other systems, feeding (uneaten, decaying food and ammonia generated from food consumption or dead fish) is the largest source of ammonia in a commercial pond. Ammonia toxicity is most likely to occur near sunset, when pH, temperature, and thus unionized ammonia are at their peak (Fig. II-1, D; see "**Diagnosis of Ammonia Poisoning**" below). In most ponds, algae, as well as *Nitrosomonas* bacteria, are major consumers of ammonia. Most ponds, especially commercial aquaculture ponds, have large algae populations. Ammonia also tends to increase during fall and winter, possibly because of a decrease in algal and bacterial metabolism at low temperatures.

Ammonia may also rise after an algae crash or massive die-off; this not only reduces ammonia assimilation but also adds to ammonia buildup caused by the decaying algae. Algae die-offs can occur spontaneously or may be caused by algicidal chemicals (see PROBLEM 1).

Flow-Through Systems

Oxygen is usually the most limiting factor in flow-through systems. However, ammonia levels can become toxic if supplemental aeration increases the maximum fish densities that can be held. Ammonia is lowest at the inflow and highest at the outflow.

Clinical Signs of Ammonia Poisoning

Acute ammonia toxicity can cause behavioral abnormalities, such as those that occur in mammals, including hyperexcitability (Daoust and Ferguson 1985). Fish often stop feeding. Chronic ammonia poisoning has been associated with hyperplasia and hypertrophy of gill tissue, although it is unclear as to whether this nonspecific pathology is due directly to ammonia poisoning or rather to other aspects of poor water quality that frequently accompany chronically high ammonia (Daoust

and Ferguson 1985). The precise mechanism of ammonia poisoning in fish is unknown, but high aqueous ammonia increases blood and tissue ammonia levels, causing elevated blood pH, osmoregulatory disturbance, increased tissue oxygen consumption, and decreased blood oxygen transport (Schwedler et al. 1985). Chronic ammonia poisoning slows growth (Colt and Armstrong 1979) and lowers disease resistance (Walters and Plumb 1980).

Diagnosis of Ammonia Poisoning

Ammonia levels are easily determined using commercially available kits. These kits measure the nitrogen present as ammonia, also known as total ammonia nitrogen (TAN). An ion-specific electrode can also be used to measure ammonia (e.g., Hach Chemical).

Ammonia is present in two forms: unionized (NH_3) and ionized (NH_4+). Unionized ammonia (UIA) is toxic to fish, while ammonium (NH_4+) is much less toxic (Russo 1985; Meade 1985). The amount of UIA in water depends mainly upon the pH, and also on temperature and salinity. High pH and temperature and low salinity favor the presence of UIA (Emerson et al. 1975; Meade 1985).

The concentration of toxic UIA is determined from a standard chart. For example, if the TAN of a freshwater sample that was measured with the water-quality test kit was 1.0 mg/l, the pH of the water was 8.5, and the water temperature was 25°C, 15.3% of the total ammonia would be present as UIA (NH_3) (Table II-4, A). Thus, the amount of UIA in the water would be 1.0 mg TAN/l × 0.153 = 0.153 mg of UIA/l. In low salinities, the fraction of total ammonia nitrogen that is present as UIA is virtually the same as for freshwater. In full-strength seawater (32–40 ppt), there is as much as 20% less UIA at the same temperature and pH as in freshwater. This variation is usually not important in making a clinical diagnosis of ammonia poisoning. Bower and Bidwell (1978) provide tables for highly accurate determination of UIA in seawater.

Ammonia toxicity varies with environmental conditions (e.g., pH, temperature, salinity, water hardness) and other stressors present. Exposure to sublethal ammonia levels also increases tolerance to ammonia toxicity (Thurston et al. 1981). Sublethal levels that influence growth are especially difficult to determine, so keeping ammonia levels as low as possible is advisable. Unionized ammonia levels greater than ~1.00–2.00 mg/l are usually lethal within 1–4 days (Meade 1985). Below this level, fish might not die, but they will be stressed. If UIA is greater than 0.05 mg/l, it should be reduced as quickly as possible.

When measuring ammonia via the Nessler method, an intense yellow color and erroneously high value (false positive) occurs in the presence of formaldehyde (formalin). The salicylate method fails to measure ammonia (false negative) in the presence of formalin. If ammonia must be measured during formalin treatment, one should use an ion probe (Heinen et al. 1995).

Treatment of Ammonia Poisoning: Aquaria

Ammonia levels can be reduced with frequent water changes; but, in a long-established tank, the clinician must be careful not to cause environmental shock (see PROBLEM 97). Adding zeolite is a safe and effective way of reducing ammonia quickly. However, zeolite's efficacy decreases with increased salinity (see "**Pharmacopoeia**"). Reducing the pH will reduce the percentage of ammonia that is present as NH_3. For every 1 unit decrease in pH, there is a ten-fold decrease in UIA (Table II-4, A); this should be done with caution because a rapid drop in pH can cause other problems (see PROBLEM 7). Ammonia can also be chemically controlled via the addition of commercial ammonia-neutralizing products (e.g., Ammonia Detox [Kent Marine]) that contain sodium hydroxymethanesulfonate (Riche et al. 2006). Note also that reducing ammonia levels during the early stages of establishing a biological filter might prolong the time required for the AOB to reach peak efficiency. However, high ammonia also inhibits the bacteria responsible for nitrite oxidation (see PROBLEM 5).

Any immediate ammonia control measures (e.g., water changes, zeolite addition, pH treatment, chemical neutralization) are useful but must be part of a plan to increase biological filtration capacity of the aquarium. In a new tank, adding a commercial preparation of nitrifying bacteria might speed up the process of establishing an effective filter but usually will not instantly result in a well-established biofilter. Also, the diversity of AOB and their varying presence under different environmental conditions (Rowan et al. 2003) calls into question the utility of commercial nitrifying bacterial preparations (Kolcott 2004). Biofilter establishment is often quicker when filter material (gravel, filter floss) from a healthy established tank is used; however, there is the risk of introducing pathogens with such material.

In an established tank, ammonia poisoning arises when more fish are in the tank than the biological filtration can sustain. In this case, either some fish must be removed or biological filtration improved.

Many medications can be toxic to the nitrifying bacteria (Table II-4, B). Use of such medications can cause "new tank syndrome" in an established tank. If chemical damage to the biological filter occurs, a filter with activated carbon should be added to the tank to remove all traces of the drug. The tank should then be treated as a new tank and appropriate measures taken as described above.

Treatment of Ammonia Poisoning: Ponds, Cages, Flow-Through Systems

In ponds, prevention of ammonia toxicity is preferable to therapy, since ammonia cannot be rapidly removed

from most ponds. Over the short term, adding freshwater will dilute the ammonia. Ponds over 0.5 ha (1 ac) cannot be rapidly flushed, but adding freshwater will create a haven where fish can avoid the toxin.

It is advisable to feed no more than 110 kg of feed/ha/day (100 lb of feed/ac/day) to avoid ammonia accumulation in channel catfish ponds (Noga and Francis-Floyd 1991). However, many producers resist this recommendation, because fish grow more slowly, lengthening the production cycle. The TAN concentration in ponds usually increases slowly; therefore, biweekly monitoring is usually adequate for commercial producers. If TAN exceeds 0.50 mg/l, it should be monitored daily until it returns to 0. Using a high-quality feed that is high in digestible protein will also reduce ammonia production.

In flow-through systems, reducing stocking densities or feeding rates or increasing water flows are the most common treatments for ammonia buildup. Treating water with zeolite is another option. Since the majority of ammonia in a flow-through system comes from fish metabolism (relatively little from the water source or from uneaten feed), an ammonia factor (AF) can be calculated as follows (Piper et al. 1982):

$$AF = \frac{TAN\ (mg/l) \times flow\ (gallons/minute)}{Pounds\ of\ food\ fed\ per\ day}$$

The AF is determined by measuring the TAN in a system several times during 1 day. When the AF is determined, the total amount of ammonia present at the outlet of that particular flow-through system under various feeding rates and water flows can be predicted from:

$$TAN = \frac{Pounds\ of\ food\ fed\ per\ day \times AF}{Flow\ (gallons/minute)}$$

PROBLEM 5
Nitrite Poisoning (Brown Blood Disease, New Tank Syndrome)

Prevalence Index

WF - 1, WM - 4, CF - 1, CM - 4

Method of Diagnosis

1. Chemical measurement of high nitrite in water
2. Measurement of high metHb in blood

History

Overcrowding; recent medication or other chemicals added; newly established aquarium; aquarium gravel recently washed or other filters recently cleaned; failure of biological filters; fall season in pond; low $Cl:NO_2^-$ ratio; acute to chronic stress response

Physical Examination

Dyspnea; light tan to brown gills; tan to brown blood; acute to chronic stress response

Treatment

AQUARIA

1. Twenty-five to 50% water change (daily to weekly, depending on nitrite concentration)
2. Add nitrifying bacteria
3. Add chloride
4. Enhance biological filtration
5. Decrease density
6. Reduce temperature
7. Reduce feeding

PONDS

1. Add chloride
2. Maintain highest DO possible

COMMENTS

Epidemiology of Nitrite Poisoning

Most circumstances causing ammonia poisoning can also lead to nitrite poisoning (see PROBLEM 4, "Aquaria"). In a newly established aquarium, nitrite buildup usually occurs after ammonia has peaked (see Fig. II-4, B). This is because the nitrite oxidizing bacteria (nitrite nitrifiers or nitrite oxidizing bacteria [NOB]) that convert nitrite (NO_2^-) to nitrate (NO_3^{-2}) require time to become active, just like the bacteria that convert ammonia to nitrite. There are a number of NOB in both freshwater and marine environments, including *Nitrobacter* and *Nitrospira* species. Sometimes, *Nitrospira* is present but not *Nitrobacter* (Hovanec and DeLong 1996).

Nitrite nitrifiers are also inhibited by ammonia. A high ammonium concentration in alkaline water (e.g., seawater) is toxic to NOB, which results in a solely AOB population in a new aquarium (Hovanec et al. 1998). In an aquarium, even adding a single fish might cause a temporary imbalance and subsequent spike in ammonia, which subsequently inhibits the nitrite-oxidizers, causing a nitrite spike. Both AOB and NOB also appear to be inhibited by strong light (Moe 1992a). Some chemicals selectively inhibit NOB (Table II-5), causing a nitrite spike.

In ponds, nitrite poisoning is common in fall because the temperature optima of AOB and NOB are different, resulting in nitrite accumulation. Nitrite can rise quickly (<24 hours) in catfish ponds (Johnson 1993b). Nitrite is not a problem in flow-through systems because there is no significant conversion of ammonia to nitrite during the short time that water is present in the system.

Clinical Signs of Nitrite Poisoning

Nitrite is actively transported across the gill, where it enters the bloodstream (Lewis and Morrios 1986) and oxidizes hemoglobin (Hb) to methemoglobin (MetHb). Methemoglobin cannot transport oxygen efficiently, so tissues are deprived of oxygen. While oxygenated Hb is

Table II-5. Effect of various drugs on nitrite detoxification when used as prolonged immersions at recommended therapeutic levels.

Agent	Effect on nitrite concentration	Reference
Chloramphenicol	FW: None	Collins et al. (1976b)
	SW: None	Bower and Turner (1982b)
Chloroquine diphosphate	SW: None	C.E. Bower (unpublished data)
Copper sulfate	FW: None	Collins et al. (1975)
	SW: Slight to moderate increase	Bower and Turner (1982b); Kabasawa and Yamada (1972)
Erythromycin	FW: Substantial increase	Collins et al. (1976b)
Formalin	FW: None	Collins et al. (1975)
Gentamicin sulfate	SW: None	Bower and Turner (1982b)
Malachite green	FW: None	Collins et al. (1976b)
Methylene blue	FW: None	Collins et al. (1976b)
	SW: None	Bower and Turner (1982b)
Metronidazole	FW: None	Halling-Sørensen (2001)
Neomycin sulfate	SW: Substantial increase	Bower and Turner (1982b)
Nifurpirinol	FW: None	Collins et al. (1976b)
	SW: None	Bower and Turner (1982b)
Oxolinic acid	FW: None	Skjølstrup et al. (2000)
Oxytetracycline	FW: None	Collins et al. (1976b)
Potassium permanganate	FW: None	Collins et al. (1975)
Quinacrine hydrochloride	SW: None	Bower and Turner (1982b)
Sulfadiazine	FW: None	Halling-Sørensen (2001)
Sulfamerazine	SW: None	Collins et al. (1976b)

FW = freshwater; SW = seawater.

red, MetHb is brown. So fish with nitrite poisoning often have pale tan or brown gills. Methemoglobin concentrations of 25–30% usually give the blood a slightly brown color, but MetHb concentrations must usually be around 40% to cause grossly visible chocolate brown blood and pale tan to brown gills. Fish with anemia may also have pale gills but with a red tinge. Fish with severe (i.e., 80% or greater) methemoglobinemia are dyspneic even with adequate oxygen.

Behavioral changes noted with nitrite poisoning are characteristic of hypoxia, including lethargy and congregating near the water surface. Fish with nitrite poisoning should be disturbed as little as possible, since even minor exertion may cause acute mortality.

Diagnosis of Nitrite Poisoning

Definitive diagnosis of nitrite poisoning requires measuring the MetHb concentration in the blood (resting MetHb levels vary considerably, but >25% is considered abnormal), combined with measuring the nitrite concentration in water. However, routine clinical diagnosis of nitrite toxicosis relies solely on measuring nitrite levels. This has its limitations, because fish vary greatly in susceptibility to nitrite poisoning. At least gross evidence of methemoglobinemia (Fig. II-5) should be sought to strengthen the diagnosis.

Colorimetic kits can be used for nitrite measurement. Analyses measure nitrite-nitrogen, which can be converted to total nitrite, using a conversion factor of 3.3. For example, if the nitrite-nitrogen (NO_2-N) measurement of the kit is 0.10 mg/l, the amount of nitrite present is 0.33 mg/l.

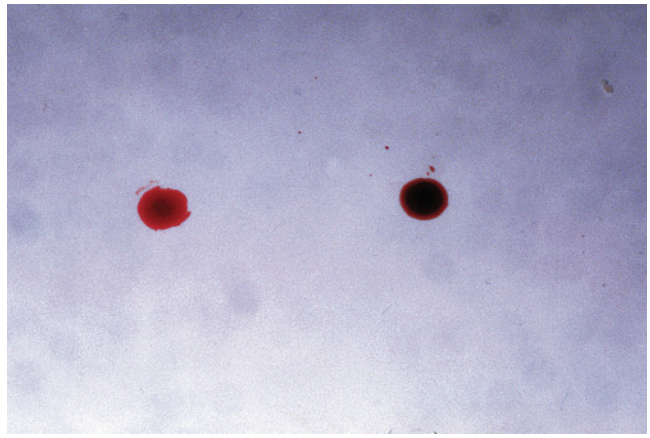


Fig. II-5. Normal drop of blood (left) and blood with high concentration of methemoglobin or brown blood (right).

Nitrite poisoning has been most extensively studied in channel catfish, where firm recommendations can be made regarding toxic levels. Data also exist for other species, especially salmonids, but for most species there are no data on toxicity. Susceptibility to nitrite poisoning varies tremendously among species and some fish are resistant (Tomasso 1986). For channel catfish in pure freshwater, nitrite should be undetectable by commercial

test kits (<0.10 mg/l of nitrite nitrogen). In contrast, sunfish tolerate high levels (96-hour LC_{50} often >50 mg/l) because they do not actively take up nitrite from water. The recommended level for salmonids is <0.50 mg/l. The 96-hour LC_{50} values for freshwater fish range from 0.60 to 200 mg/l. While marine fish are susceptible to nitrite poisoning, extremely high levels are required. For example, the 24-hour LC_{50} for spotted sea trout at 14 ppt salinity is 980 mg/l NO_2-N (Daniels and Boyd 1987). For European sea bass at 36 ppt salinity, the 96-hour LC_{50} is 90–100 mg/l NO_2-N, and induction of methemoglobinemia requires exposure to over 25 mg/l NO_2-N for 96 hours (Scarano et al. 1984). Studies in other fish have failed to demonstrate acute toxicity at as high as 1,750 mg/l (Brownell 1981). Such high nitrite levels would never be encountered in aquaculture systems. However, the 48-hour median lethal concentration of nitrite for red drum was only 87.5 mg/l at 36 ppt salinity and only 2.8 mg/l at 0.6 ppt (Wise and Tomasso 1989). Thus, chloride was not as effective in preventing nitrite toxicity as it is in other fish species, indicating that nitrite might be a problem in some fish even if cultured in high-salinity waters.

The susceptibility of tropical aquarium fish to nitrite is unknown; however, it is best to keep levels low (<0.10 mg/l) to avoid any possible toxicity. Long-term (over 6 months) exposure to even very low nitrite levels (0.015–0.060 mg/l NO_2-N) can result in mild methemoglobinemia in some fish (Wedemeyer and Yasutake 1978).

If the water is naturally high in chloride (e.g., coastal aquifers) or chloride has been added, diagnosis of nitrite poisoning also requires measurement of Cl^-. Colorimetric tests and electronic probes are available. Nitrite toxicity is affected by many other factors, including pH, fish size, previous exposure, nutritional status, and dissolved oxygen level. Thus, it is best to keep levels as low as possible, especially for species with unknown susceptibility.

Note also that grossly brown gills or blood is not always evident when nitrite poisoning occurs. Nitrite-poisoned fish can die with pink gills and blood (Scarano et al. 1984). This might be due to the fact that the fish may die from nitrite-induced hemolytic anemia rather than nitrite toxicity and because nitrite can damage not only hemoglobin but other vital, porphyrin-containing proteins, such as cytochromes.

Exposure of fish to very high nitrite concentrations is also associated with the accumulation in the spleen of foci of iron-containing (Prussian blue positive staining) macrophages caused by increased erythrocyte destruction (Scarano et al. 1984).

Treatment of Nitrite Poisoning

Nitrite is much less toxic when chloride is present, possibly since Cl^- competitively inhibits nitrite uptake across the gills (Bowser et al. 1983). In channel catfish, chloride ion prevents mortality caused by methemoglobin-associated nitrite toxicity when present in a ratio (wt:wt) of at least 3 mg chloride to 1 mg nitrite (Bowser et al. 1983). Thus, a water sample with 1.2 mg/l chloride and 0.30 mg/l nitrite (= 4 mg Cl:1 mg NO_2 ratio) would not be acutely lethal to channel catfish. However, this ratio does not prevent chronic erythrocyte damage that can lead to anemia. This adverse effect is not seen when the chloride:nitrite ratio is 6:1 (Tucker et al. 1989). Molar ratios of 6 (rainbow trout) to 16 (channel catfish) completely inhibit nitrite toxicity (Wise and Tomasso 1989). Similar guidelines may be satisfactory for treating nitrite toxicosis in other fish, although, as mentioned previously, most fish species have not been examined.

Sodium chloride is the least expensive and most readily available form of chloride, but calcium chloride is equally effective (Tomasso et al. 1979). The low level of salt needed to treat nitrite toxicosis (usually <50 mg/l) is nontoxic to freshwater fish.

Once treatment is instituted, reduced hemoglobin levels usually return to normal within 12–24 hours, and fish will begin eating. However, secondary infections may be a sequela of sublethal nitrite exposure (Hanson and Grizzle 1985) and anemia caused by low hemoglobin can take days to return to normal (Scarano and Saroglia 1984).

Prevention of Nitrite Poisoning

Prevention is preferable to treatment. In channel catfish ponds, at least 20 mg/l chloride should always be present to prevent nitrite toxicity. Many natural waters have this chloride level, often obviating the need for prophylactic chloride addition. Even dilute brackish water probably has enough chloride to prevent nitrite toxicosis in most euryhaline species. For example, 1 ppt seawater contains over 500 mg/l chloride. Clinically encountered nitrite levels have never been shown to be toxic to fish in seawater, probably because of seawater's high chloride content. However, there are few studies on nitrite's effect on tropical marine reef fish, so it is advisable to keep nitrite levels in marine aquaria low.

Bicarbonate is also somewhat protective against nitrite but considerably less than chloride. High dietary ascorbate levels also protect against nitrite-induced MetHb formation (Wise et al. 1988).

PROBLEM 6
Nitrate Poisoning ("Old Tank" Syndrome)

Prevalence Index

WF - 1, WM - 4, CF - 1, CM - 1

Method of Diagnosis

1. Chemical measurement of high nitrate in water
2. Measurement of high metHb in blood

History
Overcrowding; inadequate water changes
Physical Examination
Dyspnea; light tan to brown gills; tan to brown blood; acute to chronic stress response
Treatment
1. Appropriate percentage water change (daily to weekly, depending on nitrate concentration)
2. Denitrification apparatus
3. Decrease density

COMMENTS

Causes of Nitrate Accumulation
The end product of nitrite oxidation is nitrate. In a newly established aquarium, nitrate buildup occurs after nitrite has peaked (see Fig. II-4, B). If not actively removed (via water changes or denitrification), nitrate will continue to increase over time. How quickly it rises depends mainly upon the amount of ammonia entering the system, which in turn is primarily dependent upon the fish biomass and feeding rate. Thus, while ammonia and nitrite poisoning often occur with a new tank, nitrate increase (and risk of intoxication) typically occurs after an aquaculture system is established ("old tank").

In natural surface waters, nitrate concentrations are increasing around the world. Nitrate, like ammonia and nitrite, can enter aquatic ecosystems via animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents (wastewater treatment plants that are not performing tertiary treatment). Atmospheric deposition of inorganic nitrogen can originate from use of nitrogen fertilizers and combustion of fossil fuels.

Clinical Signs of Nitrate Poisoning
As with nitrite, the major toxic mechanism of nitrate in aquatic animals is the conversion of oxygen-carrying pigments (e.g., hemoglobin) to forms that are incapable of carrying oxygen (metHb). But, due to low gill permeability to nitrate, its uptake in fish is more limited than that of ammonia or nitrite, resulting in its relatively low toxicity (Stormer et al. 1996). There is some suggestion that nitrate toxicity might be less in larger fish, at higher salinity (freshwater fish appear more sensitive than marine fish), and with environmental adaptation (Camargo et al. 2005). However, others have observed greater susceptibility in larger individuals (Hamlin 2006).

Almost no studies have examined the clinical effects of nitrate on fish (except mortality rate), but effects appear to reflect damage to hemoglobin. Exposure of rainbow trout fry to 5–6 mg NO_3-N/l for several days caused increased ferrihemoglobin, alteration in peripheral blood and hematopoetic centers, and liver damage (Grabda et al. 1974).

Diagnosis of Nitrate Poisoning
Routine clinical diagnosis of nitrate toxicosis relies solely on measuring nitrate levels. This has its limitations, since fish vary greatly in susceptibility to nitrate poisoning and effects are probably much more subtle.

Colorimetic kits can be used for nitrate measurement. Analyses measure nitrate-nitrogen, which can be converted to total nitrate by using a conversion factor of 4.4. For example, if the nitrate-nitrogen (NO_3-N) measurement of the kit is 5.0 mg/l, the amount of nitrate present is 22 mg/l.

The U.S. federal maximum limit of nitrate for drinking water is 10 mg NO_3-N/l. The toxic level for the majority of fish tested appears well above this level, even with prolonged chronic exposure (Camargo et al. 2005). While nitrate is certainly much less toxic than either ammonia or nitrite, some fish are highly sensitive to relatively low nitrate levels. Nitrate poisoning has been most extensively studied in salmonids, where a wide range of toxic levels have been observed in various species and life stages. The eggs and fry of rainbow trout and cutthroat trout are adversely affected, and in some cases can die, after exposure for 30 days to as little as 1.1–7.6 mg NO_3-N/l (Kincheloe et al. 1979). These levels are well below what is typically considered to be toxic to fish, as a level of 50 mg/l nitrate is generally considered safe. Most fish species tolerate very high levels; toxic levels for bluegills, guppies, and channel catfish range from 200 to 2,000 mg NO_3-N/l. However, almost all studies have only examined acute toxicity (24–96-hour exposures), while nitrate is more of a chronic problem.

As with nitrite, susceptibility to nitrate poisoning varies tremendously among species, and some fish are resistant. The susceptibility of the few tropical marine fish tested is also quite low in acute exposures (>1,000 mg NO_3^{-2} N/l). However, it is best to keep levels as low as possible, especially for species with unknown susceptibility. Reef corals (and possibly marine reef fish) are very sensitive; levels should be <20 mg NO_3^{-2}/l in marine reef aquaria (Frakes and Hoff 1982; Moe 1992a).

Nitrate level is also a surrogate indicator of the overall water quality in a culture system. The buildup of nitrate occurs concurrently with accumulation of other deleterious compounds (e.g., increased organic loading) that are less easy to measure, thus keeping nitrite levels low via water changes also reduces the levels of these compounds.

Treatment and Prevention of Nitrate Poisoning
The most common means of reducing/controlling nitrate levels is to perform water changes at intervals and amounts that keep the nitrate concentration within acceptable limits. While this is not feasible in ponds, nitrate does not appear to reach toxic levels under typical pond aquaculture situations. For example, exposure of channel catfish to 90 mg NO_3^--nitrogen/l for nearly 6 months does not affect their health or growth (Camargo et al. 2005).

Clinically encountered nitrate levels have never been shown to be directly toxic to marine fish. However, given

the high sensitivity of some freshwater species, it is advisable to keep nitrate levels in tropical marine aquaria low since the natural reef environment has very low nitrate levels. Also, there is evidence that elevated nitrate can prevent the uptake of iodine, predisposing marine fish to goiter (Crow et al. 1998).

PROBLEM 7
Too Low (Too Acidic) pH

Prevalence Index
WF - 1, WM - 3, CF - 2, CM - 4
Method of Diagnosis
Chemical measurement of low pH
History
Acutely low pH: Acute mortality with tremors and hyperactivity; dyspnea; acute stress response
Chronically low pH: Increased mucus production; chronic stress response
Physical Examination
See "**History**"
Treatment
AQUARIA
1. Change water
2. Add buffer
3. Adjust pH only if ammonia levels are safe

PONDS
1. Add buffer
2. Reduce density

FLOW-THROUGH SYSTEMS
1. Pretreat incoming water with buffer
2. Add base

COMMENTS
Fish species differ in their optimal pH range. A pH range of 6.5–9.0 is generally recommended for freshwater fish (Swingle 1969). Values outside this range are stressful (Swingle 1961). A pH of <4.0 or >11.0 is lethal (Swingle 1961; Tucker 1985). However, this is a wide range. This range is generally considered satisfactory for salmonids and channel catfish and most other freshwater food fish. While some freshwater aquarium fish (generally, the "hardy" species most commonly sold in pet shops) can do well within this entire range, most do considerably better if maintained within a narrower range.

Many freshwater aquarium fish come from poorly buffered waters that are high in tannins or other organic acids (e.g., Amazon River basin) and thus do best in neutral to slightly acidic (pH ~6.5–6.8) conditions (see PROBLEM 8). Notable exceptions include the following: African rift lake cichlids and brackish water fish (e.g., mollies, guppies, platies, swordtails) do best in hard (>100 mg/l), alkaline (pH 7.6–8.0) water. Marine aquarium fish require a stable, alkaline pH. The tolerable pH for marine aquaria is generally 7.8–8.4 (Moe 1992a). Optimal limits are much narrower and the pH is best kept between 8.1 and 8.3 (Bower 1983). Fromm (1980), Leivestad (1982), Schwedler et al. (1985), and Evans and Claiborne (2006) review the physiological effects and pathology of suboptimal pH.

Fish acclimated to a relatively low pH can survive a drop in pH better than the same species maintained at a higher pH. Fish routinely exposed to wide pH fluctuations (e.g., in ponds) are probably more tolerant of rapid pH change than fish kept under more stable conditions (e.g., typical aquarium).

Primary Sources of Low-pH Water
Most ground (well or spring) water has dissolved carbonates and carbon dioxide and a pH somewhere between 5 and 8 (Boyd 1990). Ground water in contact with silicate minerals is poorly buffered and typically has a low pH and a large amount of CO_2 compared to ground water taken from carbonate substrate (e.g., limestone) that is thus well buffered. In a pond, pH is highly influenced by the soil type. Acid sulfate soils may have a pH less than 4 because of the oxidation of sulfide to sulfuric acid (Boyd 1990), making them unsuitable for fish culture unless neutralized (see "**Treatment**" below). Waters impacted by acid rain or that drain acidic soils may have low pH (Callinan et al. 2005). This can be a problem in a raceway culture after a rain, when large amounts of acids are washed into a stream water supply. The latter is a problem in trout farms in the eastern United States. Inadequately cured silicone aquarium sealants release acetic acid.

Secondary Sources of Low-pH Water
The metabolic activity of fish and other aquatic organisms produces acids. In a closed system, such as an aquarium or pond, these acids tend to gradually reduce pH. If water changes are not regularly performed or if the pH is not otherwise adjusted, it can drop low (e.g., to pH 5 in a freshwater aquarium). A pH below 5.5 is very stressful; if too low, it is lethal. Acute exposure of fish to such a low pH (such as by adding a new fish to such a tank) can be fatal (see PROBLEM 97).

Buffering Capacity and pH
The bicarbonate-carbonate buffer system (Fig. II-7) is the major moderator of pH in aquatic ecosystems. Alkalinity is the buffering capacity in water, as measured by the amount of bicarbonate (HCO_3^-) and/or carbonate ($CO_3^=$) present (see PROBLEM 9). Alkalinity is usually expressed as mg/l of calcium carbonate equivalents. Thus, water with high alkalinity resists pH change from acids produced by the aquatic organism's respiration (i.e., CO_2) and other metabolites.

Low pH is most common in waters with low alkalinity (i.e., less than 50 mg/l as $CaCO_3$) because the lower the alkalinity, the less the buffering capacity of the water and its ability to buffer acid production. However, given

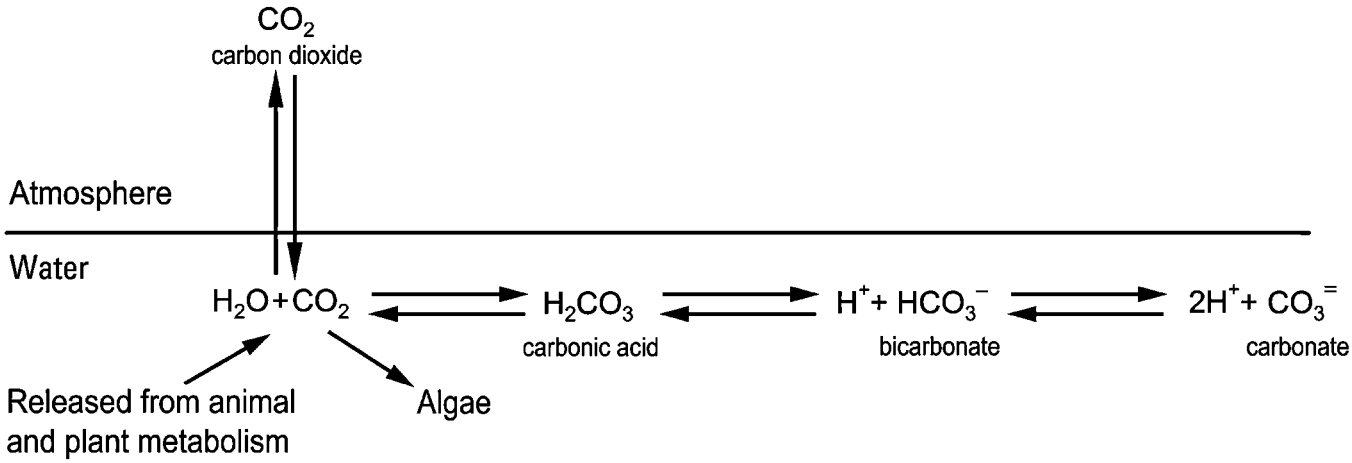


Fig. II-7. The carbonate-bicarbonate buffer equilibrium.

enough time, the pH can drop in even highly buffered waters, such as seawater.

Pond pH is influenced not only by the amount of bicarbonate present but also by photosynthesis.

Plant photosynthesis uses CO_2, raising the pH and causing it to peak near sunset (see Fig. II-1, D). At night, cessation of photosynthesis results in a net accumulation of CO_2, causing a drop in pH. It is not unusual for pH to vary diurnally from 6.5 to 9.0 within a commercial aquaculture pond (Boyd 1979). Diurnal pH variation can also occur in a heavily planted freshwater aquarium.

In low-alkalinity waters, the pH may fall considerably as water passes through a flow-through system, caused primarily by fish metabolism. Thus, the pH is highest at the inflow and lowest at the outflow.

Diagnosis

A good colorimetric test is adequate for routine clinical measurements of pH, but low-cost, portable pH meters (e.g., Aquatic Ecosystems; Fisher) are more convenient.

Diagnosis of acute or chronic acid stress must take into consideration the optimal pH of the species, the rate of pH change observed, and the magnitude of the change. Acclimation is also an important consideration: the pH of catfish ponds often fluctuates diurnally from 6.5 to 9.0 (Swingle 1969). However, if this large a pH change were to occur in a typically pH-stable marine aquarium, it would cause major stress.

The toxicity of low pH is also complicated by its influence on so many water quality variables, especially ammonia (see PROBLEM 4) and other toxins (see PROBLEMS 91, 93, and 95). Many toxins are highly affected by pH, especially metals, which become more toxic at low pH. Aluminum is one of the most common metals in soil. Aluminum ion is solubilized and is more toxic in acid pH and thus aluminum toxicity can occur concurrently (see PROBLEM 93) and may be the primary cause of death during acid runoff into streams. Low pH also increases the proportion of the bicarbonate buffer system that is present as free CO_2 (see PROBLEM 90). Thus, rapid acidification of high-alkalinity water can increase the free CO_2 concentration, causing hypercarbia rather than acidosis (EIFAC 1969).

Pure water saturated with carbon dioxide has a pH of 5.6. If the pH is <5.6, the water must have other acids that are stronger than carbonic acid (e.g., nonmetallic oxides, hydrides of halogens, organic acids). This may suggest the possible source of the low pH.

Acute acid poisoning, characterized by tremors and hyperactivity (Schwedler et al. 1985), is much less common than chronic acid stress. Gill tissue is the primary target of acid stress (Leivestad 1982). Low pH stimulates increased mucus production, which interferes with gas and ion exchange. Failure in acid-base balance (causing respiratory stress) and low sodium and chloride (causing osmotic stress) are the primary clinical signs. Chronic low pH stress is associated with poor growth, reproductive failure, and increased accumulation of heavy metals (Haines 1981). Fish recovering from acute acid stress are more susceptible to infections (Jones et al. 1987). Acute acid exposure can also cause skin loss (Iger and Wendelaar Bonga 1994; Davis et al. 2008) and lead to secondary microbial infection (Callinan et al. 2005).

Treatment

It is difficult to give exact recommendations for allowable pH change because it varies with species, environment, and prior acclimation conditions. When acclimating fish to a specific pH, a rule of thumb is that pH should not be changed more than about 0.2–0.5 pH units/day, unless the level is life-threatening. Fish are rarely stressed by this change and many tolerate even more rapid

changes. For example, rainbow trout tolerate immediate transfer from pH 7.2 to pH 8.5 (Witschi and Ziebel 1979).

Note that ammonia toxicity increases greatly with pH, so ammonia levels should be low enough to prevent possible toxic side effects before the pH is adjusted (see PROBLEM 4). High calcium increases the tolerance to low pH, presumably by reducing the ionic permeability of the gills (Haines 1981).

AQUARIA

Many commercial preparations are available for adjusting the pH of aquaria. These consist of carbonate, bicarbonate, and/or phosphate buffers. Carbonate-bicarbonate buffers are preferable; they are the major source of buffer in natural waters. Frequent routine water changes (10–25% every 2–4 weeks) will prevent the drop in pH and can be used to adjust improper pH. Note that carbonate filtrants (i.e., limestone) will buffer acids but will not maintain pH over 7.5, which is outside the range required for tropical marine fish (Bower et al. 1981). Total alkalinity in marine aquaria should be 200 mg/l (4 mEq/l) and should not exceed a range of 100–300 mg/l (2–6 mEq/l) (Moe 1992a).

PONDS

In ponds, increasing the alkalinity with buffer will also solve the pH problem. In warm water fish ponds, if pond alkalinity is less than ~50 mg/l as $CaCO_3$, buffer should be added. Some acid-sulfate soils need extremely large amounts of buffer to be neutralized. They are best managed by using buffer in combination with other management techniques (Boyd 1990). Boyd (1990) provides detailed techniques for adding buffer to ponds.

FLOW-THROUGH SYSTEMS

Trout farms susceptible to low-pH runoff may need to lime the water supply during low-pH episodes. Agricultural lime and slaked lime do not react quickly enough to raise the pH in flow-through systems. Thus, some farms add sodium hydroxide (NaOH) solutions, using a metering device to instantaneously neutralize acidity (Boyd 1990).

PROBLEM 8
Too High (Too Alkaline) pH

Prevalence Index

WF - 3, WM - 4, CF - 4, CM - 4

Method of Diagnosis

Chemical measurement of too high pH

History

Acutely high pH: Cloudiness of skin and gills; improper lime treatment of pond; acute stress response

Chronically high pH: Chronic stress response

Physical Examination

See "**History**"

Treatment

AQUARIA

1. Add buffer
2. Add deionized water
3. Add peat
4. Mechanically remove excess plants

PONDS

1. Add buffer (low-alkalinity ponds)
2. Add calcium (high-alkalinity ponds)
3. Add alum (high-alkalinity ponds)
4. Treat algae with herbicide

COMMENTS

Alkaline pH stress is much less common than acid stress because, first, most closed culture systems tend to decrease in pH over time; and, second, acids are much more common environmental contaminants than alkalis.

Acutely high pH may be caused by high levels of alkalis leaching out of inadequately cured concrete (Hine 1982). Concrete containers should be allowed to leach all alkali before using for fish culture. Concrete can be cured with muriatic (hydrochloric) acid to speed up the process. Improper use of slaked or hydrated lime will rapidly raise the pH to 11, killing all fish (see "**Pharmacopoeia**"). The owner must then wait several weeks for the pH to return to normal before restocking.

Many fish do poorly in even moderately alkaline water and should be kept in soft, moderately acid conditions (Table II-8). Alkaline pH can also increase the mortality of incubating eggs of some species, possibly because acid waters are somewhat bacteriostatic.

Chronically high diurnal pH in ponds is almost always caused by excessive phytoplankton or vascular plant photosynthesis, which drives up the pH during the day as

Table II-8. Some tropical, freshwater aquarium fish that do best in soft, slightly acid water (pH~6.5–6.8; hardness ~20–40 mg/l).

KILLIFISH	TETRAS
Aphyosemion	*Cheirodon*
Aplocheilus	*Crenuchus*
Nothobranchius	*Hemigrammus*
Cynolebias	*Hyphessbrycon*
Epiplatys	*Megalamphodus*
Pterolebias	*Moenkhausia*
Rivulus	*Paracheirodon*
SOUTH AMERICAN CICHLIDS	LOACHES
Apistogramma	*Botia*
Symphysodon	
GOURAMIES	BARBS
Trichogaster	*Barbodes*
	Capoeta
	Puntius

CO_2 is consumed (see Fig. II-1, D). This occurs in ponds with either low alkalinity or low calcium levels (relative to the amount of alkalinity) (Table II-9, A). Wide pH swings occur in low-alkalinity waters because there is not enough buffering capacity to moderate the plant-associated metabolic alkalosis. In ponds with high alkalinity and low calcium hardness, the pH can rise high during the day, sometimes over 10. This episodically high pH can be lethal to fry (Wu and Boyd 1990). High pH can occur because the precipitation of calcium carbonate normally inhibits the rise in pH, since carbonate hydrolysis is the source of the high pH (Swingle 1961) (see Fig. II-7).

Because most natural waters have the proper amounts and proportions of hardness and alkalinity, rising pH is an uncommon stress in pond fish. High pH in ponds is mostly important because it increases the amount of toxic, unionized ammonia (see PROBLEM 4).

Diagnosis

Diagnosis of alkaline pH stress should take into consideration the same factors used in diagnosing acid stress (see PROBLEM 7). At high pH, gill mucus cells and epithelial cells are hypertrophic (Daye and Garside 1976). Corneal damage may also occur. These clinical signs are nonspecific. Note that alkalinity and alkaline pH are not the same (see PROBLEMS 7 and 9 for a general discussion of acid-base balance in water).

Treatment

To correct alkaline pH stress, the clinician should take into consideration the same factors used to correct acid stress (see PROBLEM 7). Note especially that heavy metals are mobilized and more toxic as pH is lowered. Rapid pH decrease can also cause shock (see PROBLEM 97). As a general rule it is better not to lower the pH more than ~0.20–0.50 pH unit per day, although fish often tolerate much larger changes.

Aquaria

Many commercial preparations are available for adjusting the pH of aquaria. Phosphate buffers are typically used to lower pH. Filtering water through peat will also reduce the pH, as well as the hardness, and is commonly used by aquarists to condition water for certain species (see Table II-8). Adding deionized water will also reduce the pH by diluting out carbonate buffers that maintain neutrality.

PONDS

In ponds with low alkalinity, adding buffer will reduce the high diurnal pH peak. Well-buffered, calcium-poor ponds can be treated with calcium; alum has also been used successfully (Boyd 1990). Killing some of the plants with an appropriate herbicide will also dampen the daily pH spike, but this is not usually recommended because of adverse side effects (low oxygen from an algae crash [see PROBLEM 1] and possible herbicide toxicity to the fish).

PROBLEM 9
Improper Hardness

Prevalence Index

WF - 2, WM - 2, CF - 3, CM - 3

Method of Diagnosis

Chemical measurement of improper hardness

History

Acute to chronic stress response

Physical Examination

See "**History**"

Treatment

HARDNESS TOO LOW

Add calcium

HARDNESS TOO HIGH (AQUARIA)

1. Do water change with deionized water or other low-hardness water
2. Filter water through peat

COMMENTS

Hardness vs. Alkalinity

Hardness is a measure of the divalent metal cation (e.g., calcium, iron, zinc, magnesium) concentration in water. In most waters, it is composed almost entirely of Ca^{++} and Mg^{++}.

Hardness and alkalinity usually are closely related but measure different activities. Total hardness values (in mg/l as $CaCO_3$) usually will be similar to alkalinity values (in mg/l as $CaCO_3$) because the alkalinity of most

Table II-9, A. Primary contributors to hardness and alkalinity in various natural waters.

		Hardness	
		Low	High
Alkalinity	Low	Low in both heavy metal salts and carbonate	Ca and Mg salts of sulfate, nitrate chloride, silicate
	High	Na_2CO_3, K_2CO_3	$CaCO_3$, $MgCO_3$

Table II-9, B. Ranges of hardness using the carbonate and °dH scales.

Water	Hardness as:	
	Calcium carbonate equivalents (mg/l)*	German hardness (°dH)
Soft	0–75	0°–4°
Moderately hard	75–150	4°–8°
Hard	150–300	8°–16°
Very hard	>300	16°

*Calcium carbonate equivalents (mg/l) = °dH × 17.9 (Moe 1992a).

natural waters comes primarily from the carbonate salts of calcium and magnesium. Other divalent or trivalent metal ions are relatively uncommon in natural waters. The hardness derived from carbonate salts is termed temporary hardness, since it is precipitated by boiling (e.g., this causes "scale" on aquaria or other items exposed to water with high temporary hardness).

Noncarbonate metal salts—sulfate (SO_4^{-2}), nitrate (NO_3^{-2}), chloride (Cl^-), and silicate (SiO_3^{-2})—comprise the permanent hardness, which is a less common component of total hardness. Waters where the hardness is mainly a permanent hardness are very hard but are low in alkalinity. In some waters, alkalinity is due primarily to sodium or potassium carbonate and thus may have low hardness with high alkalinity (Table II-9). The total hardness of seawater is very high (Boyd 1990). Thus, even dilute estuarine water has considerable hardness.

Hardness Requirements

Hardness requirements vary greatly among species and somewhat with environmental conditions. Once acclimated, many fish do well over a wide range of hardness. For example, while at least 100 mg/l total hardness is considered optimal for freshwater salmonid culture, rainbow trout are successfully cultured in southern Appalachian mountain waters that have less than 10 mg/l total hardness. A total hardness of at least 50 mg/l is recommended for most warm water, freshwater, food fish (e.g., channel catfish, hybrid striped bass) (Wedemeyer et al. 1976; Piper et al. 1982).

Many freshwater aquarium fish do poorly in even moderately soft water and should be kept in waters with high calcium content (see PROBLEM 7 for aquarium fish that do best in hard, alkaline water). Conversely, some fish do poorly in even moderately alkaline water and should be kept in soft, moderately acid conditions (see Table II-8 for aquarium fish that do best in soft, acid water). The hardness of full-strength seawater is about 6,600 mg/l as $CaCO_3$, with ~1,000 mg/l as $CaCO_3$ coming from calcium (~400 mg Ca/l) and ~5,500 mg/l as $CaCO_3$ coming from magnesium (~1350 mg Mg/l). Calcium levels in tropical marine aquaria should be ~400 mg/l and should not exceed 200–450 mg/l (Moe 1992a).

It is often easier for fish to adapt to hard water from soft water rather than vice versa. Fish that are transferred from hard to soft water also appear to be more prone to environmental shock (Grizzle et al. 1985) (see PROBLEM 97). Transporting fish in too soft water can also cause chronic losses (Jensen 1990). Calcium and magnesium are needed for osmoregulation. Calcium reduces the permeability of the gills to water, thus reducing water and electrolyte flux.

It is important to realize that hardness includes all divalent cations. For example, a hardness of at least 20 mg/l as $CaCO_3$ is needed for channel catfish health (Tucker 1987). However, catfish in the yolk sac stage need water with at least 20 mg/l calcium, since the primary source of calcium is the water, not the diet, at this life stage. Thus, since hardness readings do not measure which metals actually constitute the hardness, it is often important to determine which minerals are contributing to the hardness.

Diagnosis of Improper Hardness

Hardness is usually expressed as mg/l equivalents of calcium carbonate, although the German hardness scale (degrees of hardness or °dH) is used extensively in the aquarium hobby (Sterba 1983; Ruff 1995) (see Table II-9, B). Commercial kits are available for both measurements (e.g., Hach Chemical; Tetra). A °dH of 3–10° is considered appropriate for most aquarium fish, while °dH >10° is best for African rift lake cichlids.

In marine or brackish water pond systems, calcium levels increase with increasing salinity; thus, if the salinity is optimal for growth, calcium levels usually will be satisfactory. However, hardness reportedly can significantly decrease in marine aquaria that contain corals, crustaceans, or other invertebrates that use large amounts of calcium during growth (Moe 1992a).

Treatment of Improper Hardness

Lime (see "**Buffers—Ponds**" in "**Pharmacopoeia**") or other calcium salts are excellent sources of supplemental calcium for pond fish. Salt mixtures are commercially available for increasing hardness in aquaria. If aquarium hardness must be reduced, this can be done by adding distilled water, available at groceries or pharmacies. Small reverse osmosis or ion exchange deionization units are available for the home aquarist (see "**Deionized Water**" in "**Pharmacopoeia**"). Filtering water over peat will also soften it (Sterba 1983). Sedimentary rocks (e.g., schist, sandstone) may increase hardness because of the release of calcium and magnesium salts. Limestone substrates (e.g., coral, oyster shell) can be used in marine aquaria but are not advisable for aquaria where fish that need soft, acid waters are maintained. Metamorphic or volcanic rocks (e.g., basalt, granite, gneiss), as well as quartz, do not release divalent cations.

PROBLEM 10
Improper Salinity

Prevalence Index

WM - 3, CM - 4

Method of Diagnosis

MEASUREMENT OF SALINITY

In marine aquaria, <30 or >35 ppt salinity (<~1.020 or >~1.026 specific gravity at 25°C)

History

Maintaining salt-requiring fish in freshwater; incorrect calculation of seawater mixture; replacing seawater with freshwater during water changes; failure to replace

evaporative loss of freshwater; acute to chronic stress response

Physical Examination

See "**History**"

Treatment

Add salt or freshwater to correct salinity

COMMENTS

Definition

Salinity is the amount (mass) of all ions in water and is most commonly expressed as parts of ions per thousand parts water (abbreviated as ppt or ‰). Freshwater has less than 0.5 ppt salinity, while natural, full-strength seawater ranges from 30 to 40 ppt salinity. Between these two extremes are various concentrations of brackish (estuarine) water, including oligohaline, mesohaline, and polyhaline. As with other water-quality variables, salinity tolerance of fish varies (i.e., with age, environment).

Salinity Requirements/Tolerance

Marine aquarium fish are adapted to a narrow salinity range, and this should be maintained in the aquarium. Aquarium salinity can rapidly increase because of evaporative loss of water. Salinity in a 35 ppt aquarium will often increase about 2 ppt (0.0005–0.001 specific gravity units) per week; it will rise more rapidly if the tank is not covered (Bower 1983). Thus, it is best to keep the salinity of the tank at the low end of the optimal range (30 ppt).

Some freshwater aquarium fish are native to either estuarine environments or other waters that have a high concentration of dissolved minerals (Table II-10). It is best to keep these fish in a dilute salt solution; this can be simple table salt (NaCl), a dilute seawater mixture, or a specialized formulation for certain species groups (e.g., Malawi® salt mix for African cichlids). It is best to use a balanced salt mixture rather than pure sodium chloride because the latter lacks valuable divalent cations that are also important for osmoregulation and other physiological functions.

Many fish from freshwater environments can tolerate salinities up to 7 ppt but may not do well (McKee and Wolf 1963). As much as 2 ppt salinity is probably safe for the great majority of freshwater fish (McKee and Wolf 1963) (see "**Pharmacopoeia**"), but some (e.g., tetras, many catfishes) are sensitive to salt. Even the latter species seem to tolerate 1 ppt salinity indefinitely (G. Lewbart, personal communication).

Salinity stress may occur if young freshwater salmonids (parr) are prematurely transferred to saltwater before they are ready to undergo transformation into marine-adapted fish (smolts). The parr-to-smolt transformation is a stressful time during the salmonid life cycle and a transfer to seawater can often be accompanied by infectious disease outbreaks (e.g., see PROBLEMS 50 and 54).

Diagnosis

Salinity is difficult to measure directly but can be measured indirectly in several ways, including conductivity, chlorinity, refractive index, or specific gravity. Salinity can be measured least expensively by using a hydrometer, which measures specific gravity. However, this method is cumbersome when compared with refractometry and needs a relatively large volume of water (usually at least 50 ml). If salinity is to be measured frequently, it is easiest to use a hand-held refractometer or electronic meter. A meter is the most accurate means of rapidly measuring salinity but is an expensive instrument and subject to mechanical breakdown.

Treatment

Abnormally high or low salinity places an osmotic stress on the fish and should be corrected as soon as possible with appropriate addition of salt or freshwater. It is generally recommended that the salinity not be changed more than 1 ppt/hour. For estuarine fish, salinity should not be adjusted more than 10 ppt in a few hours. As with other water-quality variables, rapid changes are less tolerated.

Salt is also a useful prophylactic and can be added to freshwater aquaria to reduce prevalence of many infectious diseases, many of which are inhibited by even low salt concentrations (see "**Pharmacopoeia**").

Table II-10. Tropical aquarium fish that do best with at least a small amount of salt (~1–5 ppt salinity).

Brachygobius (bumblebee goby)
Chonerinus (puffer)
Fundulus (topminnow)
Monodactylus (mono)
Periopthalmus (mudskipper)
Poecilia (molly)—many species
Scatophagus (scat)
Toxotes spp. (archerfish)
African rift lake cichlids

CHAPTER 8

PROBLEMS 11 through 43

Diagnoses made by either gross external examination of fish, wet mounts of skin/gills, or histopathology of skin/gills

11. Gas supersaturation
12. Lamprey infestation
13. Leech infestation
14. Copepod infestation/infection
15. Branchiuran infestation
16. Isopod infestation
17. Monogenean infestation
18. Turbellarian infection
19. Protozoan ectoparasites: general features
20. Ich infection
21. Marine white spot disease
22. Trichodinosis
23. *Chilodonella* infestation
24. *Brooklynella* infestation
25. Tetrahymenosis
26. Scuticociliatosis
27. Marine velvet disease
28. Freshwater velvet disease
29. Ichthyobodosis
30. Gill *Cryptobia* infestation
31. Gill amoebic infestation
32. Sessile, solitary, ectocommensal ciliate infestation
33. Sessile, colonial, ectocommensal ciliate infestation
34. Typical water mold infection
35. Epizootic ulcerative syndrome
36. Branchiomycosis
37. Columnaris infection
38. Bacterial cold water disease
39. Bacterial gill disease
40. Lymphocystis
41. Epitheliocystis
42. Miscellaneous skin and gill diseases
43. Incidental findings

PROBLEM 11

Gas Supersaturation (Gas Bubble Disease [GBD])

Prevalence Index

WF - 4, WM - 4, CF - 3, CM - 4

Method of Diagnosis

1. Clinical signs
2. Measurement of percentage of total gas pressure in water

History

Rapid increase of water temperature from water source to fish culture system; water intake pipe sucking in air; long pipe run; rapid decrease in pressure from water source to fish culture system; water falling over a deep spillway; ground water (borehole or spring) source; heated water; hydro power; ice formation; heavy macrophyte growth in clear pond; behavioral abnormalities; fish floating to surface

Physical Examination

Gas emboli in blood vessels of virtually any organ, including skin, gills, eyes, viscera, and peritoneal cavity; exophthalmos caused by retrobulbar gas emboli; emphysema in dermis

Treatment

Eliminate excess gas; do not stress affected fish during recovery

COMMENTS

Causes of Gas Supersaturation

Gas supersaturation (ΔP) occurs when the total pressure of gases dissolved in water is higher than the ambient atmospheric pressure. This may occur when water is pumped up from a deep (>90-meter [300-foot]) well, since such water is often supersaturated with nitrogen and/or carbon dioxide (Colt et al. 1986). Spring water may be supersaturated with nitrogen after the spring thaw because of the overwinter accumulation of nitrogen gas (N_2) produced by natural breakdown of nitrates and nitrites (Warren 1981). When the water is exposed to the atmosphere, the excess gas begins to equilibrate with air and thus leaves solution. If this occurs in the fish's blood vessels or other tissue, gas bubble disease results.

Gas supersaturation can also result from any other condition that leads to a higher than atmospheric concentration of gas in water (Colt 1986), including a leaky water pipe that can suck air under pressure, to be released at the outlet; a cavitating pump; Venturi injectors; water that is rapidly heated, such as when it is entering a heated tank or building; or water that enters a plunge pool

where air is forced into solution under pressure (e.g., hydropower generating systems). Fish transported by air have also developed gas bubble disease (Hauck 1986), but this is apparently a rare event in air-shipped fish. In deep culture systems, fish near the surface succumb more quickly because of the difference in hydrostatic pressure (Heggberget 1984). For example, ΔP decreases by 74 mm Hg for every meter depth in freshwater at 20°C (68°F; Colt 1984). Thus, fish in hatcheries are especially susceptible to gas bubble disease because they cannot escape to lower depths.

Most gas emboli are produced by excess nitrogen (Marking 1987) because oxygen is assimilated metabolically and thus less likely to form persistent bubbles. However, very high oxygen concentrations are dangerous.

In ponds that have heavy macrophyte growth (i.e., submerged aquatic weeds, such as Hydrilla), photosynthesis may be so great as to produce more oxygen than can diffuse into the water; this is most likely to occur in clear, shallow ponds with aquatic macrophytes. Such conditions allow oxygen to supersaturate the entire pond, not just the surface as would typically occur in a turbid pond where light penetrates less; thus, fish cannot escape the supersaturated conditions. Also, intensive culture systems using liquid oxygen to increase fish-carrying capacity may accidentally overdose the fish.

In ponds, oxygen levels >125% are probably not advisable, and 300% saturation is lethal (McKee and Wolf 1963). Dissolved oxygen levels >20 mg/l have caused mortality. Note that ponds with high photosynthetic activity often have ΔP > 300 mm Hg without problems if fish can escape to deeper, less saturated water (Boyd 1990). In such cases, eggs or fry at the surface with limited mobility would be most at risk.

Sequelae of Gas Supersaturation

If fish breathe supersaturated water before it equilibrates, the excess gas may leave solution in the bloodstream, forming emboli in various tissues (gas bubble disease; Fig. II-11). Histopathology of gas bubble disease (Pauley and Nakatani 1967) has been reported to include edema of the gill secondary lamellae, with accompanying degeneration of the overlying epithelium. Other lesions include edema and embolic disruption of buccal and intestinal mucosa, as well as vacuolar degeneration of the renal tubular epithelium. Lesions may also occur in the liver and muscle. Tissue hemorrhage and brain damage have been postulated to cause death (Ferguson 1988), but the mechanism of tissue damage is uncertain. The severity of the damage depends on the number of emboli formed

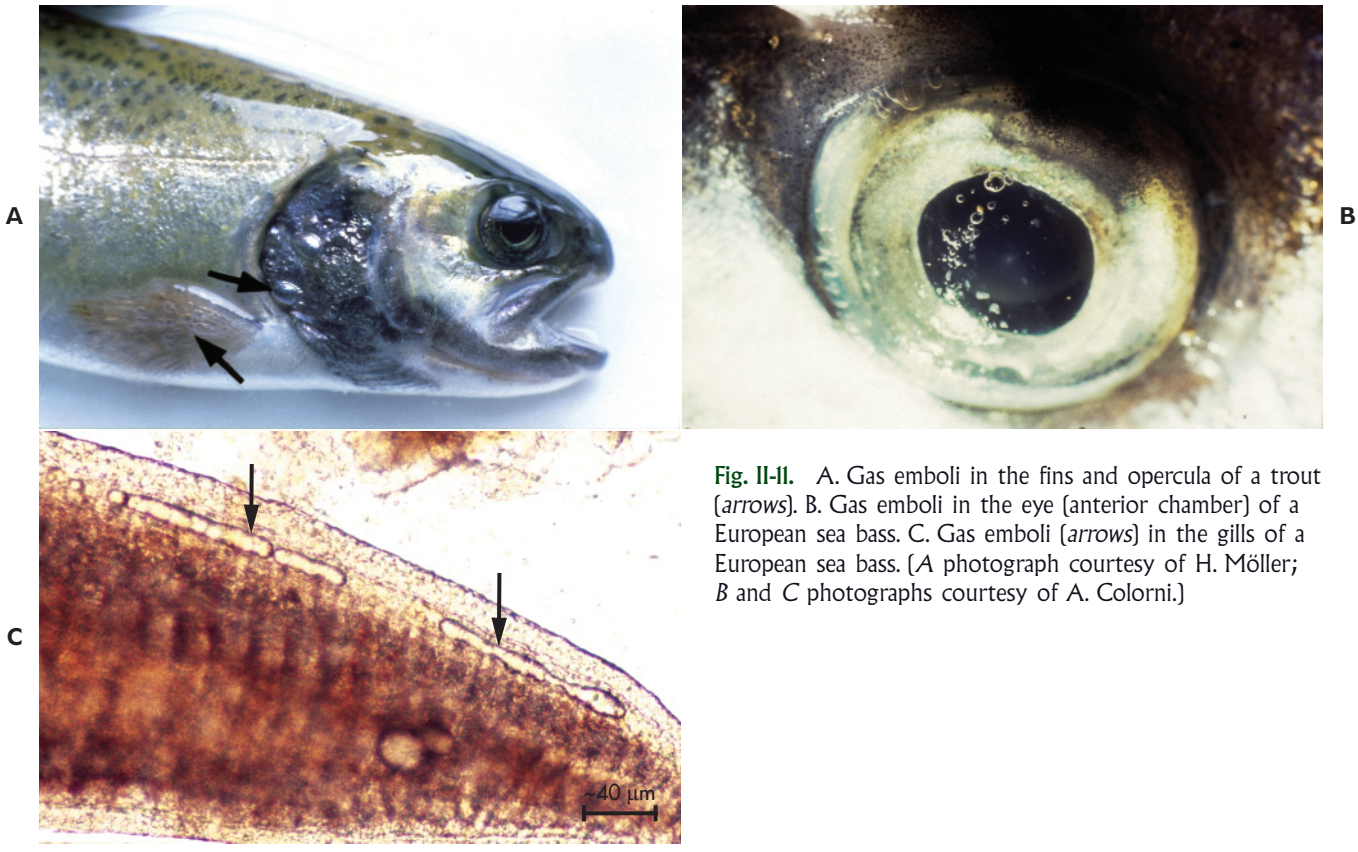


Fig. II-11. A. Gas emboli in the fins and opercula of a trout (*arrows*). B. Gas emboli in the eye (anterior chamber) of a European sea bass. C. Gas emboli (*arrows*) in the gills of a European sea bass. (*A* photograph courtesy of H. Möller; *B* and *C* photographs courtesy of A. Colorni.)

and which tissues are affected. Behavioral abnormalities that are related to the target organs (e.g., hyperactivity, loss of equilibrium) may be present.

Clinical Signs

ACUTE GAS SUPERSATURATION

Acute gas supersaturation (ΔP > 50–200 mm Hg) can cause mortalities in as little as minutes; however, most cases present less acutely, with high mortalities after a few days' exposure. Eggs float to the surface, and larvae or fry may have hyperinflation of the swim bladder, cranial swelling, exophthalmos, swollen gill lamellae, pneumoperitoneum, or gas bubbles in the yolk sac. Up to 100% mortality occurs (Colt 1986).

CHRONIC GAS SUPERSATURATION

Low supersaturation levels (ΔP < 76 mm Hg or <110% saturation at sea level) are associated with chronic low (typically <5%) mortalities, hyperinflation of the swim bladder, and extravascular emboli in the gastrointestinal tract and mouth. Low-level supersaturation rarely produces highly visible lesions; fish must be closely examined. Secondary effects (unusually high mortalities, skeletal deformities, opportunistic infections) are most evident. Anecdotal evidence suggests that fish exposed to chronic supersaturation early in life might be less resistant to stress later in the production cycle.

Diagnosis

The presence of gas emboli is pathognomonic for gas bubble disease. Holding fish up to a light source (candling) can help to visualize emboli (Ferguson 1988). Bubbles can be squeezed from fin or gill clips while the fish is held under water, confirming the diagnosis. Do not confuse putrefaction in dead fish with gas bubble disease.

The supersaturation of just one gas may not cause gas supersaturation; for gas bubbles to form, the total gas pressure must exceed the barometric pressure. Determination of gas supersaturation is based on the measurement of the total concentration of dissolved gas in the water source. It is thus important to realize that some gases may be present in harmful concentrations in the absence of gas bubble disease (see PROBLEMS 90 and 91). Measurement of excess gases requires a saturometer (Fickeisen et al. 1975), which is commercially available (Aquatic Ecosystems).

Gas saturation can be expressed as a percentage of the total barometric pressure:

$$\text{Percentage total gas pressure} = \frac{BP + \Delta P}{BP} \times 100$$

For example, if the local barometric pressure (BP) is 760 mm Hg and the ΔP measured with the saturometer is 76 mm Hg, the total gas pressure is 110% saturation of water with atmospheric gases.

In general, levels of about 110% saturation are considered dangerous for fish. However, this varies with the species and with the age of the fish. For example, even low levels (101–105%) affect salmonid sac fry, while adult salmonids often tolerate over 125% saturation (Wood 1974). Eggs are usually more tolerant. Warm water fish are generally more tolerant of supersaturation than cold water fish. Whether fish will develop GBD at low (101–102%) levels of supersaturation depends upon the water vapor pressure. For a bubble to form in the blood (or in water), the gas pressure has to be greater than 100% *plus* the partial pressure exerted by water vapor, which is a function of water temperature. At 8°C (46°F), the partial pressure of water vapor is equal to 7.5 mm Hg, which is equivalent to a partial pressure of 1% of the total gas pressure. Thus, *at the surface,* the total gas pressure has to be over 101% supersaturation for air bubbles to form. At a gas pressure of 101.1%, gas bubbles will form and fish at the surface will be vulnerable. The hydrostatic head pressure gives an equivalent compensation of 1% gas pressure. Thus, the total pressure must exceed 102% before bubble formation can occur. In this case, the fish will be vulnerable if the pressure is ≥102.1% (Dryden 1994).

Treatment

Treatment of gas bubble disease requires eliminating the excess gas in the water source. This can involve first aerating the water source in a reservoir to allow it to equilibrate with air, but in many cases this is not practical. In flow-through systems that use large volumes of water, the water can be stripped of excess gas by using a packed column degasser. Packed columns are commercially available (Aquatic Ecosystems). Construction of degassers is described by Colt (1986). Passive degassers can be used to return the gas concentration to 100%, but vacuum degassing provides a greater margin of safety.

PROBLEM 12
Lamprey Infestation

Prevalence Index

Not seen in cultured fish

Method of Diagnosis

1. Presence of lamprey on host fish
2. Lamprey lesions on host fish

History

Wild-caught fish from lamprey-endemic area

Physical Examination

Anemia; circular skin ulcers

Treatment

TFM + Bayluscide

COMMENTS

Lampreys are eel-like, jawless fish in the class Agnatha. They are important parasites of freshwater and marine commercial fish (Fig. II-12, A). They feed by using a

Fig. II-12. A. American sea lamprey attached to brook trout. B. River lamprey, showing key diagnostic features, including eel-like shape and circular, rasping mouth, with chitinized teeth (*arrow*). (*B* photograph courtesy of Wydoski RS and Whitney RR, *Inland Fish of Washington*, Bethesda, MD: American Fisheries Society, 2003.)

circular suctorial mouth that has sharp, horny teeth (Fig. II-12, B), which rasp the skin and form a characteristic, circular ulcer. In spring, lampreys spawn in freshwater; eggs hatch into small, worm-like, ammocoete larvae, which filter-feed in the mud. After several years the larvae metamorphose into adult lampreys, which, depending on the species, may migrate into the ocean or remain in freshwater. The American sea lamprey has become a serious problem in the Great Lakes, where it causes hemorrhagic anemia and mortality in lake trout (Wooten 1989). Lampreys are controlled with TFM (3-trifluoromethyl-4-nitrophenol), a lampricide that is selective for the ammocoete larva. Bayluscide potentiates the effectiveness of TFM.

PROBLEM 13
Leech Infestation

Prevalence Index
WF - 4, WM - 4, CF - 4, CM - 4
Method of Diagnosis
1. Parasite on skin, gills, or in oral cavity
2. Histology of skin, gills, or oral cavity with parasite
History
Wild-caught or pond-raised fish
Physical Examination
Anemia; small red or white lesions on skin
Treatment
Organophosphate prolonged immersion

COMMENTS
Epidemiology/Pathogenesis
Leeches are rare in cultured fish but are occasionally seen in wild or pond-raised fish. They have a direct life cycle, with juveniles hatching from cocoons laid by the hermaphroditic adults. Some species have a relatively wide host range, while others are restricted to only a few fish species. Both mature and immature leeches are hematophagous, with pathology depending on the amount of blood taken (i.e., number and size of worms and the duration of feeding). Heavily infested fish (Fig. II-13, A) often have a chronic anemia. Leeches can also transmit microbes and hemoparasites (see PROBLEMS 44 and 80) during feeding. Some can cause large ulcers on the skin or in the mouth (Noga et al. 1990a).
Diagnosis
Leech infestation can be diagnosed by histopathology (Fig. II-13, C). Leeches should preferably be removed from the fish and then fixed, especially if species identification is desired. Leeches can be differentiated from monogeneans (see PROBLEM 17) by the presence of body segmentation. They are also much larger than the great majority of monogeneans. Leeches are annelids. They differ from typical free-living annelids in having anterior and posterior suckers (Fig. II-13, B and D). However, some aquatic leeches are free-living (i.e., do not feed on fish). They require examination by an expert to distinguish them from parasitic species. Leeches resemble large digenean trematodes but have a complete digestive tract, with a mouth in the anterior sucker and an anus in the posterior sucker.
Treatment
Leeches are usually easily treated with a single dose of organophosphate, although fish should be watched closely for 3 weeks to monitor for possible reinfestation. Another suggested method for reducing the number of leeches in a pond is to place a piece of meat or raw liver in a plastic container with several small holes. This is weighted down with rocks so that it remains on the

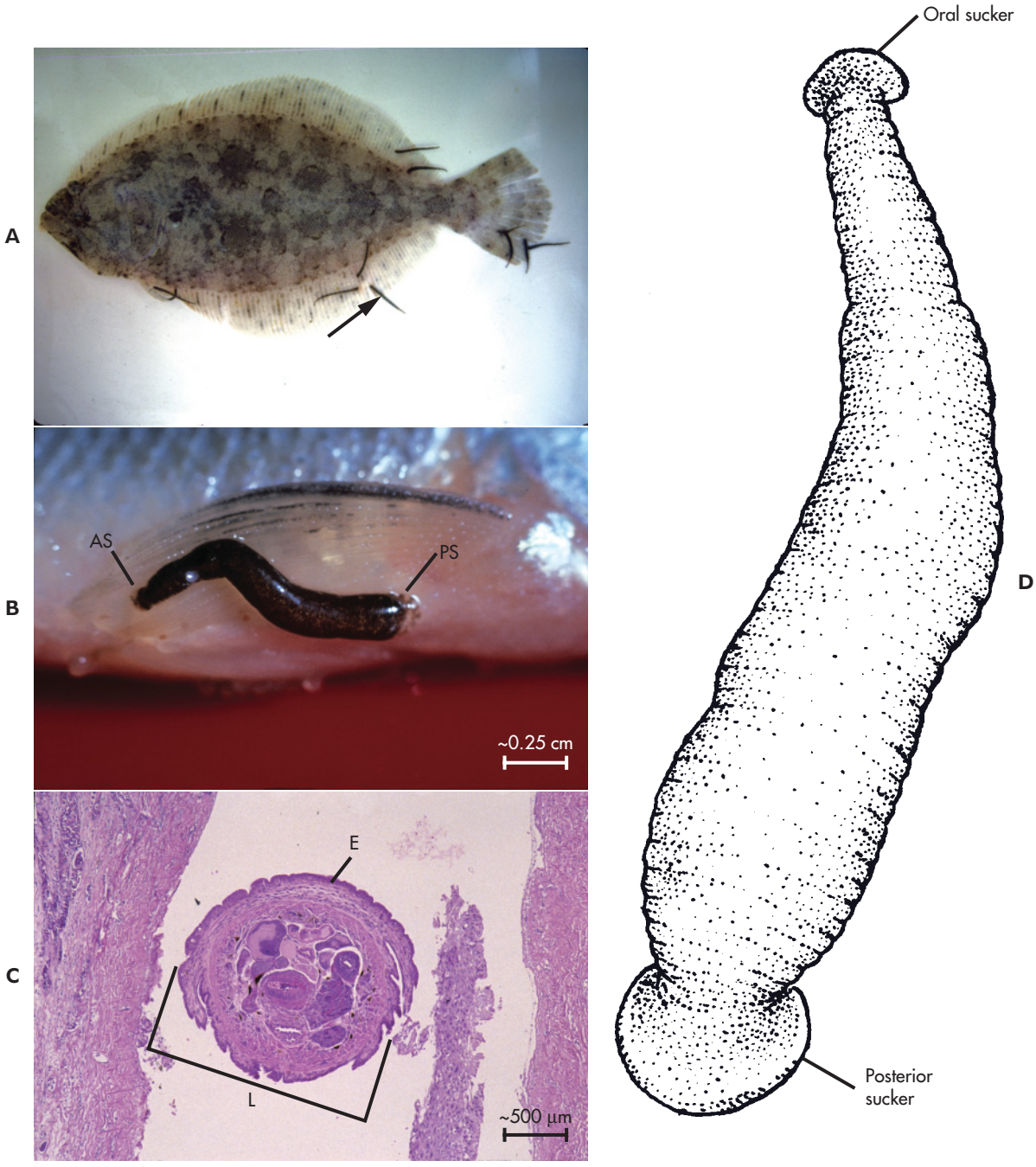


Fig. II-13. A. Southern flounder with a heavy leech (*Myzobdella*) infestation (*arrow*). B. Wet mount of a leech (*Piscicola*) on a smelt. *AS* = anterior sucker; *PS* = posterior sucker. C. Histological cross-section of a leech (*L*), *Myzobdella lugubris*, in the mouth of a largemouth bass. Key diagnostic features are epithelium (*E*) on the surface of the body, circular shape, and various organs (e.g., digestive, reproductive) suspended in a true coelomic space. Hematoxylin and eosin. D. Diagram of a leech showing diagnostic characteristics, including suckers and segmentation. (*B* photograph courtesy of H. Möller; *C* photograph by L. Khoo and E. Noga.)

bottom of the pond. Leeches are attracted to the meat and can be removed regularly (Wildgoose and Lewbart 2001).

PROBLEM 14

Copepod Infestation/Infection (Sea Louse, Fish Maggot, Anchor Worm)

Prevalence Index

WF - 2, WM - 4, CF - 3, CM - 1

Method of Diagnosis

1. Wet mount of gills, skin, or mouth with parasite
2. Histopathology of gills, skin, or mouth with parasite

History

Wild-caught, pond-raised, or cage-cultured fish; skin sores

Physical Examination

Various-sized (barely visible to ~25 mm) copepods attached to gill arches, oral cavity, or skin; erosion and/or ulceration; red areas on skin, may be raised up to 5 mm in height

Treatment

SEA LICE

1. Enamectin oral
2. Teflubenzuron oral
3. Organophosphate bath
4. Pyrethroid bath
5. Hydrogen peroxide bath
6. Diflubenzuron oral
7. Ivermectin oral
8. Freshwater bath (*C. elongatus* only)

ANCHOR WORM

1. Organophosphate prolonged immersion
2. Difluorobenzuron prolonged immersion
3. Salt prolonged immersion (freshwater copepods only)

Class Copepoda

Orders of parasitic copepods follow, including selected families (after Kabata 1984 and Martin and Davis 2001):

Order Poecilostomatoida: almost all are marine species
Grasp and anchor to gill and skin surfaces:
Ergasilidae (*Ergasilus*)—both freshwater and marine

Order Siphonostomatoida: almost all are marine species—includes >75% of all fish-parasitic copepod infestations/infections
Families
Grasp and anchor to gill and skin surfaces:
Cecropidae
Caligidae (*Caligus*, *Pseudocaligus*, *Lepeophtheirus*)
Hatschekiidae
Lernanthropidae
Penetrate skin or gill and burrow deeply into tissues:
Pennellidae (*Lernaeenicus*)
Sphyriidae
Lernaeopodidae (*Salmincola*, *Achtheres*)
Chondracanthidae

Order Cyclopoida: all but one is a freshwater species
Penetrate skin and burrow deeply into tissues
Lernaeidae (*Lernaea*)

COMMENTS

General

Parasitic copepods are increasingly serious problems in cultured fish and can also impact wild populations (Lester and Hayward 2006). Most of the approximately 10,000 copepods are free-living, but about 1,700 species are parasites, and there are likely many thousands more yet to be described (Thatcher 1998). Most parasites affect marine fish, but there are some important freshwater pathogens. Parasites vary from organisms that morphologically resemble free-living copepods to others that are highly modified for parasitism (Fig. II-14, B). Most are skin or gill parasites. A few penetrate deep into host tissues, such as the heart; endoparasites are not a problem in cultured fish.

The life cycle of clinically important parasitic copepods (Fig. II-14, A) typically comprises 1–5 free-living nau-

A

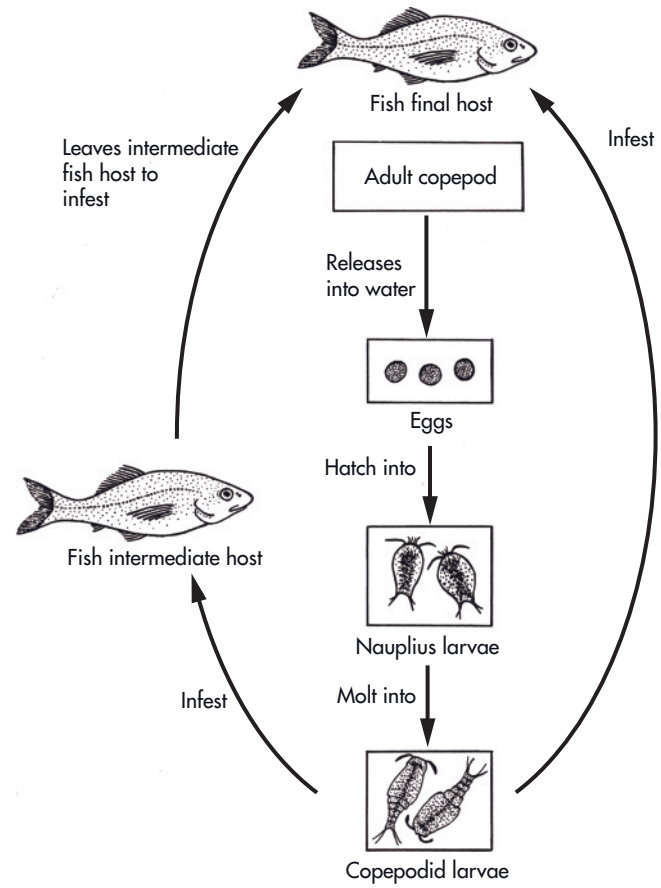


Fig. II-14. A. Life cycles of fish-parasitic copepods.

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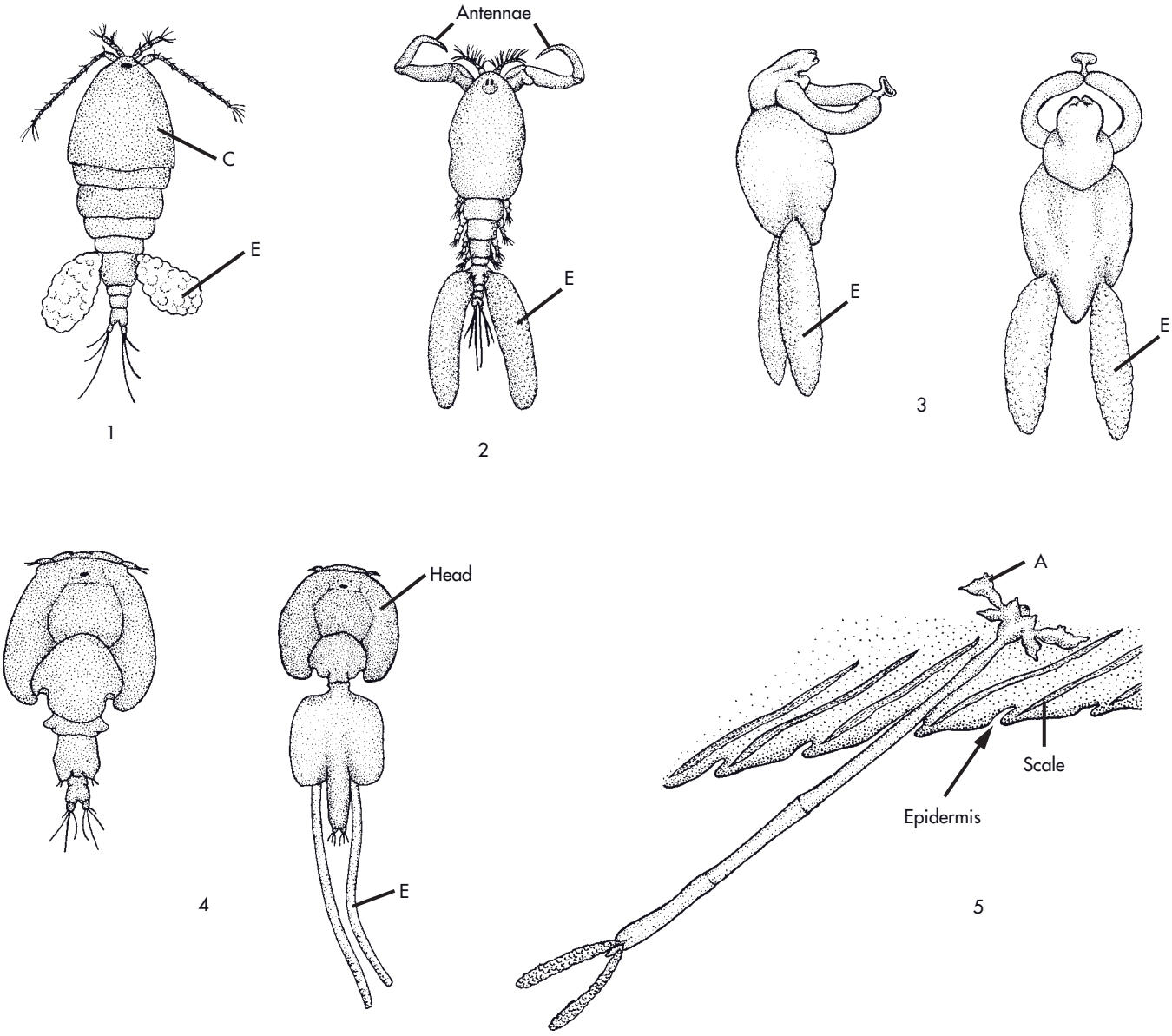

Fig. II-14.—cont'd. B. Diagrams of major types of parasitic copepods affecting cultured fish, including key diagnostic features. Egg sacs (*E*) are only present in mature females. If the adult has only recently attached to the fish, it may not be visible grossly. B_1. Typical free-living copepod (*Cyclops* sp.). C=cephalothorax. B_2. Ergasiliform type: body is divided into cephalothorax and abdomen, and has a shape similar to that of free-living copepods, but has grasping antennae. Egg sacs (*E*) are only present in mature females. B_3. Lernaeopodid type (lateral and dorsal views): body more grub-like than ergasilids. B_4. Caligiform type (male on left; female on right): adult sea louse (5–10 mm), flat, broad head. Egg sacs (*E*) are only present in mature females. B_5. Lernaeid type: mature female (~5–25 mm), long thin body, vestigal appendages, head with anchors (*A*).

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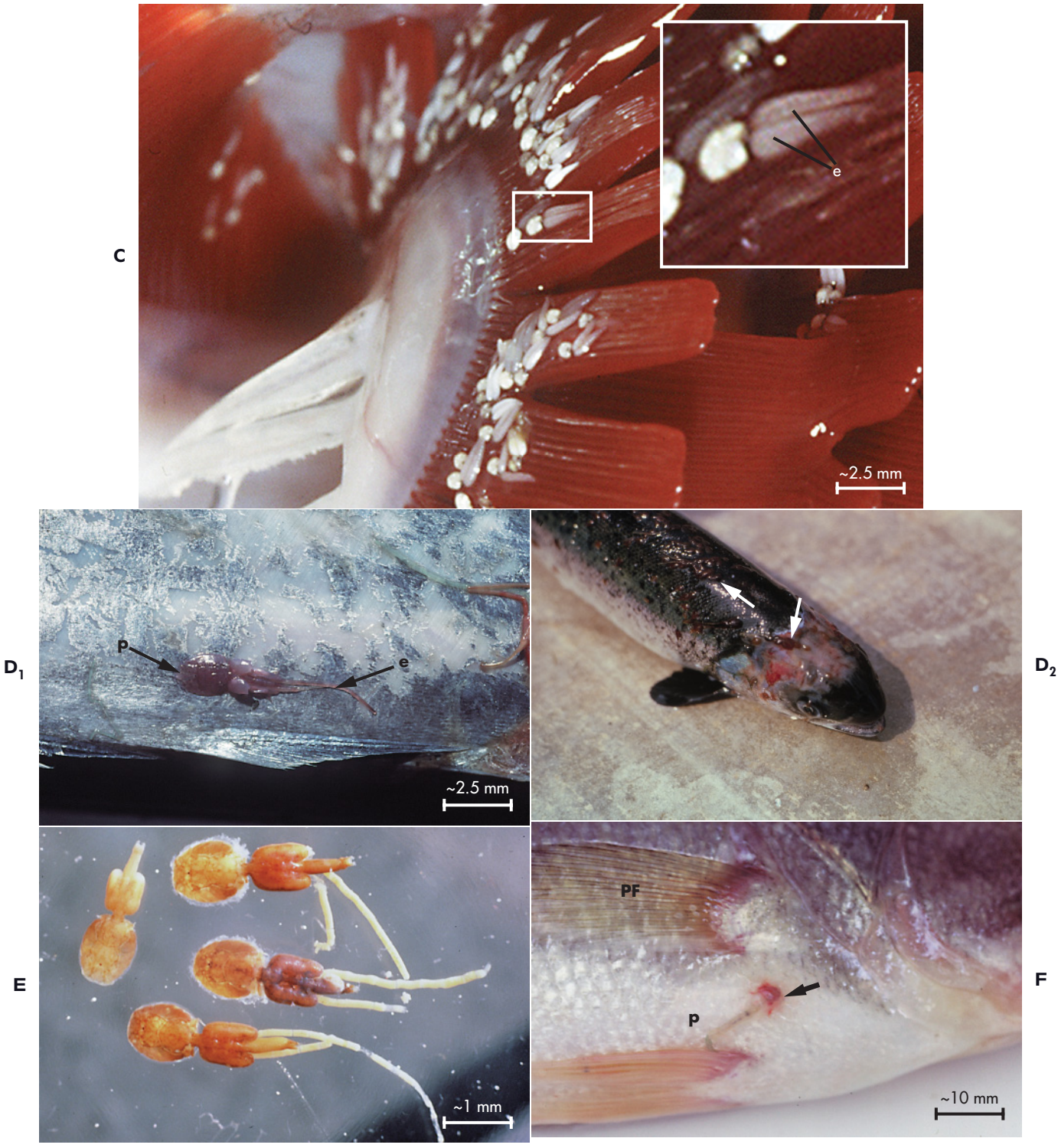

Fig. II-14.—cont'd. C. Severe gill maggot (*Ergasilus*) infestation of a striped bass. The parasites are attached to the primary lamellae of the gills by their modified antennae. Note that the egg sacs (*E*) vary from white to grey, depending on the developmental stage of the larvae in the egg sacs. D_1. Sea louse infestation of wahoo. Note parasite (*P*) with egg sacs (*E*) trailing from the flat, scale-like body. D_2. Severe sea louse infestation on an Atlantic salmon. Note the numerous parasites (arrows) mainly on the dorsal surface of the body, as well as the severe ulceration of the head due to feeding activity. E. Sea lice (*Lepeophtheirus salmonis*). F. Anchor worm (*Lernaea cruciata*) infection of a largemouth bass. The head of the parasite is embedded under the skin while the body (*P*) with egg sacs protrudes. Note the hemorrhage (*arrow*) where the parasite enters the fish. *PF* = pectoral fin.

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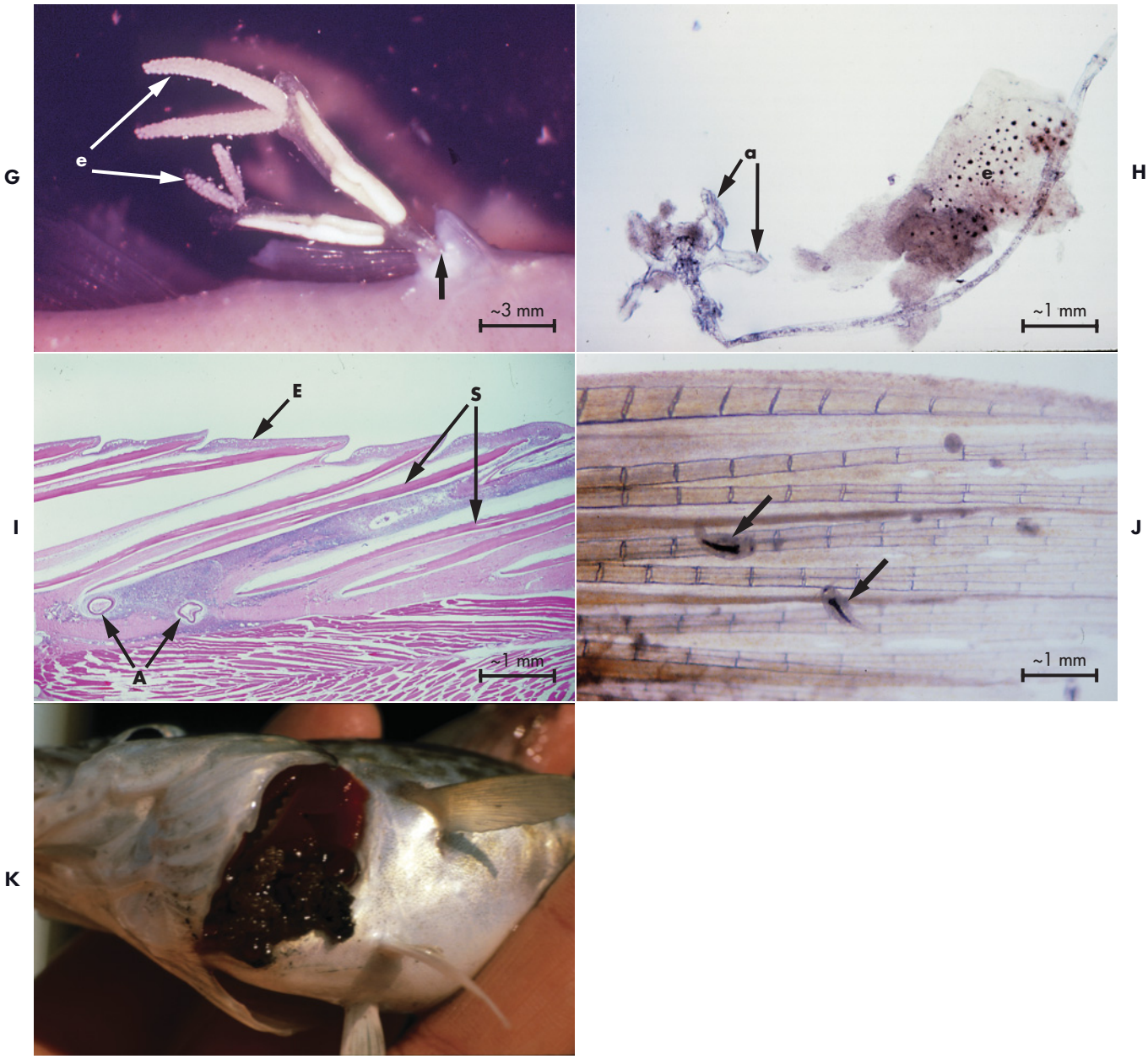

Fig. II-14.—cont'd. G. Close-up view of two anchor worms (*Lernaea cyprinacea*), which enter the fish at the *arrow*. *E* = egg sacs. H. Wet mount of a skin scraping with an immature *Lernaea cruciata* female. *A* = anchors. *E* = host epithelium. I. Histological section through an immature *Lernaea cruciata* female. Only anchors (*A*) are visible in this plane of section. The parasite penetrates between two scales (*S*), inciting inflammation. *E* = epithelium. Hematoxylin and eosin. J. Copepodid infestation (*Lernaea cyprinacea; arrows*) on the fin of a goldfish. K. *Lernaeocera branchialis*, a pennellid, attached to and penetrating the base of the branchial cavity of an Atlantic cod. (D_1 photograph courtesy of T. Wenzel; D_2 photograph courtesy of A. Pike; *E* and *K* photographs by H. Möller; *G* photograph by G Hoffman.)

plius stages, 1–5 free-living or parasitic copepodid stages (Fig. II-14, J), 1 pre-adult, and finally the adult. Parasitic copepods attached to the host by a frontal filament are known as chalimus larvae. The life cycle is typically faster with higher temperature.

Classification of parasitic copepods is based on the structure of the buccal region (Kabata 1981). Yamaguti (1963) provides comprehensive but outdated keys to all parasitic copepods. Boxhall (2004) provides keys to copepod genera. Keys to various species of parasitic copepods are referenced in Lester and Hayward (2006). Three major types are most commonly found on cultured fish: ergasiliform, caligiform, and lernaeiform types.

Ergasilids

Ergasilids (family Ergasilidae) appear similar to free-living copepods in having division of the body into distinct cephalothorax and abdomen and presence of paired locomotory appendages (Fig. II-14, B_2 and C). However, they are also modified for parasitism as indicated by the presence of antennae modified for grasping the host and a large trunk for reproductive products (Kabata 1988). Ergasilids are usually ~2 mm long, with a conical, segmented body (Fig. II-14, B_2 and C). Most infest the gills (rarely the skin) of freshwater fish (Kabata 1979). Some have a wide host range (e.g., *Neoergasilus japonicus* and *Ergasilus sieboldi* on temperate freshwater fish; *E. labracis* on temperate marine fish). Rarely causing epidemics in cultured fish, they are often incidental findings on wild or pond-raised fish in summer (Wooten 1989) and probably cause few problems in small numbers. However, their feeding activity does severe focal damage and very heavy infestations can be lethal. Adult females of some species can move from host to host. Several ergasilids have been inadvertently spread across continents by movement of infested fish (Lester and Hayward 2006).

Lernaeopodids

Lernaeopodids (family Lernaeopodidae) are mainly parasites of marine fish. They still retain some of the typical copepod shape (Fig. II-14, B_3) but are more grub-like compared with ergasilids. *Salminicola* infests the gills of older salmonids in freshwater. Fish that return to the sea after spawning may retain the parasite; thus, individuals returning to freshwater to spawn for a second time may have severe gill damage (Hoffman 1967). Some have rarely caused disease in aquaculture; when present, they are mainly a concern because they reduce the carcass value ("grubby" fish) and such fish may be banned from stocking into reservoirs (Modin and Veek 2002).

Caligids (Sea Lice)

The sea lice *Lepeophtheirus* and *Caligus* (family Caligidae; Fig. II-14, B_4, D, and E) infest the skin. Species are usually restricted to certain groups (e.g., salmonids; Table II-14). For example, *L. salmonis* is largely confined to Atlantic salmon and sea trout. However, some species have a wide host range. For example, *C. elongatus* can parasitize over 80 different fish species, including salmonids (Atlantic salmon and others), pleuronectids, scombrids, clupeids, gadoids, and elasmobranchs (Revie et al. 2002a). Most sea lice occur in warm temperate and tropical seas; *L. salmonis* is an exception, with a circumpolar distribution in cold temperate waters (Lester and Hayward 2006). Sea lice have become a serious problem in all major salmon-producing regions of the northern hemisphere, including Norway, Scotland, Ireland, Chile, Japan, and Canada; they are also a problem in Chile, but are not yet a major problem in Australia or New Zealand (Pike 1989; Costello 2006). While mainly a problem in cultured salmonids, they are becoming problems in other fish that are now being cage-cultured at sea.

Once established in a cultured population, parasite numbers slowly increase over time, eventually causing an epidemic. In salmon having a 2-year production cycle in sea cages, fish in the second year of production usually have the most severe *L. salmonis* infestations (Revie et al. 2002a); however, the most severe *C. elongatus* infestations occur in the first year (Revie et al. 2002c). Sea lice are most serious where salmon farming has been established for many years. Cultured fish become infested by wild fish, which usually carry low parasite burdens. Although epidemics have rarely occurred in wild populations (Panasenko et al. 1986; Berland 1993), there is still considerable controversy about the possible transfer of sea lice from cultured to wild salmonids (Pike and Wadsworth 1999) and its relationship to the severe decline in natural sea trout stocks along the Scottish and Irish coasts (Harder 2005).

Grossly resembling the fish louse (see PROBLEM 15), sea lice are dorsoventrally flattened copepods that adhere to fish; they scrape the epithelium while feeding, causing erosions and at times deep ulcers extending to bone. They attach to the host by pressing their shield-like cephalothorax onto the skin like a sucker. The second antennae and maxillipeds are used as clamps.

The life cycle of sea lice includes nauplius (free-swimming) → copepodid (~1 mm, infective) → chalimus (~1–3 mm, parasitic, sessile) → pre-adult → adult (to 10 mm; Kabata 1988). The pre-adult and adult stages are commonly called "mobiles" since they can move on the fish and among fish (Bron et al. 1993). The life cycle is temperature-dependent; for example, *L. salmonis* requires as little as 4 weeks or as long as 9 weeks, depending upon the temperature (6–18°C [43–64°F]; Wootten et al. 1982; Kent 1992). Thus, multiple generations occur in a single year. Warm water species can complete their life cycle in ~2 weeks. Infestations can be established by copepodids, pre-adults, or adults. The pre-adult is active, scurrying on the fish's skin, where it may cause small petechial hemorrhages at feeding sites. Heavy feeding of sea lice causes deep ulcers, even exposing the cranium (Roth et al. 1993). Death is believed to be due to

Table II-14. Geographic and taxonomic distribution of parasitic copepods in marine fish farming with reference to the genera *Caligus*, *Lepeoptheirus*, *Pseudocaligus*, and *Ergasilus* (reprinted with permission from Roth et al. [1993] with additional data from Ho [2000]).

Country	Species present	Host	Reference
EUROPE			
Norway	*C. elongatus, L. salmonis*	Salmonids	Johannessen 1974; Hastein and Bergsjo 1976; Brandal and Egidius 1977, 1979; Hoy and Horsberg 1991
Sweden	*Caligus sp.*	Salmonids	Lundbjorg and Ljungberg 1977
Scotland	*C. elongatus, L. salmonis*	Salmonids	Rae 1979; Wootten et al. 1982
Ireland	*C. elongatus, L. salmonis*	Salmonids	Tully and Morrissey 1989
France	*C. minimus*	European sea bass	Paperna 1980
Israel	*C. pageti, E. lizae, P. apodus*	Mullet	Paperna 1975
NORTH AMERICA			
Eastern N. America	*C. curtus, C. elongatus, E. labracis, L. salmonis*	Salmonids, red drum	Hogans and Trudeau 1989a, b; Landsberg et al. 1991; Richard 1991
Western N. America	*C. clemensi, L. cuneifer, L. salmonis*	Salmonids	Johnson and Albright 1991a, b; Richard 1991
SOUTH AMERICA			
Chile	*C. teres* *C. rogercresseyi*	Salmonids	Reyes and Bravo 1983 Gonzálex and Carvajal 2003
ASIA			
New Zealand	*C. longicaudatus*	Salmonids	Jones 1988
Malaysia	*Caligus sp., E. borneoensis*	Grouper	Leong and Wong 1988
Philippines	*C. patulus*	Milkfish	Jones 1980
Taiwan	*C. acanthopagri*	Schlegeli black seabream, Malabar rock cod, scat, Mozambique tilapia	Ho 2000
Taiwan	*C. rontundigenitalis*	Schlegeli black seabream, Malabar rock cod, scat	Ho 2000
Taiwan	*C. punctatus* *C. epidemicus*	Schlegeli black seabream, Malabar rock cod, milkfish, Japanese sea bass, barramundi, tilapia, large scale mullet, grey mullet, three-striped tigerfish, snubnose pompano	Ho 2000
Taiwan and China	*C. orientalis*	Schlegeli black seabream, Malabar rock cod, milkfish, large scale mullet, tilapia, grey mullet	Ho 2000
Japan	*L. longiventris*	Spotted halibut	Ho 2000
Japan	*L. paralichthydis*	Olive flounder	Ho 2000
Japan	*C. orientalis, C. spinosus*	Yellowtail, salmonids	Fujita et al. 1968; Izawa 1969; Urawa and Kato 1991

osmoregulatory failure, possibly complicated by anemia or secondary bacterial infection of wounds in some cases (Wootten et al. 1982). *L. salmonis* might also transmit *Aeromonas salmonicida* or infectious salmon anemia virus (Nylund et al. 1993). Sea lice can survive at least 1 week in freshwater if attached to the fish.

Lernaeids (Anchor Worms)

Anchor worm is a general term for species of highly modified copepods that possess anchor-like processes for securing themselves to the host (Fig. II-14, B_5 and F through I). Anchor worms may be introduced into an aquarium from wild or pond-raised fish. Goldfish, koi, or wild native fish are most commonly affected. Marine aquarium fish may rarely be infected with morphologically similar but taxonomically unrelated species; such individuals are usually culled before shipment to wholesalers.

Lernaea is the most important genus of lernaeid copepods, but other genera (e.g., *Opistolernaea*, *Lernaeagiraffa*) are also important in tropical environments (Paperna 1991). *Lernaea* and related genera infect freshwater fish. In temperate climates, *Lernaea* is most likely to be seen in summer, when reproduction usually occurs. For example, *Lernaea cyprinacea*, a cosmopolitan species that infects a wide range of fish and even tadpoles, does not reproduce at less than 14°C (57°F). A single female may produce several hundred larvae about every 2 weeks for up to 16 weeks at optimal temperatures (>25°C = 77°F). After several nonparasitic stages, the terminal copepodid stage (Fig. II-14, J) attaches to a fish, mates, and the male dies. The female then penetrates under the skin of the fish and differentiates into an adult.

Single lernaeid parasites are usually not life-threatening, unless they are infecting a small fish or when they

penetrate near vital organs. Heavy infections can lead to debilitation and secondary bacterial or water mold infection (Noga 1986b). Hemorrhage at the site of attachment is common (Fig. II-14, F), and in some cases, considerable hyperplasia or fibrosis may develop at the attachment site, which may remain even after the parasite has died. Consumers may reject disfigured fish.

Pennellids

Pennellids (family Pennellidae) parasitize a number of feral marine fish and have very rarely caused epidemics in cultured marine fish (Khan et al. 1990). Some cause relatively superficial infestations (i.e., skin) while others penetrate deeply into internal organs, such as the heart or kidney. They can cause loss of condition (especially when infecting vital organs) and reduced reproductive capacity. Like lernaeids, they are highly modified for parasitism (Fig. II-14, K). Some require an intermediate host (another fish species).

Diagnosis

Diagnosis of copepod infestation/infection is based on identification of typical parasitic life stages on fish. Large, mature females are often pathognomonic (Fig. II-14, C through G). Small immature stages, such as copepodids (Fig. II-14, J) or even immature adults (Fig. II-14, H), may not be grossly visible (Noga 1986), so microscopic examination of skin scrapings is advisable. When the skin is scraped for lernaeids, the parasite's head may remain embedded in the fish, leaving only the thin vestigal body. Histopathology may also be used to identify permanently attached forms (Fig. II-14, I). On dead fish, sea lice can sometimes be detected as small bumps by running a hand over the surface of the skin (Lester and Hayward 2006).

While the above methods can be used to identify the major category to which a copepod belongs (e.g., caligid, lernaeid, etc.), identification to species requires expert taxonomic assistance using classical taxonomic keys. For caligids, it is important to determine the species involved in the outbreak because species vary in their pathogenicity, host specificity, life cycle, and possibly drug susceptibility. Gene probes have been developed for some caligids and have shown that some species also may be comprised of more than one distinct population (Costello 2006).

Treatment

ERGASILIDS

Studies are limited but have shown sensitivity to organophosphates. For example, Neguvon® (0.25 mg/L) controlled *Ergasilus labracis* on Atlantic salmon parr (Hogans 1989). There is no evidence of resistance in fish recovering from natural infestations.

SEA LICE

The inevitable increase in the numbers of sea lice over time makes treatment essential for both production and animal welfare. However, no ideal treatment has been devised. A number of drugs have been used with varying success. Organophosphate (trichlorphon or dichlorvos, and recently azimethiphos) baths have been commonly used but carry potential risks to both the handler and the environment. Also, resistance has developed at some geographic sites (Roth et al. 1993; Fallang et al. 2004). Their use has been increasingly supplanted by other drugs including hydrogen peroxide, which is considered more environmentally friendly. However, while hydrogen peroxide can detach up to 100% of mobiles, a high proportion recover. There is also some evidence of resistance on farms using it (Treasurer et al. 2000). Insect growth regulators (diflubenzuron, teflubezuon, and enamectin) and pyrethroids are also used. Currently, the most popular drug is enamectin.

Many other drugs have been examined as sea lice controls, including other organophosphates, formalin, carbaryl, and natural remedies (onions and garlic; Roth et al. 1993). None of them are completely satisfactory, either because of a narrow margin of safety (to fish and/or farmer) or potential damage to marine life. Nonetheless, treatment must be done to keep the parasite burden manageable. This might involve as little as one or two treatments per year in first year fish or as many as eight or more treatments per year in second year fish (Revie et al. 2002a). Effective treatments typically cause an immediate large drop in attached parasites, but the population can rebound very quickly (as soon as 3 weeks for *L. salmonis*), requiring retreatment (Revie et al. 2002a). An increased number of treatments also seem to be associated with lower parasite burden (Revie et al. 2002a). Timing of treatment is critical: if done too early, it is ineffective, but if done too late, significant losses can ensue.

Drugs should not be used alone, but rather should be part of an integrated health management strategy to mitigate losses (Mordue and Pike 2002; Anonymous 2003; Costello 2004). This strategy includes:

- Keeping only one generation of fish on a site (and often within an entire loch, fjord, or bay) at one time. This allows fallowing of sites after the two year production cycle. Fallowing should be for 4–6 weeks.
- Management of fish densities
- Keeping nets clean to allow adequate water circulation
- Rapid removal of dead and moribund fish
- Use of cleaner fish (see below) where possible (and where experience has been good)
- Use of health-promoting diets
- Stress reduction
- Preventing escape of fish from the farm

When drugs must be used:

- Treatment should be coordinated and synchronous (treating all cages on the entire farm within 1–2 days)
- They should be selected for their suitability for that specific louse population, and should be strategically used based upon clinical need.

- The entire site should be treated (and immediately followed by a lice count) and the relevant withdrawal period for that product must be followed.
- If lice are present in winter, fish should be treated then, to remove egg-bearing females before spring.
- Products should be alternated/rotated.
- A drug product should no longer be used if efficacy declines.

Many of these recommendations are applicable to overall health management, not just sea lice control. Environmental factors, including temperature, salinity, proximity to other farms and tidal flow, probably all affect the course of infestations (Tucker et al. 2000; Revie et al. 2002b) and can sometimes be used to advantage. For example, freshwater controls *Caligus elongatus* infestations on red drum (Landsberg et al. 1991). Unfortunately, *L. salmonis* tolerates freshwater for 1 week. Biocontrol using parasite-eating ("cleaner") fish, especially goldsinny and rockcook wrasses, have been used successfully on many farms (Bjordal 1991; Sayer et al 1996), reducing or eliminating the need to use drugs (Lester and Hayward 2006). Other possible controls under study include vaccines and parasite attractants (Lester and Hayward 2006).

ANCHOR WORMS

For lernaeid infestations in aquarium fish, some advocate removing individual parasites with forceps (even if the head remains embedded, the parasite will die). Wounds should be watched closely for secondary infections but usually heal uneventfully within 48 hours, which is faster than if the dead parasite is expelled (G. Lewbart, personal communication). Note that larval stages may still remain on the fish or in the water; thus, fish must still be treated and then placed in uncontaminated water. Treating with potassium permanganate after removal of adults can be curative (Faisal et al. 1988).

Organophosphate is usually effective; prolonged immersion treatment should be repeated every 7 days for 28 days. Copepodids are more sensitive than naplii or adults. Resistant strains have been detected on some commercial farms (Goven et al. 1980). Diflubenzuron is less toxic to fish and is highly effective (Hoffman 1985). It is also not inactivated at high temperatures, as are organophosphates. However, diflubenzuron can be damaging to nontarget arthropods and is not legally approved for this use. Sodium chlorite (20–40 mg/L prolonged immersion) eradicates *L. cyprinacea* in a closed system but frequent water changes must be done for the first 2 weeks to allow the establishment of chlorite-resistant nitrifying bacteria (Dempster et al. 1988). *Lernaea cyprinacea* is also inhibited by salt (Shields and Sperber 1974). Convalescent fish are often more resistant to lernaeid reinfection (Shields and Goode 1978).

OTHER COPEPODS

Most other parasitic copepods are probably susceptible to simlar drugs as those used to treat sea lice and anchor worms. Rainbow trout infested with the lernaeopodid copepod *Salmincola californiensis* were successfully treated with oral ivermectin or by manually removing the parasites from the gills using forceps (Roberts et al. 2004).

PROBLEM 15

Branchiuran Infestation (*Argulus* Infestation, Fish Louse)

Prevalence Index

WF - 3, WM - 4, CF - 3, CM - 3

Method of Diagnosis

Wet mount of skin or buccal cavity with parasite

History

Pruritus; red sores; wild-caught or pond-raised fish

Physical Examination

Focal red lesions on skin; focal color change (especially darkening) on skin

Treatment

1. Organophosphate prolonged immersion
2. Formalin bath
3. Potassium permanganate bath
4. Enamectin oral

COMMENTS

Epidemiology

There are about 150 species of branchiuran crustaceans. The most common and by far most important genus encountered is *Argulus*. Fish lice (do not confuse with sea lice; see PROBLEM 14) are uncommon in freshwater aquarium fish but may occur if wild or pond-raised fish are introduced into the tank. Fish lice are especially common on goldfish and koi and can be prevalent on many wild freshwater fish (e.g., cyprinids, centrarchids, salmonids, among others). Common marine hosts include the mummichog, gulf killifish and sheepshead minnow. Aquacultured marine salmonids have rarely experienced epidemics (Stuart 1990). Many fish lice have a wide host range (e.g., *Argulus foliaceus*, *A. japonicus*, *A. coregoni*). *Argulus japonicus*, originally from the Orient, has been spread worldwide on goldfish.

Pathogenesis

Fish lice feed by inserting a pre-oral sting (stylet) into the host and sucking body fluids with the proboscis-like mouth (Fig. II-15, D). Fish can display violent erratic swimming or other behavioral abnormalities because of the irritation caused by the stylet. Fish are damaged by the repeated piercing of the skin by the stylet, which injects toxic enzymes, causing irritation. Also, hooks and spines are on the appendages, which may cause mechani-

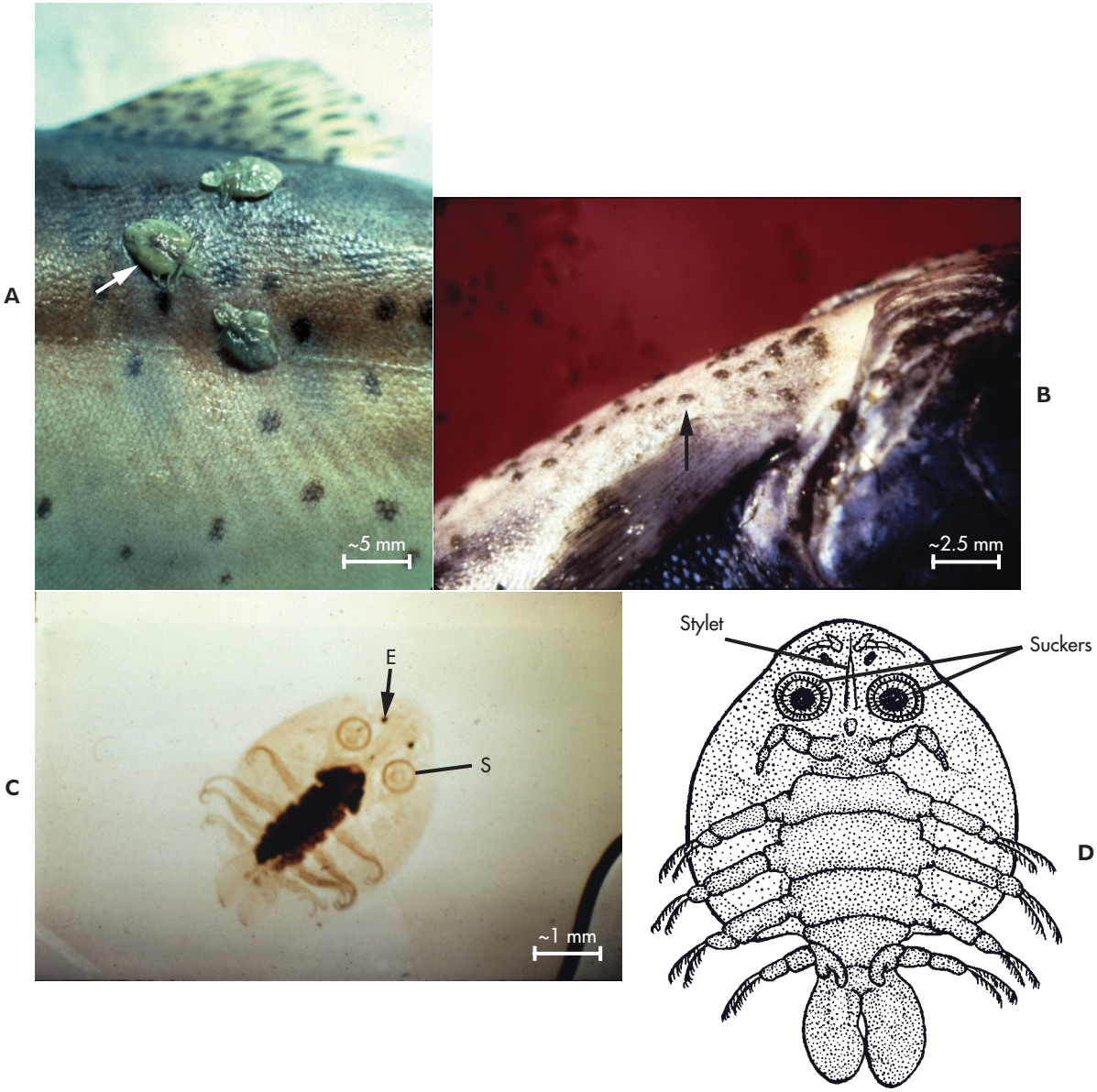

Fig. II-15. A and B. Branchiuran (*Argulus*) infestations (*arrows*). A key identifying feature is the flattened, saucer shape. C. Branchiuran (*Argulus*) infestation wet mount. Key diagnostic features include flattened shape, shell-like carapace covering the body, two suckers (*S*) that look like large eyes, eyespots (*E*), and jointed appendages. D. Diagram of a typical branchiuran (*ventral view*). Key diagnostic features include size (5–20 mm), oval body that looks like a scale, and suckers that look like large eyes. (*A* photograph courtesy of D. Mitchum; *B* photograph courtesy of P. Ghittino.)

cal damage (Kabata 1988). This irritation may cause focal hemorrhage or hyperpigmentation. Fish may be anemic. *Argulus* can also mechanically transmit bacterial or viral pathogens (Pfeil-Putzien 1978; Shimura et al. 1983). Fish lice can be intermediate hosts for several fish-parasitic nematodes, including members of the families Anguillicolidae, Skrjabillanidae, and Dracunculoidea (Lester and Hayward 2006). One or two parasites usually cause no clinical signs in large fish, but fish lice have a high reproductive rate, often resulting in rapid escalation of infestations.

Life Cycle

Speed of the life cycle depends upon parasite species and temperature, with peak abundance typically in summer

and fall. The entire life cycle is typically 30 days or more. Eggs laid on vegetation or other objects (which act as fomites) usually hatch into juveniles within 10–50 days (Paperna 1991). In cold climates, eggs can overwinter. Juveniles (1–3 mm) look like adults without suckers; they must find a host within 2–3 days or will die. Adults can also survive without a host for several days.

Diagnosis

Diagnosis is easily made by morphological identification of the parasite. Branchiurans are differentiated from caligoid copepods (see PROBLEM 14) by having suckers and large, compound eyes (Fig. II-15, C and D). Fish lice frequently move on a host and may be seen swimming when they are in an aquarium. They often remain attached when the host is removed from the water (Fig. II-15, A and B) but can be coaxed to move by gentle nudging with a blunt probe. Fish lice look like a moving fish scale.

Treatment

Individual parasites can be removed from fish by using forceps, but this does not eliminate parasites in the environment and smaller individuals might be missed. As with other crustacean parasites, organophosphates are usually an effective treatment (Paperna and Overstreet 1981). Adult fish lice continue to molt, making them susceptible to chitin synthesis inhibitors, such as diflubenzuron. Oral enamectin is also effective (Hakalahti et al. 2004). The time needed to complete the life cycle varies but typically is about 2 months; therefore, it is useful to rid tanks of egg contamination by using disinfectant or by allowing the tanks to dry thoroughly for several days. Otherwise, multiple chemical treatments may be needed. In ponds, removing all hard objects and adding a hard substrate (planks, etc.) can be used to collect eggs that should then be cleaned of all eggs every week or so. This will probably not eliminate the infestation but will reduce parasite load. Mosquitofish can reportedly be used as a biological control in ponds (Langdon 1992a); freshwater angelfish and sticklebacks also prey on them (Lester and Hayward 2006).

PROBLEM 16
Isopod Infestation

Prevalence Index

WF - 4, WM - 4, CM - 4

Method of Diagnosis

Gross observation of parasite in gill chamber or mouth, or on skin

History/Physical Examination

Isopod grossly visible on body, in mouth, or in gill chamber

Treatment

1. Remove parasite with forceps
2. Organophosphate bath

COMMENTS

Life Cycle

Parasitic isopods (~500 species) are fairly common crustacean infestations of wild tropical marine fish. They are less common in cold marine waters and rarely found on freshwater fish. They are rare in cultured fish, although infestations have caused epidemics in cage-cultured salmonids in Australia and Chile (Langdon 1992a; Thatcher and Blumenfeldt 2001) and other caged fish (e.g., McAndrew 2002), as well as aquarium fish (Marino et al. 2004). The life cycle is simple. Most are parasitic as both juveniles and adults, although some are only parasitic as juveniles (e.g., pranizae of gnathiids).

The are two categories with prominent differences in morphology and ecology (Kabata 1984; Brandt and Poore 2003). The Flabellifera (families Aegidae, Corallanidae, and Cymothoidae) have a typical isopod shape (Fig. II-16, A, B, and C_1) and are up to 6 cm in length. Nearly all fish groups are represented. The great majority of fish parasites are cymothoids.

The less common family Gnathiidae includes ~160 species that have larvae, and male and female adults, which differ in shape and behavior. Only the larva (praniza, Fig. II-16, C_2) is parasitic, living in the gastric cavity of sea anemones and tunicates or on the skin or gills of fish. Adults are nonfeeding, live in mud tubes or sponges, and produce infective larvae.

Pathogenesis

Because of their large size, single isopods can cause considerable damage with their biting and sucking mouthparts. This may include pressure necrosis of gill tissue and growth retardation. However, many have stable host-parasite relationhips and as adults cause relatively little apparent harm. Heavy infestations of parasitic larvae or juveniles can kill small fish when they first attach. Initial attack of even larger fish by the larvae (especially the manca larvae of cymothoid flabelliferans) can be extremely irritating, causing a violent escape reaction in the fish.

Diagnosis

Diagnosis of parasitic isopods is easily made from morphological characteristics. Note that free-living isopods may occasionally be seen in marine aquaria.

Treatment

Cymothoids are susceptible to organophosphates. Individuals can also be removed from fish by using forceps. Placing fish in aquaria (without a refuge, such as mud) breaks the life cycle of gnathiid isopods (Langdon 1992a). Cleaner shrimp and cleaner fish, such as the blue-lined cleaner wrasse, prey on gnathiids (Becker and Grutter 2004).

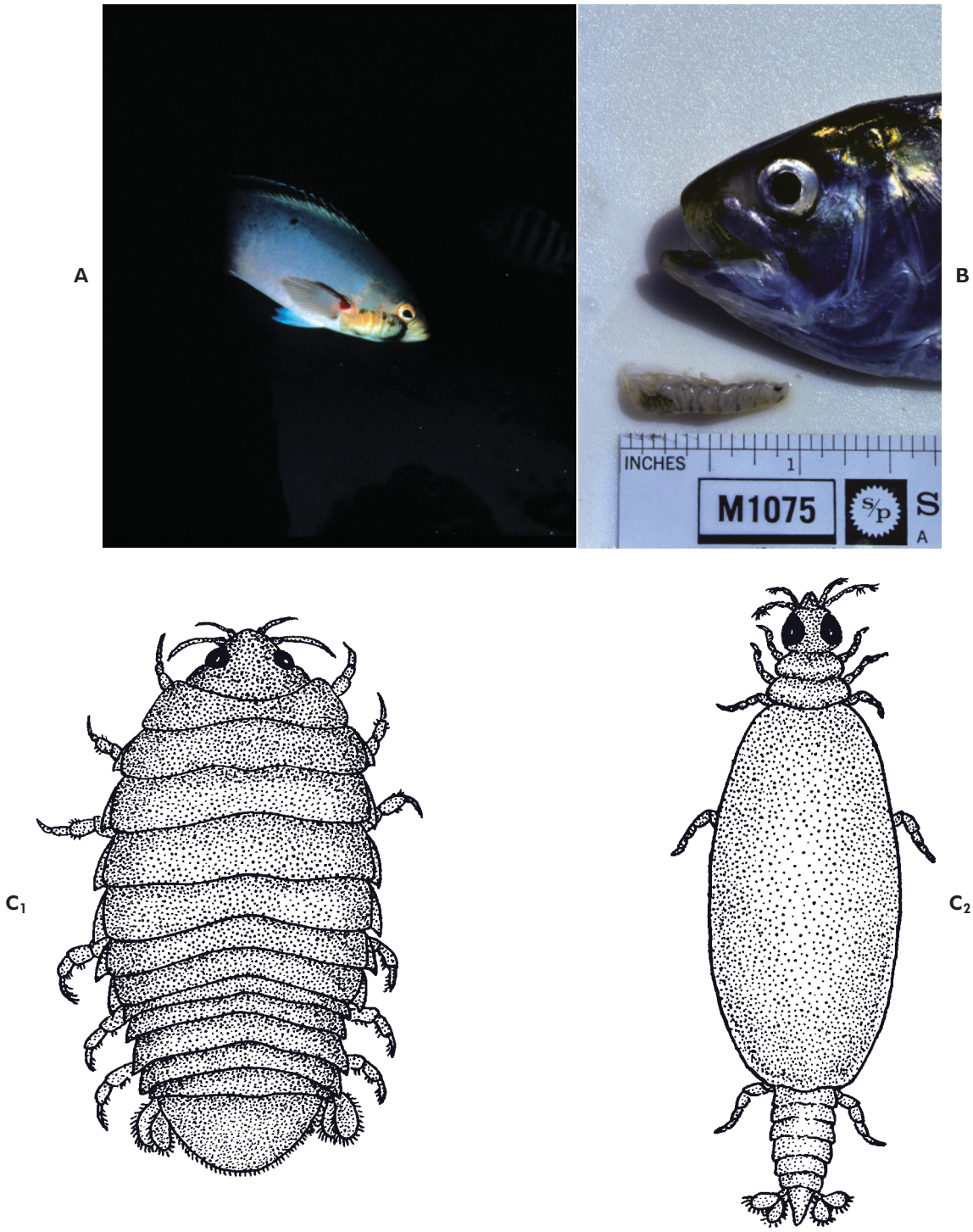

Fig. II-16. A. Flabelliferan isopod attached to the cheek of a marine reef fish. B. Flabelliferan isopod (*Olencira praegustator*) that resided in the oral cavity of an Atlantic menhaden. Head with eyes on the right (Kroger and Guthrie 1972). C. Diagram of fish-parasitic isopods showing the following diagnostic features: (*1*) Flabelliferans—size (several mm to 6 cm), body segmentation, chitinous plates over body segments, and paired, segmented appendages; (*2*) Gnathiid—insect-like body; lack of segmentation because of engorgement on blood. (*A* photograph courtesy of S. Spotte.)

PROBLEM 17
Monogenean Infestation (Skin Fluke, Gill Fluke, Eye Fluke)

Notifiable to OIE
Gyrodactylus salaris only
Prevalence Index
WF - 1, WM - 1, CF - 1, CM - 1
Method of Diagnosis
1. Wet mount of skin or gills with parasite
2. Histology of skin or gills with parasite

History/Physical Examination
Cloudiness to skin; grey-white cast or irregular areas on skin; eroded fins; focal hemorrhages on skin; pruritus; dyspnea

Treatment
1. Formalin bath
2. Formalin prolonged immersion
3. Organophosphate bath (marine capsalids only)
4. Organophosphate prolonged immersion
5. Acetic acid bath (freshwater monogeneans only)
6. Freshwater bath (marine monogeneans only)
7. Saltwater bath (freshwater monogeneans only)
8. Potassium permanganate prolonged immersion (freshwater monogeneans only)
9. Copper prolonged immersion
10. Praziquantel bath (marine monogeneans only)
11. Praziquantel prolonged immersion
12. Mebendazole bath
13. Mebendazole prolonged immersion
14. Fenbendazole bath
15. Chloramine-T bath
16. Hydrogen peroxide bath
17. Praziquantel oral

COMMENTS

Epidemiology
Monogeneans are common parasites of the skin and gills of both marine and freshwater fish (Bychowsky 1957; Hoffman 1967; Rodhe 1984; Buchmann and Bresciani 2006). There are many different species (~3,000), most of which have a narrow host range in nature (i.e., restricted to one species, genus, or family). However, this host specificity is often lost in aquaculture (Nigrelli 1940; Thoney and Hargis 1991).

Heavy monogenean infestations are usually indicators of poor sanitation and deteriorating water quality (e.g., overcrowding, high ammonia or nitrite, organic pollution, or low oxygen). They can rapidly reproduce under such conditions. The doubling time for viviparous monogeneans can be as little as 24 hours. Reproductive rate is also controlled by temperature, which, although not variable in a tropical aquarium (which should have a narrow range of temperature), is important in less controlled environments (e.g., ponds, raceways). Monogeneans often bloom in spring.

Types of Monogeneans
Taxonomic identification of mongeneans is based upon the morphology of the posterior attachment organ (opisthohaptor), mode of reproduction, and presence of eyespots, among other features. There are two types of monogeneans, based upon opisthohaptor morphology: in the much more common Monopisthocotylea (e.g., dactylogyrids, gyrodactylids, capsalids), there is a single unit comprising several, large, centrally located, sclerotized anchors (hooks or hamuli) and often small marginal hooklets (Fig. II-17, A_1, A_2, A_3, C, D, E, F, G, and J); in the Polyopisthocotylea, the opisthohaptor consists of a battery of small, muscular, adhesive suckers or clamps that are supported by cuticular sclerites (Fig. II-17, A_4 and I) (Egusa 1983). The monopisthocotyleans' use of anchors or hooks for attachment tends to pierce tissue, while the polyopisthocotyleans' clamps have opposing sections that grasp host tissue between them.

Pathogenesis
The monopisthocotyleans feed mainly on the superficial layers of the skin and gills. This feeding activity is irritating and thus often causes skin cloudiness (see Fig. I-9, C) or focal reddening resulting from excess mucus production, epithelial hyperplasia, or hemorrhage (Kabata 1985). Even small numbers of parasites can elicit excess mucus production or pruritus. Some species can cause deep skin wounds. Polyopisthocotyleans feed mainly on blood and can cause severe anemia (Dalgaard et al. 2003).

Individual worms cause proportionately greater damage. In large enough numbers, monogeneans can kill, especially small fish, and parasite numbers have been correlated with mortality (Busch et al. 2003). Monogeneans might transmit bacteria or other pathogens (Cusack and Cone 1986; Justine and Bonami 1993), although evidence for this is not strong. There is some evidence for development of partial resistance to reinfection in both monopisthocotyleans and polyopisthocotyleans (Nigrelli, 1937; Evans and Gratzek, 1989; Buchmann 1999).

Exotic Monogeneans
Many monogeneans have been accidentally introduced with infested fish to various parts of the world. For example, *Pseudodactylogyrus anguillae* and *P. bini* were introduced into European eel stocks from Asia (Buchmann et al. 1987), *Gyrodactylus cyprini* was introduced into the United States with European carp, and *Cleidodiscus pricei* was introduced into Europe on U.S. brown bullheads (Thoney and Hargis 1991). Introduction of the capsalid *Nitzschia sturionis* into the Aral Sea decimated the spiny sturgeon population (Buchmann and Bresciani 2006). The introduction of *Gyrodactylus salaris* into native Norwegian Atlantic salmon stocks caused massive

damage to wild populations, presumably because native stocks were much less resistant to these exotic parasites (Johnsen and Jensen 1986).

Reproduction

Two major modes of reproduction are important considerations in medical management of monogeneans. Oviparous monogeneans (Fig. II-17, B) lay eggs that usually settle to the bottom to develop (Kearn 1986). A few capsalids attach egg bundles to gill filaments (Fig. II-17, H; Whittington 1990). After hatching, the free-swimming (rarely unciliated) infective stage (oncomiracidium, usually survives less than 24 hours) seeks out and attaches to a new host and crawls to its final site, where it usually stays for the rest of its life. In contrast, viviparous monogeneans give birth to living young (Fig. II-17, A_1, C, and D).

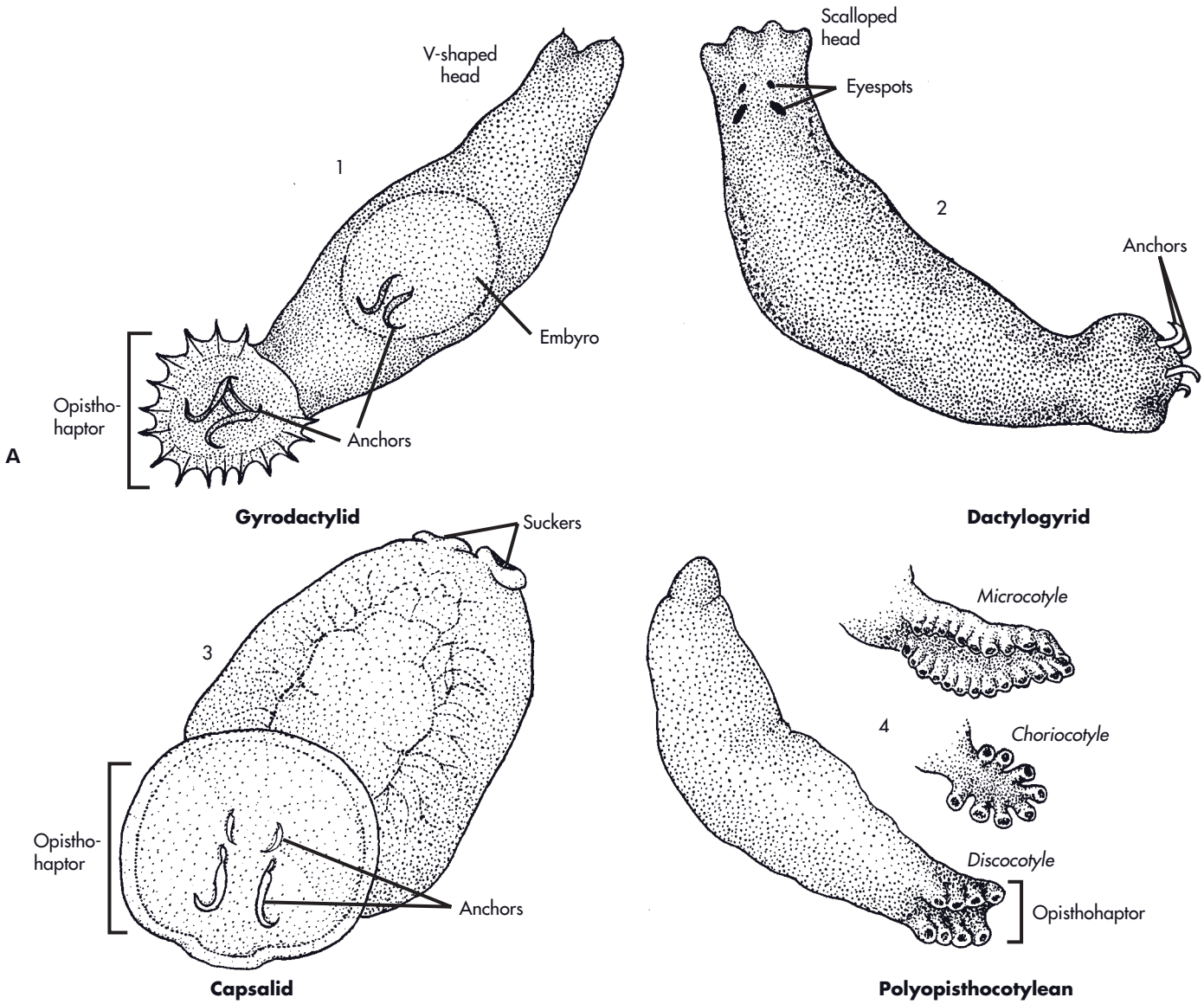

Fig. II-17. Diagrams of major types of monogeneans affecting cultured fish, including key diagnostic features. A_1. Gyrodactylid type. Note size (0.3–1 mm), V-shaped head, lack of eyespots, developing embryo with anchors, single pair of anchors. A_2. Dactylogyrid type. Note size (to 2 mm), scalloped head, one or more pairs of eyespots, ovary without embryo, 1–2 pair of anchors; primarily on gills. A_3. Capsalid type. Note size (often >4 mm), anchors; some also have anterior suckers. A_4. Polyopisthocotylean type. Note clamps and lack of anchors on various opisthohaptors.

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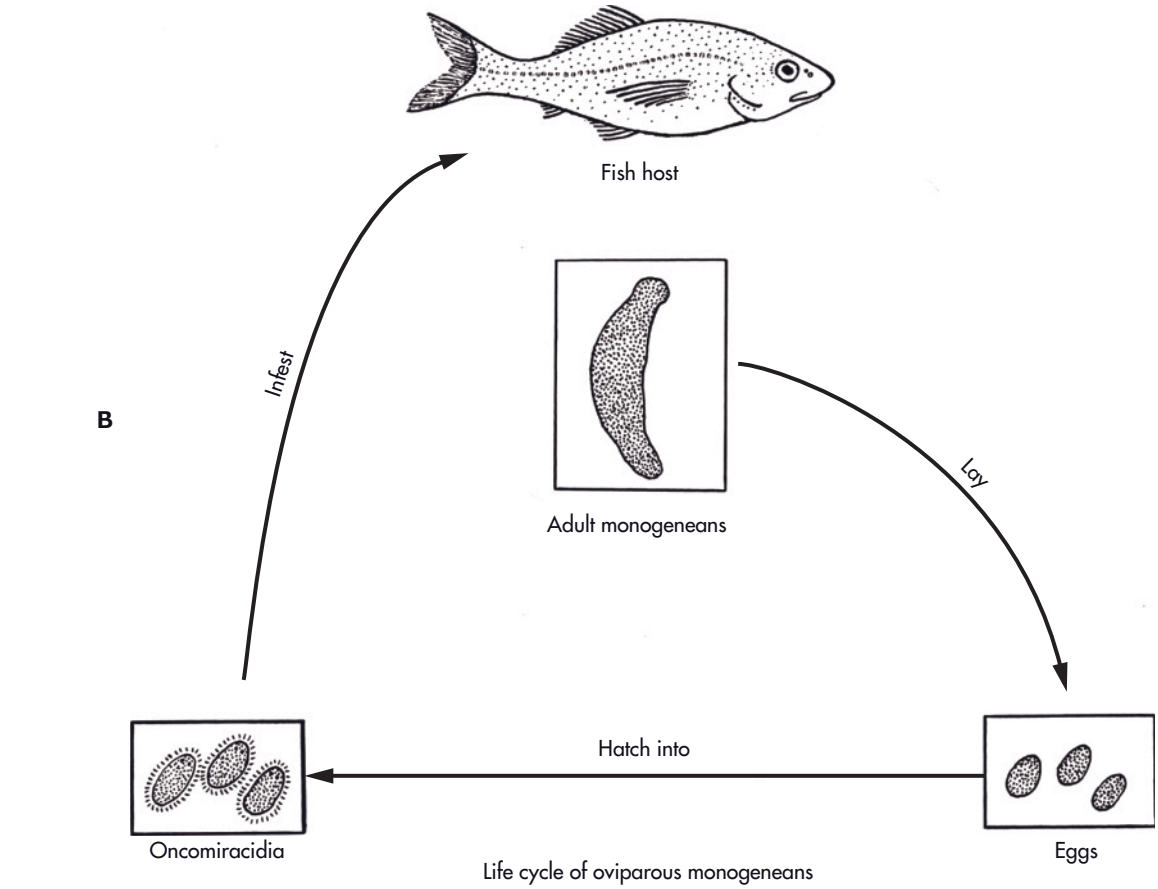

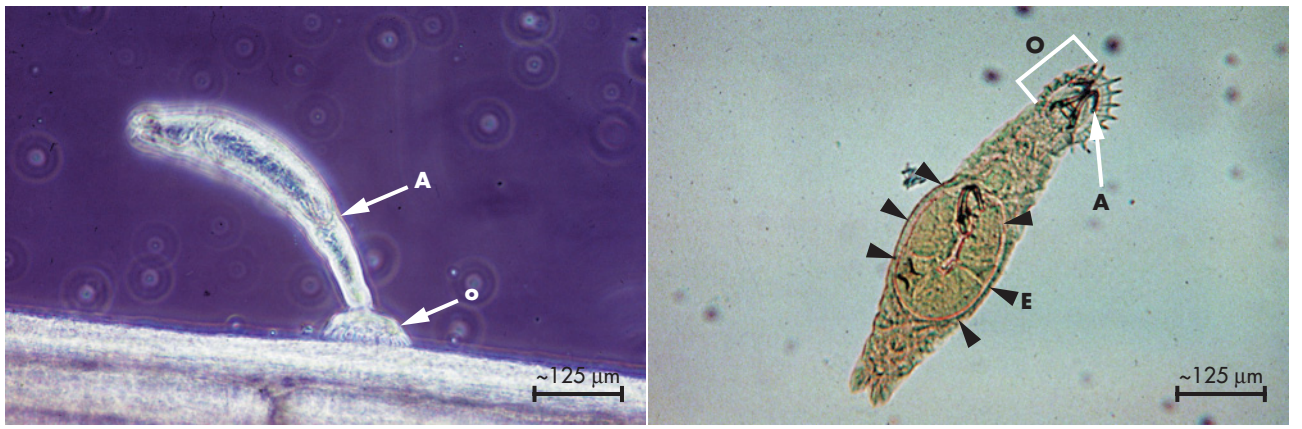

Fig. II-17.—cont'd. B. Life cycle of oviparous monogeneans. C. Wet mount of a gyrodactylid monogenean attached to goldfish fin (*F*). *O* = opisthohaptor; *A* = anchors of embryo's opisthohaptor. D. Wet mount of a typical monopisthocotylean monogene (*Gyrodactylus*). Key identifying features include size, worm-like appearance, and anchors (*A*). Note the embryo (*E, arrows*), which differentiates it from oviparous monopisthocotyleans. *O* = opisthohaptor.

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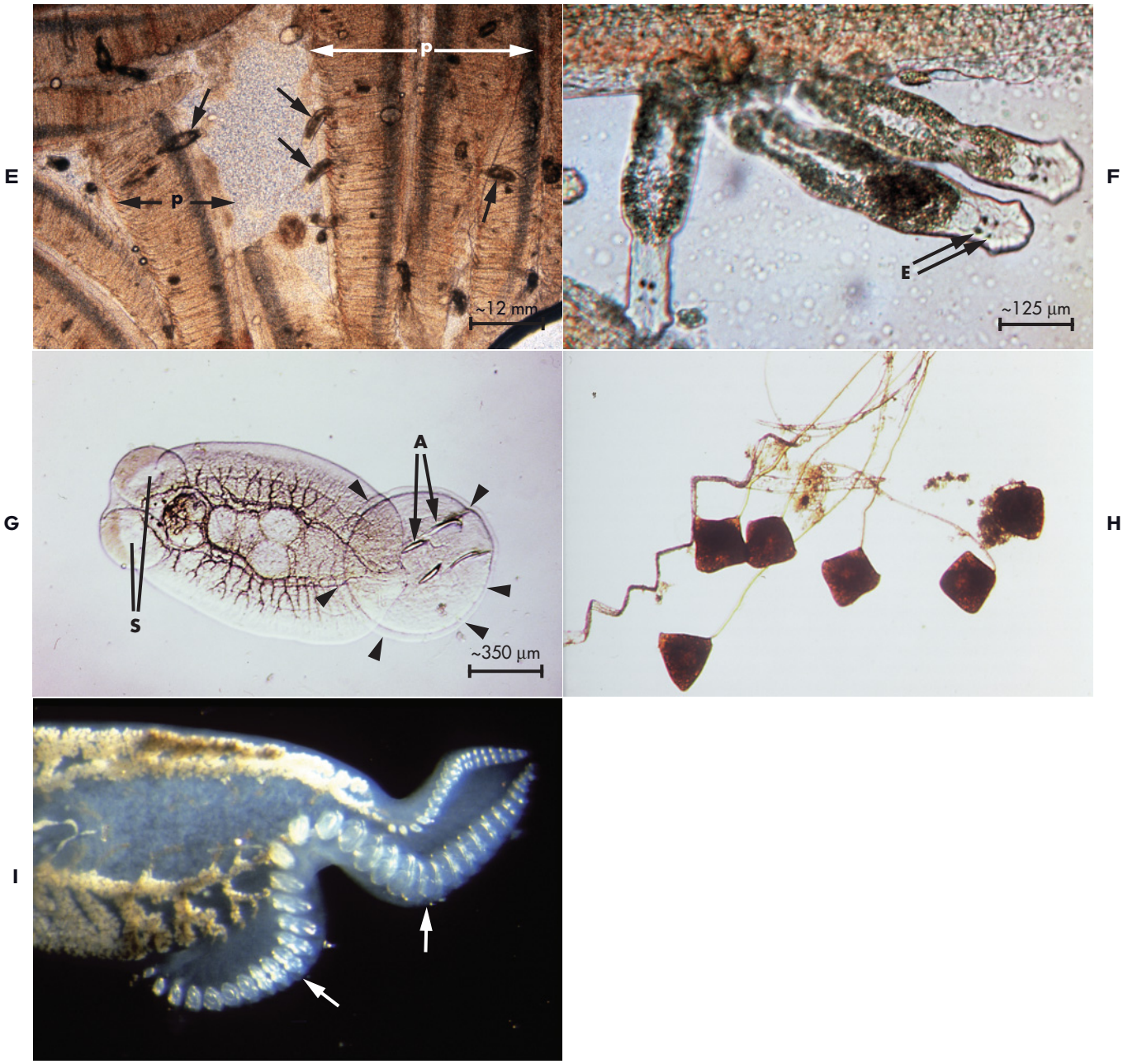

Fig. II-17.—cont'd. E. Wet mount of a heavy dactylogyrid (*Cleidodiscus*) infestation (*arrows*) of channel catfish gills. *P* = primary lamella. F. Wet mount of a typical dactylogyrid monogenean (*Cleidodiscus*) attached to gill. *E* = eyespots. G. Wet mount of capsalid monogenean (*Benedenia*). Note the two pairs of tightly apposed, curved anchors (*A*) in the opisthohaptor (arrowheads), which differentiate it from *Neobenedenia*, which has three pairs of tightly apposed, curved anchors. Both genera have two anterior suckers (*S*). H. Wet mount of *Benedinia* eggs, with threads used for attachment to fish or other objects. I. Wet mount of a typical polyopisthocotylean monogenean (*Allencotyla mcintoshi* on amberjack). Note the row of clamps (*arrows*) on the opisthohaptor.

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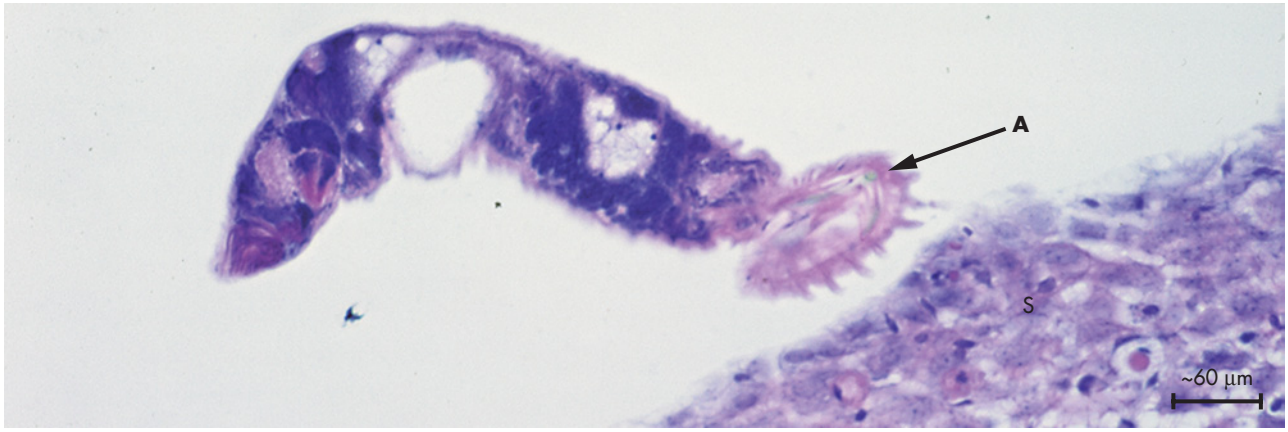

Fig. II-17.—cont'd. J. Histological section through a monopisthocotylean monogenean infesting the skin [*S*] [detachment is an artifact]. Note the refractile anchors [*A*]. Hematoxylin and eosin. [*D* photograph courtesy of G. Hoffman; *F* photograph courtesy of A. Mitchell; *G* and *H* photographs courtesy of A. Colorni; *I* photograph courtesy of R Goldstein.]

Important Pathogens

A summary of some of the most important monogeneans affecting cultured fish is presented by Buchmann and Bresciani (2006).

MONOPISTHOCOTYLEA

Gyrodactyloidea

The most economically important monogeneans in cultured fish are in the monopisthocotylean Superfamilies Gyrodactyloidea and Dactylogyroidea (Wooten 1989). The viviparous gyrodactylids (Fig. II-17, C and D) are skin and gill parasites of both freshwater and marine fish (Yamaguti 1968). Various species of *Gyrodactylus* are pathogenic to eels, salmonids, cyprinids, ictalurids, clariids, fundulids, poeciliids, gasterosteids, cyclopterids, cichlids, and pleuronectids (Thoney and Hargis 1991).

Dactylogyroidea

The oviparous dactylodyrids are primarily gill parasites of freshwater fish (Yamaguti 1968). There are many species in various genera, especially *Dactylogyrus*, *Pseudodactylogyrus*, and *Cleidodiscus* (Fig. II-17, E and F).

Capsaloidea

Some capsalids (Fig. II-17, G) can be important pathogens of marine fish. They are large, monopisthocotylean worms (up to ~3–10 mm) that concomitantly can cause large wounds on the skin or eyes (erosion, ulceration). Feeding activity of the capsalids can induce hyperplasia, which may partly enclose the flukes. Skin infestations induce flashing and resultant long scratches on lightly scaled fish (e.g., pompano, kingfish, dolphin). Capsalids can produce large numbers of eggs (over 80/day in some species; Thoney 1990).

Neobenedenia melleni causes serious skin damage and has a predilection for the eye (i.e., eye fluke; don't confuse with digenean eye flukes; see PROBLEM 58); its large hooks cause ophthalmic lesions leading to blindness (Nigrelli 1940). It infests numerous species, including various tropical reef fish, including members of the Acanthuridae, Ariidae, Balistidae, Diodontidae, Carangidae, Chaetodontidae, Holocentridae, Labridae, Lutjanidae, Malacanthidae, Ostraciidae, Pomadasyidae, Percichthyidae, Pomatomidae, Psettidae, Scatophagidae, Sciaenidae, Serranidae, Sparidae, and Triglidae (Thoney and Hargis 1991).

Other pathogens in cultured fish include *Entobdella solea* on dover sole (Anderson and Conroy 1968), *Benedenia seriolae* on yellowtail (Egusa 1983), *Benedenia* sp. on pompano (Lawler 1977b), and *Dermophthirius* or *Dermophthioides* spp. on various elasmobranchs (Thoney and Hargis 1991).

POLYOPISTHOCOTYLEA

Relatively few polyopisthocotyleans are problems in cultured fish; most that affect cultured fish have a wide host range. They feed on blood and do not browse like monopisthocotyleans. Thus, they usually elicit a less severe host response compared to monopisthocotyleans (Thoney and Hargis 1991). Reported pathogens in cultured fish include *Discocotyle* in European salmonids (Wooten 1989; Rubio-Godoy and Tinsley 2004), *Allobivagina* on *Siganus* spp. in the Mediterranean Sea (Paperna et al. 1984), *Heteraxine* on yellowtail in Japan (Egusa 1983), and *Polylabroides* on *Acanthopagrus* in Australia (Diggles et al. 1993). *Microcotyle* is common on the gills of tropical butterflyfish and angelfish (Blasiola 1992). Heavy infestations of some polyopisthocotyleans may cause few clinical signs, but sublethal effects are not well studied.

Diagnosis

Worms are easily identified as monogeneans by using wet mounts of skin or gills. They often have a characteristic jerking or caterpillar-like motion, in which the parasite will repetitively stretch and recoil. Other key features

include the presence of hooks (most commonly), suckers, or clamps. In viviparous species, a single embryo is easily seen developing within the adult worm (Fig. II-17, C and D). Capsalids are large but transparent flukes. Fixation in formalin or alcohol causes them to turn white, rendering them more visible (Fig. III-7). The great majority of monogeneans do not exceed 4 mm, although species from large marine fish (e.g., sailfish) may be up to 3 cm (Möller and Anders 1986). Monogenean infestation can also be diagnosed via histopathology (Bruno et al. 2006; Fig. II-17, J).

Identification to species or even genus is not essential for successful treatment but can be useful, since species vary considerably in pathogenicity (Cone and Odense 1984) and in response to treatment. Live or preserved samples (Table I-5) are best sent to a reference laboratory for specific taxonomic identification. Nucleic acid probes have been designed to definitively identify some species (Hansen et al. 2003) but are not yet used for routine diagnoses.

Monogeneans may be present in low numbers without causing disease; for example, the presence of a single parasite in a skin scraping or gill clip of a 10 cm long fish would not be compatible with a history of mortalities in a fish population, and thus, other causes should be sought under those circumstances.

Treatment

GENERAL CONSIDERATIONS

Several therapies have been successfully used to control monogenean infestations, but it is important to realize that monogenean species and even populations differ in their sensitivity to treatments, so the clinician must often try different therapies to determine which works best in a particular situation. For example, toltrazuril has shown experimental effectiveness against pseudogyrodactylosis in some cases (Schmahl et al. 1988) but not others (Buchmann et al. 1990). Formalin is effective for many monogeneans, but adult and juvenile polyopisthocotyleans were only removed by high-dose formalin treatment (400 ppm for 25 minutes; Langdon 1992a), a dose not tolerated by many fish.

Gill monogeneans are often more resistant to treatment than skin parasites, possibly because the gill provides protection from drug exposure (Thoney and Hargis 1991). Another important consideration in designing an effective treatment is whether the monogenean is viviparous or oviparous because the eggs of some monogeneans are resistant to treatment and thus several drug applications may be required for control. In capsalids, resistant eggs may take from 4 to 21 days to hatch, mainly depending upon parasite species and temperature; thus, control requires applying repeated treatments (usually at least three treatments) at appropriate intervals.

Freshwater or saltwater baths usually work best on small monogenean species. Some large species (e.g., certain large capsalids) may require follow-up treatment 48 hours later with formalin or organophosphate treatment (Langdon 1992a). However, some larger monogeneans are quite susceptible to freshwater baths (A. Colorni, personal communication); this must be determined for each species/strain. Monogeneans on estuarine species are also usually more resistant to freshwater or saltwater baths. However, if feasible, long-term exposure to suboptimal salinity may be highly effective. For example, salinities less than 20 ppt significantly reduce the egg viability of *Neobenedenia melleni* (Mueller et al. 1992) or *Polylabroides multispinosus* (Diggles et al. 1993). Only long (1 hour) freshwater baths cured fish of *P. multispinosus* (Diggles et al. 1993). This is much longer than normally recommended for marine fish. Marine monogeneans can vary greatly in their tolerance of low salinity. *Neobenedinia girellae* reproduction is severely inhibited at 17 ppt or less, while egg hatching rates of *Heterobothrium okamotoi* and *Neoeterobothrium hirame* are not affected at 11 ppt (Umeda and Hirazawa 2004).

Organophosphate is one of the most useful treatments for monogeneans. However, resistance to organophosphates can develop; it has been most commonly seen in farms that regularly use this agent (Goven et al. 1980). Copper has been used with some success. While copper is thought to affect the oncomiracidia more than the adults, this is not always true (Thoney 1990).

Mebendazole and praziquantel are also effective against several marine or freshwater monogeneans (Székely and Molnár 1987; Thoney and Hargis 1991; Buchmann and Bresciani 2006). Hydrogen peroxide delivered as sodium percarbonate has also been successful (Buchmann and Kristensson 2003). Aluminum has been used to control *Gyrodactylus* populations in both wild and cultured salmonids (Larsen and Buchmann 2003). Interestingly a bath of 80 mg/l benzocaine has successfully treated some monogenean infestations (Svendsen and Haug 1991).

Biological control may be feasible, especially in tropical marine aquaria, since cleaner fish, such as French angelfish, neon gobies, and blue-lined cleaner wrasse, pick monogeneans off other fish (Moe 1992a; Grutter et al. 2002).

ENVIRONMENTAL CONSIDERATIONS

Chemical treatments often only control and do not eradicate monogeneans, emphasizing the need for environmental management. Monogeneans cannot survive for more than 2 weeks off a host (unless present as overwintering eggs); many will die much more quickly (some within minutes). Because of their reproductive cycle, the offspring of viviparous monogeneans remain on the same host; thus, transmission can occur via fish-to-fish contact. Reducing crowding may be more significant in reducing such transmission than in oviparous monogeneans, where

a free-swimming larva may attach to any host. Cage placement is important since some monogenean larvae can be spread by water currents (Chambers and Ernst 2003).

Filtering out eggs and oncomiracidia using 80 µm pore size nylon mesh has controlled pseudodactylogyrosis in closed systems (Buchmann and Bresciani 2006). Eggs having long filaments (Fig. II-17, H) can be trapped by placing ropes and nets into net-pens and regularly removing these traps. However, this is very labor-intensive (requires removal every few days) (Ogawa 2002).

All efforts should be made to prevent the introduction of exotic mongeneans into new areas.

PROBLEM 18
Turbellarian Infection (Tang Turbellarian, Black Ich)

Prevalence Index
WM - 3
Method of Diagnosis
1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination
Black (rarely white) skin lesions up to ~1 mm; white lesions may interconnect into larger foci
Treatment
1. Freshwater bath
2. Formalin bath
3. Organophosphate prolonged immersion

COMMENTS
Epidemiology/Pathogenesis
Turbellarians are a phylum of mainly free-living worms related to trematodes. *Ichthyophaga*, *Paravortex*, and several other genera have been reported from free-ranging marine fish (Cannon and Lester 1988).

Most of what is known about these parasites is based on studies of the tang turbellarian (tentatively identified as a *Paravortex* sp.; Kent and Olson 1986) (Fig. II-18, A through C). The tang turbellarian has been most commonly observed on yellow tangs but also infects at least 16 tropical marine species in 5 families, including butterflyfish, angelfish, gobies, opisthognathids, and other tangs. Less well-described turbellarian infections on other marine species have also been suspected to be caused by this organism. The tang turbellarian induces a hypermelanization reaction, resulting in dark foci on the skin, which are best seen on light-colored fish; there may be acute, focal dermatitis and hemorrhage. Parasites less commonly infect gill epithelium.

The life cycle is direct and is analogous in many ways to marine white spot disease (see PROBLEM 21), with a proliferative stage off the host. After feeding for about 6 days on the fish (growing from 77 µm to 450 µm), the parasite leaves the host and falls to the sediment, where it continues to increase in size (to ~750 µm) over the next 3–4 days. During this time, progeny form and the young are brooded internally. An adult can produce as many as 160 juveniles at once. The adult's body wall ruptures, releasing juveniles that can immediately infect a host. The life cycle takes ~10 days at 24.5°C (76°F; Kent and Olson 1986).

Because of the high reproductive rate, fish can harbor up to 4500 parasites in as little as 20 days. Death can ensue in 10–23 days. Infestations can also spread from fish to fish when worms in the parasitic phase change hosts. Fish-to-fish transmission takes less than 24 hours.

Another turbellarian, tentatively identified as an *Ichthyophaga* sp., has caused epidemics in lookdowns and other cultured carangids in North Carolina (Noga et al. 1999). This organism induces a proliferative epithelial response (Fig. II-18, D and E).
Diagnosis
Lesions produced by the tang turbellarian look grossly similar to those caused by digenean metacercariae (see PROBLEM 58) and lesions caused by the lookdown turbellarian look grossly similar to cryptocaryonosis (see PROBLEM 21). Both are easily identified using wet mounts or histopathology. Note that the lookdown turbellarian is easily crushed when covered with a coverslip (E. Noga, unpublished data). Thus, wet mounts should be examined without coverslips when suspect skin lesions are seen but no parasites are detected. Brooding adults of the tang turbellarian can also be detected in detritus samples from the tank bottom. Do not mistake the parasite for free-living turbellarians, which are common, nonpathogenic pests in freshwater and marine aquaria.
Treatment
Formalin or organophosphate controls the tang turbellarian. Two to three treatments are usually advisable (Kent and Olson 1986). The lookdown turbellarian is resistant to both copper and formalin but responds well to a freshwater bath (J. Camper, personal communication).

PROBLEM 19
Protozoan Ectoparasites: General Features

Prevalence Index
WF - 1, CF - 1, WM - 1, CM - 1
Method of Diagnosis
1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination
All caused by feeding activity of the parasite; typical signs include pruritus ("flashing"), dyspnea, "cloudy" skin, secondary microbial infections
Treatment
Usually antiseptic-type treatment

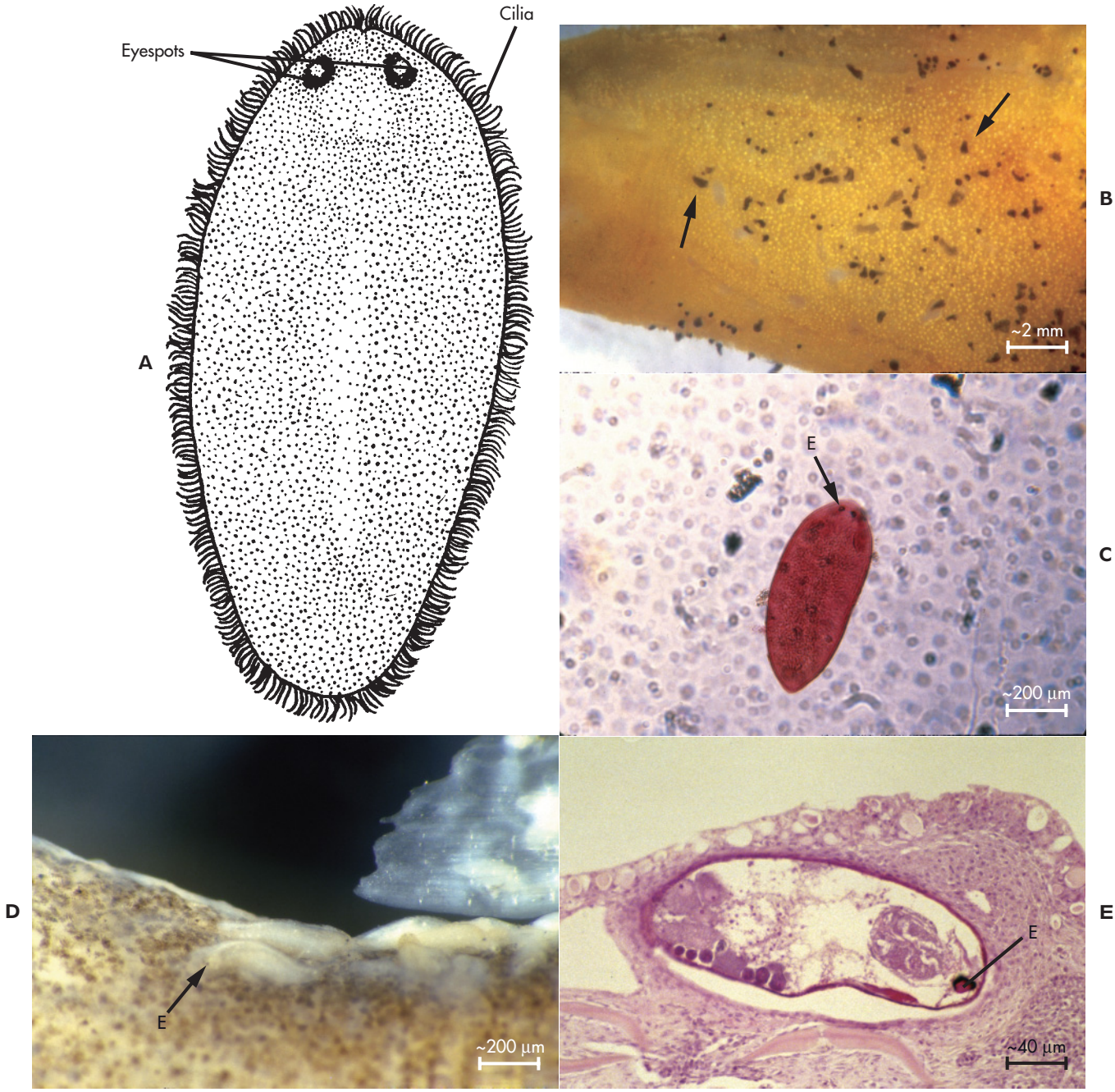

Fig. II-18. A. Diagram of turbellarian with diagnostic features: cilia, eyespots, ovoid shape. B. Yellow tang with black foci on the skin surface (*arrows*), each of which corresponds to a single turbellarian. C. Wet mount of a skin biopsy showing *Paravortex* sp. from a yellow tang. Stained specimen. *E* = eyespots. D. Close-up view of a lookdown with turbellarian infection. Note the epithelial hyperplasia covering the parasite. *E* = eyespot. E. Histological section through skin of a lookdown showing turbellarian. Key diagnostic features include complete ciliation, two eyespots, size ~100–500 µm, and ovoid shape. (*A* from Cannon and Lester 1988; *B* and *C* photographs courtesy of G. Blasiola.)

COMMENTS

Protozoan ectoparasites are the most common parasites encountered in cultured fish (MacMillan 1991); they are also frequently found on wild fish. The term "protozoa" does not define a distinct taxonomic group but rather is a general term for a diverse array of single-celled orgnisms tht actually belong to one of several distinct taxa. The protozoan ectoparasites are mainly ciliates and flagellates that feed on the most superficial skin layer (i.e., epithelium). Most feed only on the epithelium's surface, but a few (e.g., *Ichthyophthirius*, *Cryptocaryon*) penetrate into the epithelium.

Clinical signs are due to damage caused by parasite feeding activity. Parasites are irritating, which can cause behavioral signs such as hyperactivity (tremors, sudden darting movements, etc.). This irritation often causes a reactive hyperplasia of the epithelium and/or increased mucus production. When the hyperplasia is severe, this response appears as cloudiness of the skin or eyes (see Figs. I-36 and I-9, C). The same response can occur on the gills and leads to hypoxia. Note that not all ectoparasites cause all these clinical signs and their severity will depend upon the intensity and chronicity of the infestation/infection.

All protozoan ectoparasites have a direct life cycle, which is faster at higher temperatures. Generation time of some species may be as little as 24 hours under optimal conditions. Thus, these parasites can quickly overwhelm a host population. All are easily diagnosed via wet mount of live material or from histopathology.

Effective treatment of protozoan ectoparasites depends on an understanding of the two major types of life styles: nonencysting and encysting. Nonencysting protozoans (e.g., *Trichodina*, *Ichthyobodo*) complete their life cycle on the host and are easily treated, usually with a single, short-term drug application. Encysting protozoans (*Ichthyophthirius*, *Cryptocaryon*, *Amyloodinium*, *Piscinoodinium*) produce a reproductive cyst off the host. Both the fish-feeding stage and the reproductive cyst are resistant to treatment, so therapy must be directed at the free-swimming, infective stage (see Fig. II-20, A). This requires that chemicals be present for a long time or that several treatments be applied to ensure that all infective stages are killed.

PROBLEM 20

Ich Infection (Freshwater White Spot Disease, *Ichthyophthirius multifiliis* Infection, Ichthyophthiriosis)

Prevalence Index

WF - 1, CF - 2

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also white nodules up to 1 mm on skin or gills that may interconnect into larger foci

Treatment

1. Formalin prolonged immersion
2. Formalin/malachite green prolonged immersion
3. Salt prolonged immersion
4. Copper prolonged immersion (ponds only)
5. Raise temperature to >30°C (86°F) for 10 days
6. Formalin bath weekly until cured
7. Transfer fish to new aquarium daily for 7 days at 25°C (77°F)

COMMENTS

Life Cycle

Ich is one of the most common diseases of freshwater fish (Matthews 2005). Virtually all freshwater fish are susceptible to infection, although scaleless fish, such as catfish and loaches, are especially vulnerable. Up to 100% mortality may occur (Meyer 1974; Dickerson 2006).

The ich trophozoite (feeding stage) feeds in a nodule (i.e., small cavity made and occupied by the ich cell) formed in the skin or gill epithelium (Ewing and Kocan 1986; Fig. II-20, A). After it feeds within the skin or gills, ich breaks through the epithelium, falls off the host, and forms an encapsulated dividing stage (tomont). The tomont secretes a capsule, which is sticky and adheres to plants, ornaments, nets, or other objects. It divides up to 10 times by binary fission, producing tomites that break through the nodule wall to form motile, infective, ~20 × 50 µm theronts (Fig. II-20, A). A single trophont may produce over 1000 theronts. Thus, ich can overwhelm a population quickly.

Epidemiology

Ich is typically a warm water disease and a common temperature for ich outbreaks is 15–25°C (59–77°F). Parasites complete their life cycle in 3–6 days at 25°C (77°F), 10 days at 15°C (59°F), and a month or more at 10°C (50°F), when the disease is typically less serious (Meyer 1974). However, outbreaks often develop at low temperatures (<10°C [<50°F]) in spring, when fish are stressed from overwintering (J. Lom, personal communication). Many epidemics in salmonids are during summer, when fish are heat-stressed (>17–19°C [>63–66°F]), but it can also infect salmonids or other cold water fish at as low as 4°C (39°F). Along with temperature, stress plays a major role in epidemics. Outbreaks are also more severe at high fish density. Epidemics occasionally occur in feral fish.

Considerable acquired immunity is present in fish that recover from infection (Dickerson et al. 1986; Dickerson 2006).

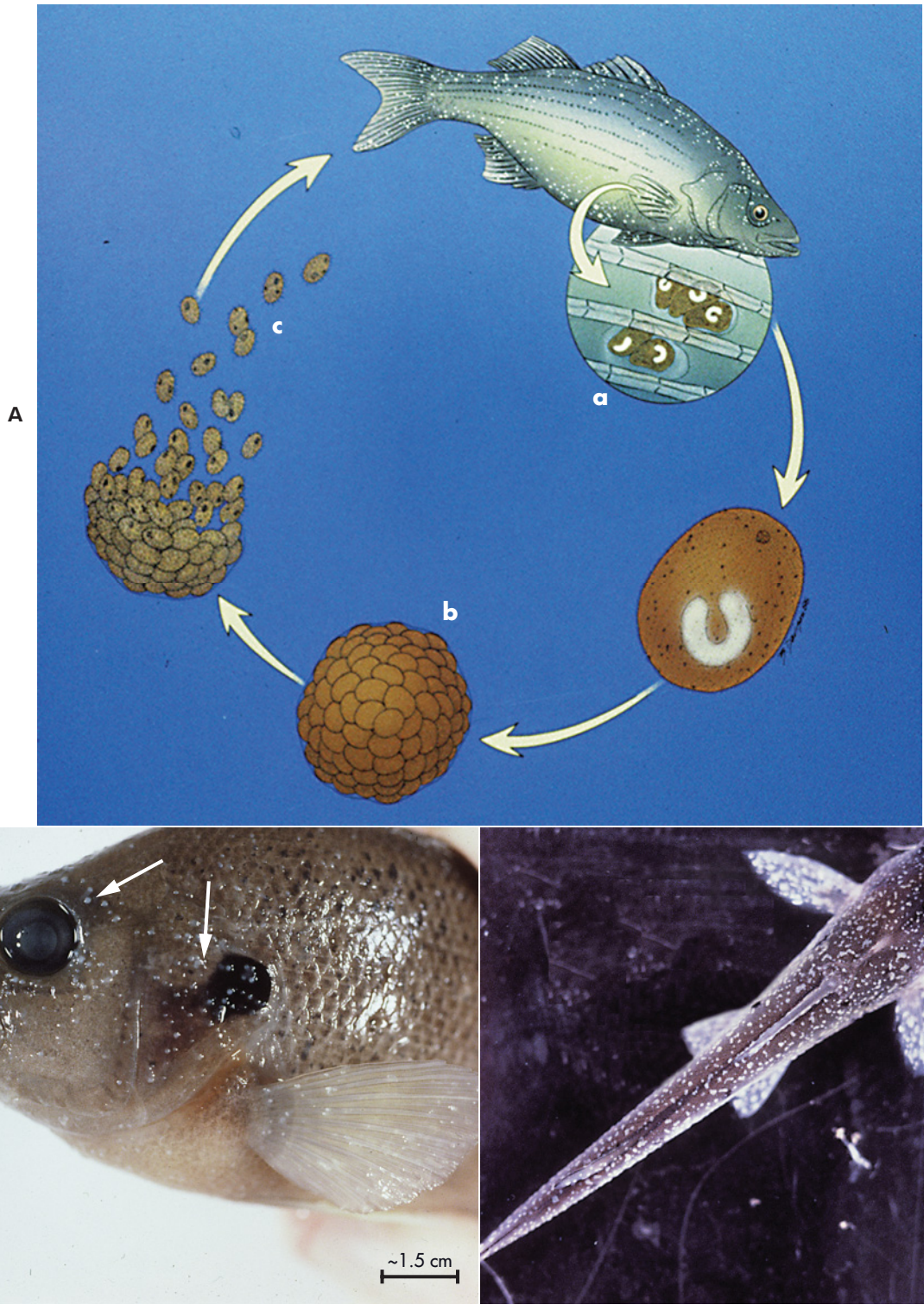

Fig. II-20. A. *Ichthyophthirius multifiliis* life cycle. *a* = trophonts; *b* = dividing tomont; *c* = tomites/theronts. B. Close-up view of a bluegill with ich. Note that the parasite nodules (*arrows*) protrude slightly above the skin surface. C. Channel catfish with a heavy ich infection.

Continued.

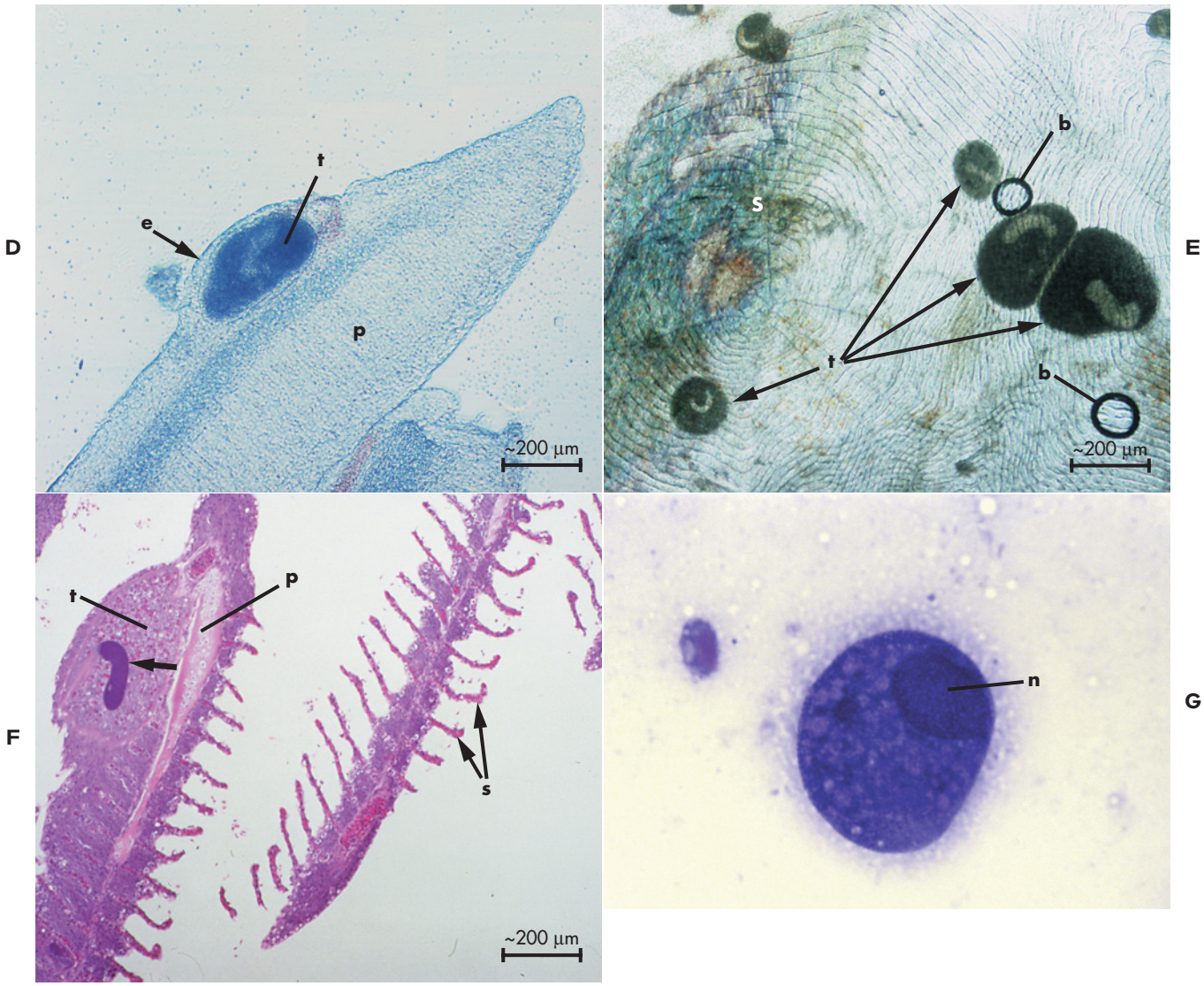

Fig. II-20.—cont'd. D. Wet mount of a gill biopsy showing *I. multifiliis* trophont (*t*) encysted within the epithelium (*e*) of the primary lamella (*p*). E. Wet mount of a skin scraping showing *I. multifiliis* trophonts (*t*). Key features include the size variation of the pleomorphic parasites and the C-shaped macronucleus. *s* = fish scale; *b* = air bubble. F. Histological section through trophont (*t*). Note the macronucleus (*arrow*): the C shape is not apparent in every section through a parasite. *p* = primary gill lamella; *s* = secondary gill lamellae. Multiple trophonts at the same site might be due to multiplication while in the fish (Ewing et al. 1988). G. Stained smear of an *I. multifiliis* trophont. Note that the nucleus (*n*) is not C-shaped in this immature individual. In larger individuals, the nucleus is usually not visible on a stained smear. Modified Wright's. (*A* figure by B. Davison-DeGraves and E. Noga; *C* photograph by R. Bullis and E. Noga; *F* photograph courtesy of L. Khoo.)

Pathogenesis

Ich trophonts appear grossly as small white nodules that produce a salt-like dusting (Fig. II-20, B). The nodules protrude slightly from the surface (Fig. II-20, B), being from ~0.10 to 1.0 mm in size. In advanced, heavy infections, individual vesicles may appear to coalesce, forming mucoid masses on the skin (Fig. II-20, C). Fish having such heavy infections are not likely to survive, even if they are treated.

The epithelial erosion and ulceration that result from the parasite's entrance into and exit from the host are probably at least as damaging as its feeding activity while it is on the host. Lesions produced by the parasites may also lead to secondary microbial infections.

Diagnosis

The presence of a ciliate encysted within the host's epithelium (Fig. II-20, D) is pathognomonic. The cilia move constantly while the trophont is within the cyst. The cilia of *Ichthyophthirius* are evenly distributed over the entire body surface (referred to as "holotrich" in older classification schemes). Other diagnostic features of the trophont include a C-shaped macronucleus (may not be easily visible on small trophonts; Figs. II-20, E and F), large size variation of trophonts (Fig. II-20, E), and pleomorphic shape.

Treatment

AQUARIA

Detection of even a single ich trophont warrants immediate treatment. Fish with extensive lesions (see Fig. II-20, B and C) have a guarded prognosis. The theront stage is most susceptible to therapy and thus drugs must remain at therapeutic levels for a sufficient time to ensure that all parasites have passed through this stage. At optimum temperatures (24–26°C), treatment must be maintained for 1 week, since the life cycle is completed in 3–7 days (Parker 1965). Watch closely for recurrence and extend treatment if parasites are still seen. At lower temperatures, the cycle takes longer. Thus, if a client has goldfish in an unheated system at 7°C, it may take 6 weeks or longer for all parasites to form theronts. Instead of treating for a longer time, it is best, if possible, to temporarily raise the temperature of the system and treat accordingly. This may not be possible in large culture systems. Ich cannot complete its life cycle at >30°C (86°F; Parker 1965), but many fish cannot tolerate such high temperatures (see PROBLEM 2).

Formalin prolonged immersion is usually effective; three treatments on alternate days (when at the typical tropical aquarium temperature) are recommended to ensure that all emerging theronts are killed. However, some cases are resistant to formalin but susceptible to copper (L. Khoo, personal communication). In advanced cases, formalin/malachite green may be preferable, because these two agents are synergistic (Gilbert et al. 1979). Oral malachite green also appears to be very effecive (Schmahl et al. 1992). A toltrazuril bath (10 µg/ml for 4 hours every day for 3 days) is reported to kill trophonts in the tissue (Melhorn et al. 1988) and is the only water-borne drug shown to be able to treat the trophont; theronts were not affected.

Early studies suggested that *Ichthyophthirius multifiliis* could not tolerate over 1 ppt salinity (Allen and Avault 1970) and thus salt can be used both as a cure and as a preventative. It is especially useful for euryhaline fish such as poeciliids, which are prone to developing ich when kept in freshwater; most fish tolerate low salt levels (see "**Pharmacopoeia**"). Salt also helps to alleviate the osmotic stress caused by the epithelial damage. However, some ich isolates can propagate for at least a limited amount of time in 1 ppt or even higher salinity (E. Noga, unpublished data). Thus, in some cases, fish might need to be treated with as high as 5 ppt salt, which is beyond the tolerance of some species.

Theronts are killed by ultraviolet light (91,900 µW/cm). However, this has not been effective in controlling an infection within an aquarium but rather preventing spread to other aquaria (Gratzek et al. 1983). Transferring fish to a new aquarium daily for 7 days will cure fish by preventing reinfection. This treatment is stressful to the fish and cumbersome. Daily vacuuming of the bottom of the aquarium has also been reported to control the infection by removing developing cysts (Brown and Gratzek 1980). At 25°C (77°F), theronts only remain infective for 30 hours after excystment, but delayed emergence of some theronts requires that aquaria be left without fish for 7 days to be rid of the infection. All stages are also killed by drying.

PONDS

For pond fish, the treatment of choice for ich is copper sulfate. Its prolonged life cycle with lower temperature necessitates increasingly longer treatment intervals. At 26°C (80°F) or higher, treatment must be applied every day at least thrice. At 20–25°C (68–77°F), treatment must be applied every other day at least thrice. At 15°C (59°F), this should be extended to every 3–4 days, while at 4°C (39°F), treatment must be applied every 7 days or longer. At cooler temperatures, ich causes more chronic outbreaks.

During outbreaks, quarantine of infected fish and disinfection of equipment (e.g., nets, aerators) are essential. Adjacent ponds should be closely monitored, since birds feeding on diseased fish or carcasses can spread the infection.

FLOW-THROUGH SYSTEMS

Fish in flow-through systems require repeated bath treatments, until no parasites are detected (usually at least three treatments). Increasing the flow in a raceway has also been reported to cure fish by sweeping away infective theronts (Brown and Gratzek 1980).

PROBLEM 21

Marine White Spot Disease (Cryptocaryonosis, Marine Ich, *Cryptocaryon irritans* Infection)

Prevalence Index

WM - 1

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, white foci up to ~0.5 mm on skin that may interconnect into larger masses; white "tags" on skin; acute mortality

Treatment

1. Hyposalinity (16 ppt or less) for 14 days
2. Hyposalinity (10 ppt) for 3 hours q 3 days × 4
3. Transfer fish to new aquarium q 3 days × 4
4. Lower temperature to <19°C (66°F)
5. Copper prolonged immersion
6. Chloroquine prolonged immersion

COMMENTS

Epidemiology/Pathogenesis

Traditionally a problem in aquarium fish, *Cryptocaryon irritans* has become a serious disease in cultured warm water marine food fish (Tookwinas 1990; Colorni and Burgess 1997) and has recently caused epidemics in wild marine fish (Bunkley-Williams and Williams 1994; Diggles and Adlard 1997). *C. irritans* has been long considered to be the "marine counterpart" of *Ichthyophthirius multifiliis*, as though the two ciliates were closely related organisms which simply lived in different aquatic habitats. While these two ciliates have a similar life cycle (see Fig. II-20, A) and pathology, this was shown to be due to convergent evolution rather than phylogenetic relatedness (Colorni and Diamant 1993; Wright and Colorni 2002). *Cryptocaryon irritans* also produces white spots on the skin (Fig. II-21, A; Brown 1951). The parasites are somewhat smaller than ich and thus produce slightly smaller nodules. Affected skin often appears like it was finely dusted with salt. Skin lesions may appear less like discrete white spots and more like multifocal white patches (Fig. II-21, B). Isolates appear to vary in pathogenicity and more than one species of *Cryptocaryon* might exist (Diamant et al. 1991; Diggles and Adlard 1997). Virtually any teleost is probably susceptible while elasmobranchs are considered resistant (Lom 1984).

Cryptocaryon is pathogenic at 20–30°C (68–86°F), with optimal reproduction at 30°C (86°F). At 21–24°C (70–75°F), the life cycle is completed in as little as 6 days, with most parasites completing their life cycle in 11–15 days (Nigrelli and Ruggieri 1966; Colorni 1985). Up to 200–300 theronts may be produced by one tomont (Colorni and Burgess 1997). Under optimal conditions, the parasite burden can increase about tenfold every 6–8 days (Burgess 1992).

Diagnosis

Even fairly heavy infections may require close examination to be grossly detectable. Shining a light on top of the fish in a darkened room can be helpful. *Cryptocaryon* is most readily diagnosed when seen under the skin or gills (Fig. II-21, D). The presence of a ciliated protozoan within the host's epithelium (Fig. II-21, D) is pathognomonic. Unlike ich, it does not have a C-shaped macronucleus. A moniliform macronucleus, consisting of four linked, bead-like segments, may be seen in histological sections or stained smears of trophonts (Kaige and Miyazaki 1985; Colorni and Diamant 1993) (Fig. II-21, C, E, and F) but not at all stages of development. The macronucleus is usually obscured in wet mounts by the many granules in the cytoplasm. Trophonts range from 48 to 450 µm × 27 to 350 µm (Nigrelli and Ruggieri 1966).

Treatment

Treatment should be prompt because the parasite reproduces quickly. Cryptocaryonosis responds relatively well to copper therapy. Formalin (25 ppm every other day for 2 weeks with a complete water change on alternate days) has also been used (Dickerson 1994), but varies in success (Colorni and Burgess 1997). The exit of trophonts from the host and of theronts from the tomonts is influenced by circadian rhythms. As the great majority exit in predawn darkness (Burgess and Matthews 1994), formalin and probably other chemical treatments are more effective if administered late at night (Colorni 2008). Some have had good experience with chloroquine, although there are no published clinical trials.

Tomonts can be lysed by hyposalinity (Colorni 1985). Thus, euryhaline fish are easily treated if the dilution of the seawater to 1/4 of its original salinity is carried out very rapidly (within 30 minutes) and maintained for at least 3 hours (Colorni 2008) (see "**Hyposalinity**" in "**Pharmacopoeia**"). Effectiveness is probably due to the osmotic shock rather than the salinity per se. The least stressful procedure is probably to lower the salinity for short time intervals, but this appears to be less effective (A. Colorni, personal communication). Alternatively, the salinity can be lowered indefinitely to 16 ppt or less (Cheung et al. 1979); even many stenohaline reef fish tolerate this well, although some fish become hyperactive or aggressive during treatment. Hypersalinity in combination with drug treatment has also been used (Huff and Burns 1981). Tomonts are killed by exposure to freshwater for 3 hours (Colorni and Burgess 1997).

Lowering the temperature below 19°C (66°F) will stop reproduction (Wilkie and Gordin 1969) but is impractical in most instances and probably not advisable for most tropical reef fish. There is also evidence that some strains might have a lower temperature optimum (Diamant et al. 1991). Transferring fish to a clean

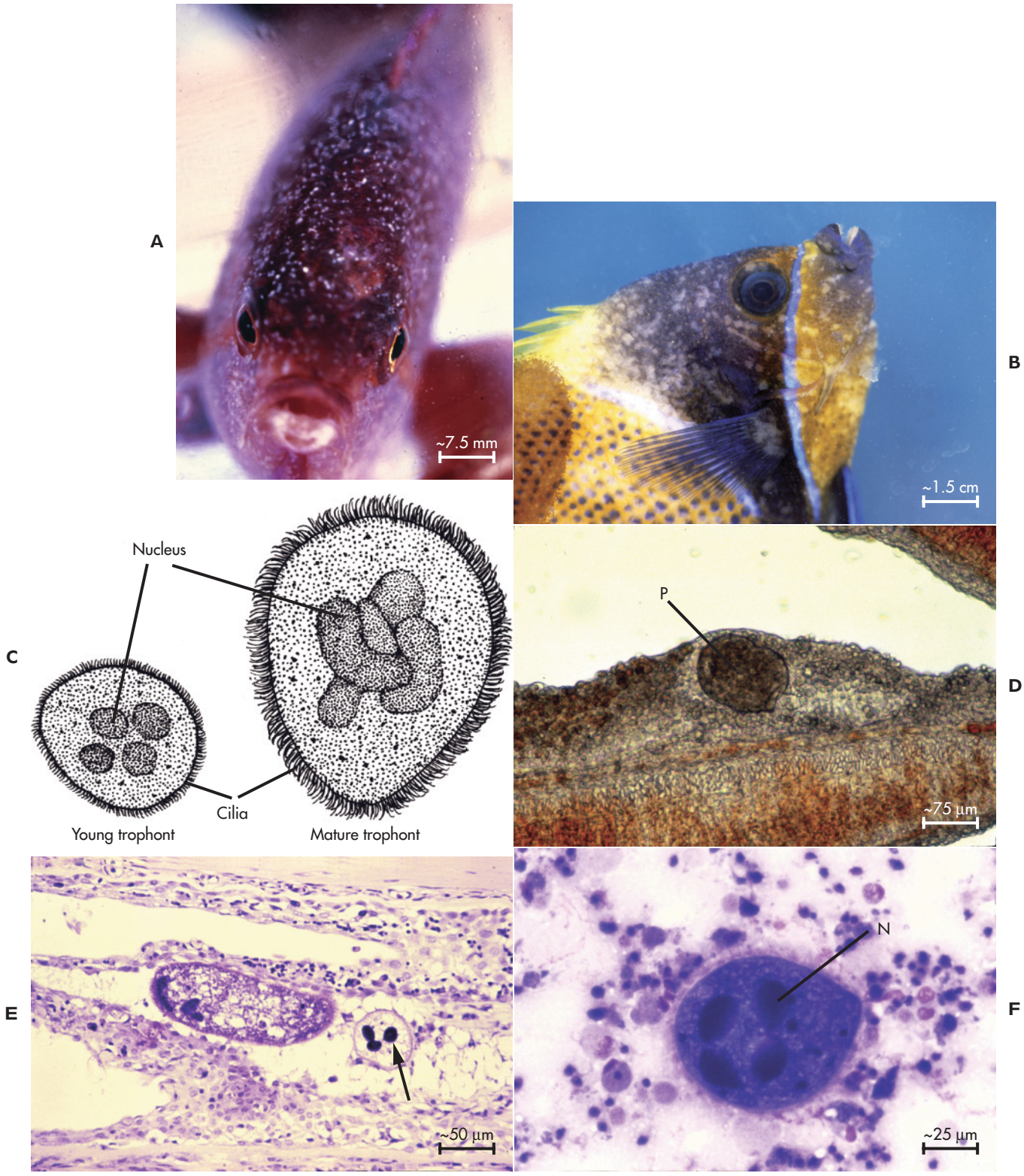

Fig. II-21. A. Red sea damselfish with a heavy *C. irritans* skin infection. The infection looks like a dusting of salt on the body. B. Queen angelfish with multifocal depigmented skin erosions affecting the entire head, caused by a prior *C. irritans* skin infection. C. *Cryptocaryon irritans*. Diagram with key characteristics of trophont: size (up to 450 µm), cilia evenly distributed over body, spherical-to-oval shape, multilobed nucleus. See ich (see *PROBLEM 20*) for life cycle. D. Gill clip with *C. irritans*. The parasite (*P*) is within the epithelium. E. Histological section of a fish infected with *Cryptocaryon irritans* (Mediterranean Sea strain). Note the presence of the two parasites *under* the epithelium, which is pathognomonic, and the lobated macronucleus (*arrow*). F. Stained smear of a *Cryptocaryon* trophont. *N* = nucleus. Modified Wright's. (*A*, *D*, and *E* photographs courtesy of A. Colorni; *B* photograph by B. Brglz and E. Noga; *F* photograph by L. Khoo, C. Harms, and E. Noga.)

aquarium four times every 3 days will also cure the fish but is stressful. Alternatively, parasites can be eliminated by regularly removing tomonts: In a bare bottom aquarium, a 1–2 cm layer of fine sand is spread over the bottom. Three days later, all the sand is siphoned out and a new layer of sand is added. This is repeated 4 times at 3 day intervals. This is useful for reef aquaria having invertebrates where drugs or hyposalinity cannot be used (Colorni and Burgess 1997). Continuous ultraviolet sterilization of the holding water might also be effective (Dickerson 1994), as it has been for freshwater ich (PROBLEM 20). The wattage would need to be high enough to kill the free-swimming theronts as they emerge from the tomont and the treatment would probably best be continued for at least one month. Similarly to freshwater ich, UV is only effective in controlling spread between aquaria and not in controlling an infection within an aquarium.

Theronts remain infective for only 24 hours after excystment at 25°C (77°F), but the long time for emergence of some theronts requires that aquaria be left without fish for at least 3 months to be rid of the parasite. Tomonts have been observed to survive and release theronts as long as 72 days after leaving the fish (Colorni and Burgess 1997). All stages are killed by drying.

Recovered fish develop a protective immunity that can last up to 6 months; however, some fish are not completely protected (Burgess and Matthews 1995). This might explain the observation of renewed outbreaks over long time intervals. Mildly susceptible fish might allow low level propagation of the parasite (at subclinical levels). When the population is stressed, reducing resistance, an outbreak can ensue (Colorni and Burgess 1997). Immunity is probably also operative when treating fish for only 2 weeks routinely cures the fish, despite the fact that theronts may continue to be present for well past this time (see above).

PROBLEM 22
Trichodinosis

Prevalence Index

WF - 1, WM - 4, CF - 1, CM - 1

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; chronic mortality

Treatment

1. Formalin bath
2. Formalin prolonged immersion
3. Potassium permanganate prolonged immersion
4. Acetic acid bath (freshwater only)
5. Salt bath (freshwater only)
6. Freshwater bath (marine only)
7. Copper prolonged immersion

COMMENTS

Epidemiology/Pathogenesis

Many trichodinid species infest marine or freshwater fish, including *Trichodina*, *Trichodinella*, *Tripartiella*, *Dipartiella*, *Paratrichodina*, *Hemitrichodina*, and *Vauchomia* species. All trichodinids have a similar morphology (Fig. II-22). All clinically important species infest the skin and/or gills. Some species infect the urinary bladder, oviducts, or gastrointestinal tract, but they are not proven pathogens. Most trichodinids have little host specificity (Basson and Van As 2006). In general, the larger (>90 µm), skin-dwelling trichodinids have a broad host range, while smaller (<30 µm), gill-dwelling parasites tend to infest one or a few fish species (Van As and Basson 1987). Many species infest both skin and gills. Other aquatic animals (e.g., amphibian larvae) can be reservoirs for some fish trichodinids (Lom and Dyková 1992).

Trichodinosis is usually a relatively mild disease that typically presents as chronic morbidity or mortality (Hoffmann 1999), but in some cases, can cause significant losses, especially in young fish. While skin/gill trichodinids only inhabit the surface of the fish, adherence to and suction on the epithelium may cause damage (Lom 1973a). Heavily infested fish are anorexic, lose condition, and usually experience low-level (1% per week) mortality. But mortalities can be much higher, especially in young fish. Secondary bacterial infections can greatly escalate mortalities. Trichodinid infestations are seen mainly in fish that are debilitated because of some other condition (e.g., poor nutrition, overcrowding, another disease). At least some trichodinids can survive off the host for 1–2 days (J. Lom, personal communication).

Diagnosis

Trichodinids are easily recognized (Fig. II-22). They often exhibit a characteristic scooting motion on tissue surfaces. All trichodinids are treated similarly, so there is no need for identification to genus (which requires silver staining of fixed samples). The observation of low numbers (e.g., 1 per 100X field of view) of trichodinids on a skin or gill biopsy is inconsequential; other problems should be sought in the clinical workup. However, because of their tenuous attachment to the tissues, they are easily lost during fixation.

Treatment

Trichodinids are easily killed with one application of appropriate treatment. Fish will often recover spontaneously if water quality is improved. Some trichodinid species can infest both freshwater and marine fish

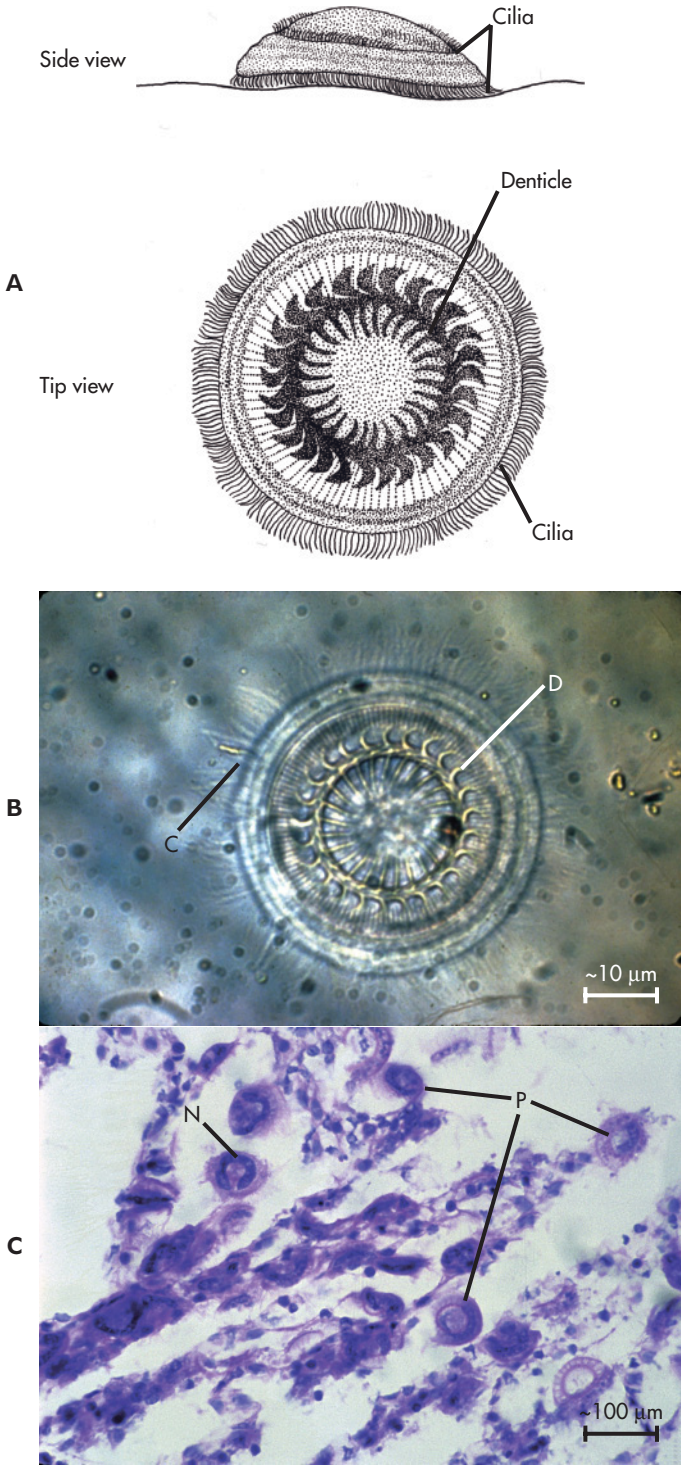

Fig. II-22. A. Diagram of a typical trichodinid parasite with key characteristics: size (15–120 µm, usually 40–60 µm in diameter); cilia for locomotion; round shape when seen from top of parasite (dorsally); and ring with hook-like denticles. B. Wet mount of a typical trichodinid parasite. *C* = cilia; *D* = denticle. C. Histological section through the gill of a goldfish with a heavy trichodinid infestation. Parasites (*P*) can be recognized by their round shape from above. *N* = nucleus. (*B* photograph courtesy of F. Meyer.)

(Lom and Dyková 1992), but virtually all common pathogens are restricted to either fresh or saltwater environments.

PROBLEM 23
Chilodonella Infestation (Chilodonellosis)

Prevalence Index

WF - 1, CF - 1, WM - 4, CM - 4

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite, especially whitish or bluish sheen on body, "tattered" appearance to skin; also, a drop in temperature or previous injury

Treatment

1. Formalin bath
2. Formalin prolonged immersion
3. Potassium permanganate prolonged immersion
4. Acetic acid bath
5. Salt bath
6. Copper prolonged immersion

COMMENTS

Epidemiology/Pathogenesis

Most *Chilodonella* species are free-living, but two species (*C. piscicola* and *C. hexasticha*) are pathogenic for fish. *Chilodonella piscicola* (formerly *C. cyprini*) infests virtually all freshwater fish, mainly fingerlings (Shulman and Jankovski 1984). *Chilodonella hexasticha* is less widely distributed but produces similar lesions, mainly in older fish. Both species can also infest fish in brackish water and appear to have been widely spread throughout the world via infested fish.

Chilodonellosis is more insidious than ich, since severe damage can occur before gross pathology is evident. *Chilodonella* elicits a strong cellular response, which suggests that it may feed directly on epithelium (Paperna and Van As 1983). It appears to feed by penetrating the host cells with its cytostome and sucking out the contents (Wiles et al. 1985). Advanced *Chilodonella* infestations are sometimes associated with skin ulcers, which like brooklynellosis, may have a tattered appearance (see Fig. II-24, A as an example of this type of lesion). High numbers can cause secondary bacterial infections and substantial mortality (10% per week). Chilodonellosis has a wide temperature tolerance. For example, outbreaks in cold water species often occur at 5–10°C (41–50°F), while tropical fish are affected when the temperature drops to 20°C (68°F). However, outbreaks have been observed at as high as 25°C (77°F) (Basson and Van As 2006). Outbreaks can also occur at higher temperatures. Mass mortalities have occurred in wild populations (Langdon et al. 1985).

Some free-living *Chilodonella* species (e.g., *C. cucullulus*, *C. uncinata*) can damage weakened fish in polluted waters (Lom and Dyková 1992). They are apparently not as widespread as the two more pathogenic *Chilodonella* species.

Diagnosis

Chilodonella is easily recognized in wet mounts or histological sections (Figs. II-23, A through C). Because of their tenuous attachment to the tissues, they are easily lost during fixation. In wet mounts, *Chilodonella* glides slowly over gill lamellae, sometimes turning in wide circles (Brown and Gratzek 1980). It is differentiated from the holotrichs ich (see PROBLEM 20) and *Tetrahymena* (see PROBLEM 25) by its flattened shape. Also characteristic are its bands of cilia on the ventral surface, which require high magnification to be seen and are best visualized with silver staining. *Chilodonella piscicola* is 30–80 × 20–60 µm, with 8–11 bands of cilia, while *C. hexasticha* is smaller (30–65 × 20–50 µm), with 5–9 cilia bands (Fig. II-23, A). Identification to species is not needed for proper treatment.

Treatment

One application of an appropriate treatment usually controls chilodonellosis. *Chilodonella piscicola* produces long-lasting cysts (Bauer and Nikolskaya 1957), but whether these are resistant to treatment is not known.

PROBLEM 24

Brooklynella Infestation (Brooklynellosis)

Prevalence Index

WM - 2

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite

Treatment

1. Formalin bath

COMMENTS

Brooklynellosis (Fig. II-24) is the marine analogue of chilodonellosis. It has been associated with acute mortalities of tropical marine fish. *Brooklynella hostilis* is morphologically similar to *Chilodonella*, having an oval shape with more numerous ciliary rows. Its most easily recognized diagnostic features are dorsoventral flattening, notched anterior end, size, and slow, *Chilodonella*-like movement. Its size range is 56–86 × 32–50 µm.

Unlike most marine fish ectoparasites, it is often not susceptible to copper, but formalin baths are effective (C.E. Bower, personal communication). While reported to be only a gill pathogen (Lom and Nigrelli 1970), it can also cause serious skin lesions (Fig. II-24, A). It commonly occurs after transport stress.

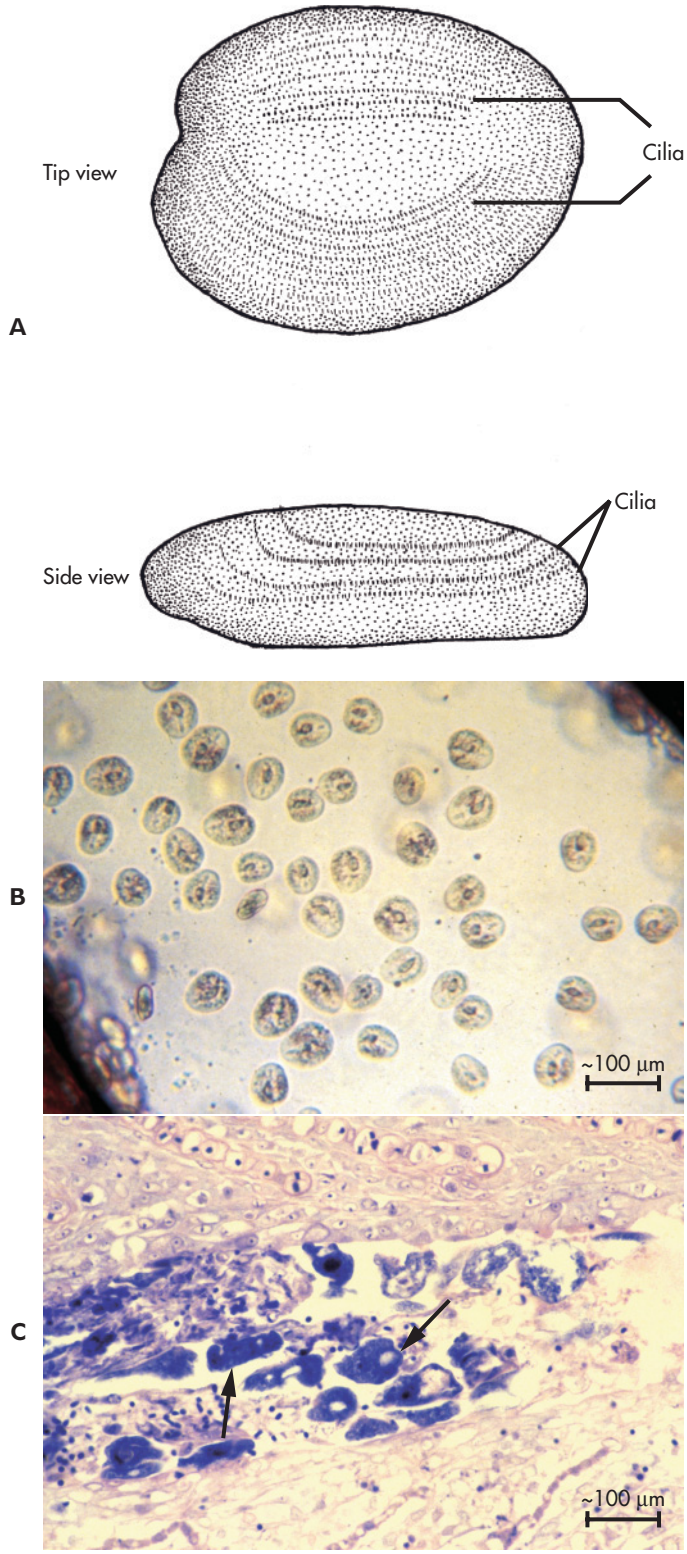

Fig. II-23. A. *Chilodonella*. Diagram of key characteristics: size (usually ~40–60 µm long); bands of cilia; when viewed from above (*top view*), oval-to-heart-shape, with notched anterior end; parasites are a flattened shape when viewed from the side (*side view*). B. Wet mount of *Chilodonella ciprini*. C. Histological section of gill with *Chilodonella* (*arrows*). Giemsa. (*B* photograph courtesy of G. Hoffman.)

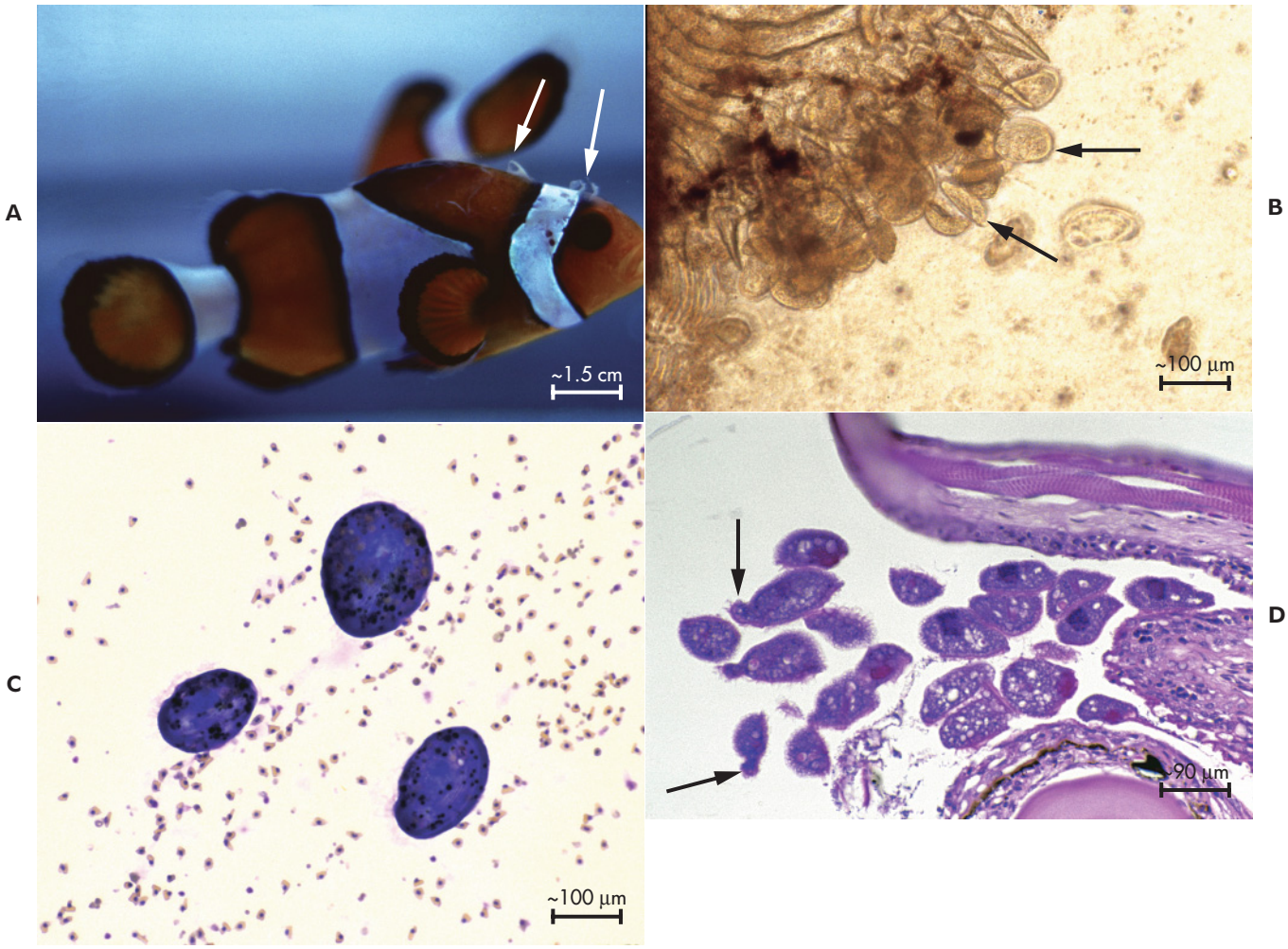

Fig. II-24. A. A percula clownfish with heavy *Brooklynella* infestation. Note the shreds of detaching skin (*arrows*). B. Wet mount of skin from a percula clownfish with brooklynellosis. Note ovoid shape on top view and flat shape on side view (*arrows*). C. Modified Wright's stained smear of the skin lesion in Fig. II-24, *A*, with three *Brooklynella* trophozoites. D. Histological section of the skin lesion in Fig. II-24, *A*, with many parasites. Key features include size, shape, and notched anterior end (*arrows*). (*C* and *D* photographs by L. Khoo and E. Noga.)

PROBLEM 25

Tetrahymenosis (*Tetrahymena* Infestation/Infection, TET Disease, Guppy Disease)

Prevalence Index

WF - 3, CF - 4

Method of Diagnosis

1. Wet mount of skin, gills, or internal organs with parasite
2. Histopathology of skin, gills, or internal organs with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, areas of muscle swelling

Treatment

1. Formalin bath

COMMENTS

Epidemiology/Pathogenesis

Tetrahymenids (Fig. II-25) are typically free-living ciliates, but some species can be highly lethal fish pathogens. In advanced cases, *Tetrahymena* may invade various internal organs, with parasite foci in muscle, kidney, or brain. Reproduction is typically by binary fission; some species (e.g., *T. corlissi*) can produce small reproductive cysts (2–8 tomites).

The species most commonly causing disease is *Tetrahymena corlissi*, which can infest/infect fish and

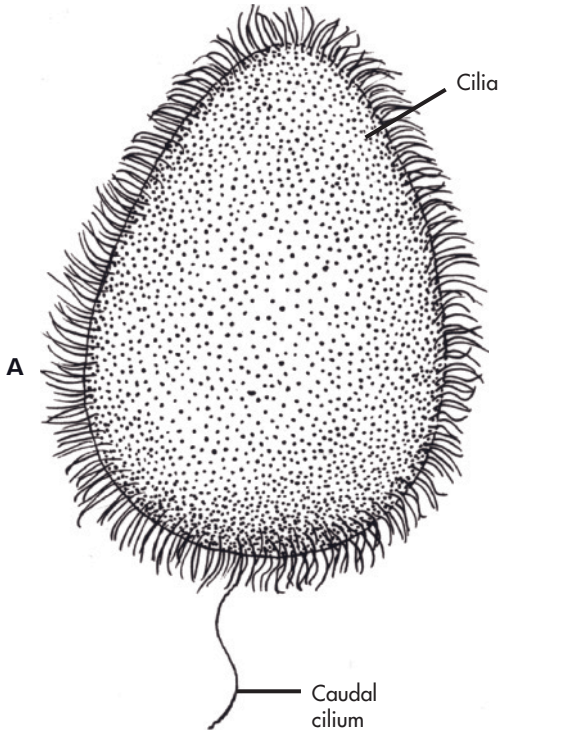

Fig. II-25. A. *Tetrahymena*. Diagram with key characteristics: size (~30–60 × 50–100 µm); pyriform or radially symmetrical, ovoid body; evenly distributed cilia; long caudal cilium [present only in some species [e.g., *T. corlissi*]). B. Wet mount of *Tetrahymena*. Note long cilia covering body. Formalin-fixed specimen. (*B* photograph courtesy of G. Hoffman.)

amphibians. Called guppy disease because of its predilection for guppies (Imai et al. 2000), the disorder also affects other livebearers, cichlids, and tetras (Hoffman et al. 1975). Clinical signs are nonspecific, but muscle swelling may be evident grossly due to parasite invasion (Ferguson 1988). Guppies can appear normal one day and be dead the next; a mass of ciliates may form a rim around the orbit (spectacle eye). In black mollies, *T. corlissi* induces white patches caused by massive numbers of ciliates in copious amounts of mucus (Johnson 1978). *T. corlissi* also causes disease in golden perch.

Tetrahymena pyriformis-like ciliates can damage the skin and invade the internal organs of common carp, catfish (*Ameiurus* sp.), and rainbow trout (Shulman and Jankovski 1984). Other *Tetrahymena* isolates cause deep ulcerative dermatitis in Atlantic salmon in freshwater (Ferguson et al. 1987). *Tetrahymena* also can cause disease in some crustaceans and turbellarians.

Diagnosis

The mucus production and epithelial damage caused by *Tetrahymena* may appear grossly similar to ich (see PROBLEM 20) but are easily differentiated by identifying the parasite. *Tetrahymena* may be confused with free-living, nonpathogenic ciliates, such as *Paramecium*, which may occasionally be found in low numbers on the skin or gills. Shape, size, movement (like a spiraling football), and presence of typical invasive lesions should be used for differentiation. Penetration of ciliates into muscle and deep tissues is highly diagnostic for *Tetrahymena*.

Treatment

Only cases without systemic disease are treatable and may require several treatments. The environment should also be improved. Feeding a diet high in the essential fatty acid arachidonic acid aided the recovery of guppies from the infection (Khozin-Goldberg et al. 2006).

PROBLEM 26
Scuticociliatosis (Uronemosis)

Prevalence Index

WM - 3, CM - 2

Method of Diagnosis

1. Histopathology of skin, gills, or internal organs with parasite
2. Wet mount of skin, gills, or internal organs with parasite

History/Physical Examination

Focal depigmentation, pitting, ulceration of skin; dyspnea; hyperactivity, then lethargy

Treatment

1. Formalin bath (useful only in early stages)
2. Freshwater bath followed after 24 hours by formalin prolonged immersion (useful only in early stages)

COMMENTS

Epidemiology/Pathogenesis

Scuticociliatosis, caused by marine ciliates of the subclass Scuticociliatia, has beeen recognized as a dangerous disease of tropical marine aquarium fish for some time (Cheung et al. 1980). Recently, it has become a serious

problem in Japanese flounder culture in Asia (Korea, Japan, China), as well as turbot and European seabass culture in the Mediterranean region. It has also caused disease in southern bluefin tuna in Australia (Munday et al. 1997).

Scuticociliates (Fig. II-26, A) seem to be the marine counterpart of *Tetrahymena* species (see PROBLEM 25). Both groups are holotrich ciliates that cause skin/gill lesions and systemic infections. *Uronema marinum*, the most common scuticociliate in tropical marine fish, appears to have a wide host range and can infect fish over a wide range of temperature (8–28°C [46–82°F]) and salinity (20–31 ppt; Cheung et al. 1980). The closely related and morphologically similar *Miamiensis avidus*

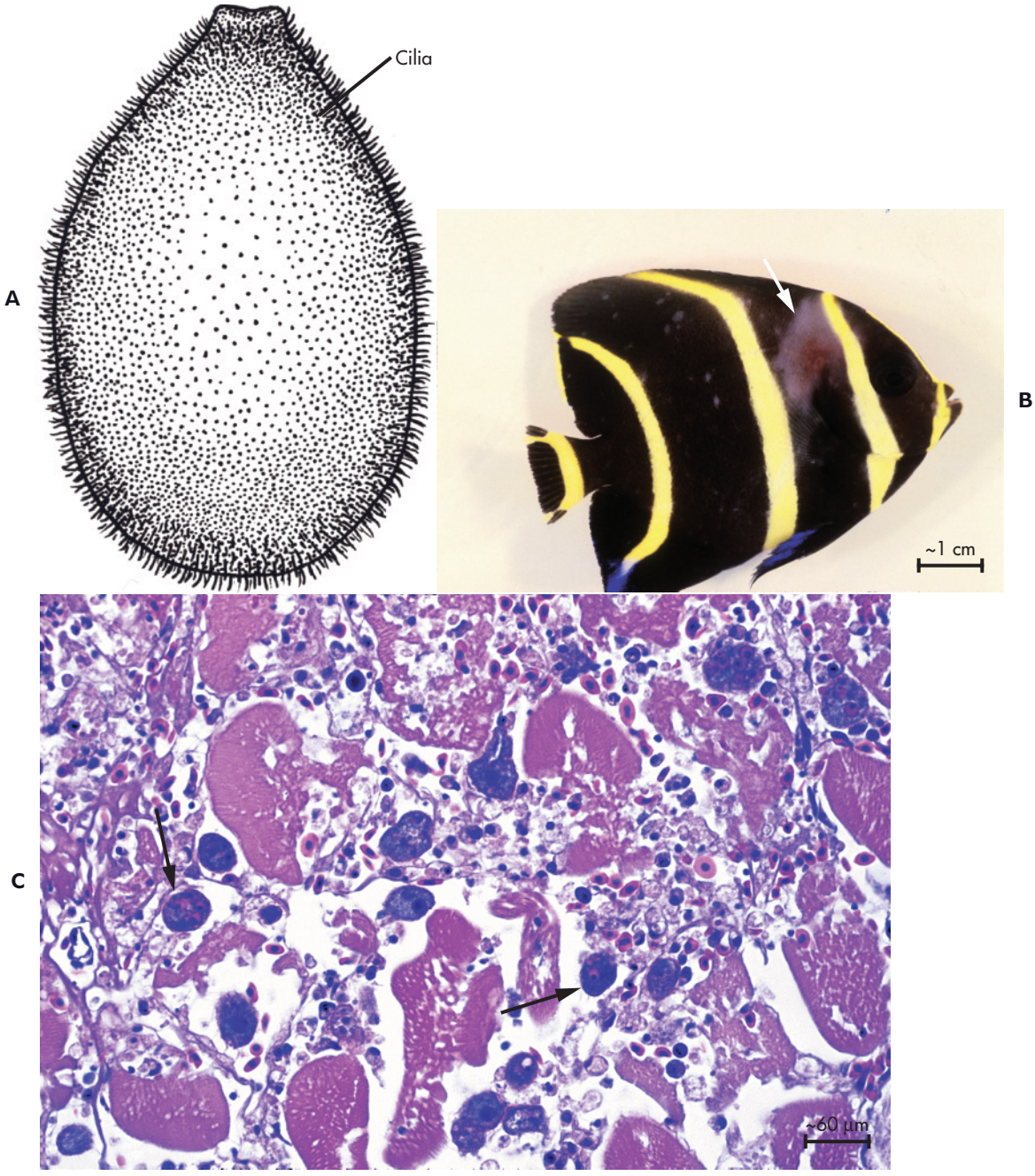

Fig. II-26. A. *Uronema marinum*. Diagram with key characteristics: size (~13–20 × 32–38 µm); tear-drop shape (narrow anteriorly); cilia (*C*) evenly distributed over body (after Lom and Dyková 1992; from Kahl). B. Immature French angelfish with large area of depigmentation (*arrow*) caused by uronemosis. C. Histological section of lesion in Fig. II-26, *B*, necrotic muscle with trophozoites, some with ingested (pink) erythrocytes (*arrows*). Hematoxylin and eosin.

was first reported from nodular lesions on seahorses (Thompson and Moewus 1964). *Philasterides dicentrarchi* has recently caused similar lesions in the closely related leafy and weedy sea dragons (Rossteuscher et al. 2008). *Uronema marinum*, *Philasterides dicentrarchi* and *Pseudocohnilembus persalinus* cause scuticociliatosis in farmed Japanese flounder (Kim et al. 2004b, 2004c). *Philasterides dicentrarchi* has also been reported to cause systemic infection in European sea bass and turbot (Iglesias et al. 2001). There is morphological evidence that *Miamiensis avidus* and *Philasterides dicentrarchi* are the same species (Jung et al. 2007). Other unidentified scuticociliates also cause disease in cultured Japanese flounder in Japan (Yoshinaga and Nakazoe 1993).

Outbreaks in food fish primarily affect younger fish, including fry and juveniles. Unlike typical ectoparasitic protozoa, scuticociliates frequently invade internal organs and cause deep ulcers (Fig. II-26, B). Muscle (Fig. II-26, C), peritoneal cavity, kidney, pancreas, liver, urinary bladder, spinal cord and brain may be affected (Cheung et al. 1980; Dyková and Figueras 1994; Ramos et al. 2007). There is typically little inflammatory response (Fig. II-26, C). Fish usually develop white skin foci, which progress to areas of depigmentation and ulceration (Bassleer 1983b; Rossteuscher et al. 2008). Some fish may show no external signs, except lethargy. There may be skin hemorrhage/necrosis and gill aneurysms. Once established in a host, death is swift. Scutiociliates are free-living protozoa and thus may exist in the environment without fish (Jee et al. 2001).

Diagnosis

Even if skin lesions are present, skin scrapings may not detect the organisms in wet mounts if they are deep in the tissues. Thus, scraping deep into the muscle, preparing wet mounts of internal organs (including brain), or examining tissues histologically should also be done for diagnosis. Unlike *Brooklynella*, scuticociliates are smaller, ellipsoid, and holotrichous (Thompson 1963; Kim et al. 2004b, 2004c). They are also smaller than *Cryptocaryon* and do not incite the typical proliferative nodule present in cryptocaryonosis (see PROBLEM 21).

Identifying a protozoan as a scuticociliate can be easily done using the morphological criteria mentioned above and shown in Fig II-26. However, species identification of scuticociliates has traditionally required the laborious preparation of silver-stained mounts of individual cells to allow observation of the cilia pattern on the cell. The recognititon that a number of highly similar-appearing ciliates are part of this disease complex prompted development of improved technologies for identifying species. Gene probes developed for some scuticociliates now allow the rapid and specific identification of infections, and some can also determine if more than one scuticociliate species is involved in an epidemic (Kim et al. 2004b).

Treatment

Early stages of *Uronema marinum* infection in tropical marine fish can reportedly be controlled by a freshwater bath followed by prolonged immersion in formalin (Blasiola 1992). Advanced lesions have reportedly responded to methylene blue or nitrofurazone (Cheung et al. 1980; Bassleer 1983b). In Japanese flounder, a 200 ppm formalin bath for 2 hours is recommended once a day for six days. However, in all fish species, systemic or deep muscle infections have a poor prognosis.

Scuticociliates are relatively resistant to UV compared with fish-pathogenic viruses or bacteria, but can be effectively eliminated from a contaminated water supply by using a high intensity treatment ($3.0 \times 10^5 \mu W \cdot sec/cm^2$) (Kasai et al. 2002).

PROBLEM 27

Marine Velvet Disease (Amyloodiniosis, Marine Oodinium Disease, Oodiniosis)

Prevalence Index

WM - 1

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, golden, dust-like sheen ("velvet") on skin

Treatment

1. Copper prolonged immersion
2. Chloroquine diphosphate prolonged immersion
3. Freshwater prolonged immersion
4. Hydrogen peroxide bath q 6 days × 2

COMMENTS

Epidemiology

Amyloodiniosis is one of the most important diseases of warm water marine fish (Paperna et al. 1981a; Noga and Levy 2006), infesting both food fish and aquarium fish worldwide (Lawler 1977a). It has also very rarely caused natural epidemics, best documented in fish of the Salton Sea, a hypersaline inland lake in eastern California (Kuperman and Matey 1999). *Amyloodinium ocellatum* is one of the few fish parasites that can infest both elasmobranchs (sharks, rays) and teleosts (Lawler 1980), and most fish that live within its ecological range are susceptible to infestations. Even freshwater fish, such as centrarchids or tilapia, are susceptible to infestation when they are in brackish water (Lawler 1980). Species most resistant to infestations tend to produce thick mucus or tolerate low oxygen levels (Lawler 1977a). It can be transmitted via aerosols (Roberts-Thomson et al. 2006).

Life Cycle

Amyloodinium is a dinoflagellate that is highly adapted to parasitism; the trophont bears little resemblance to

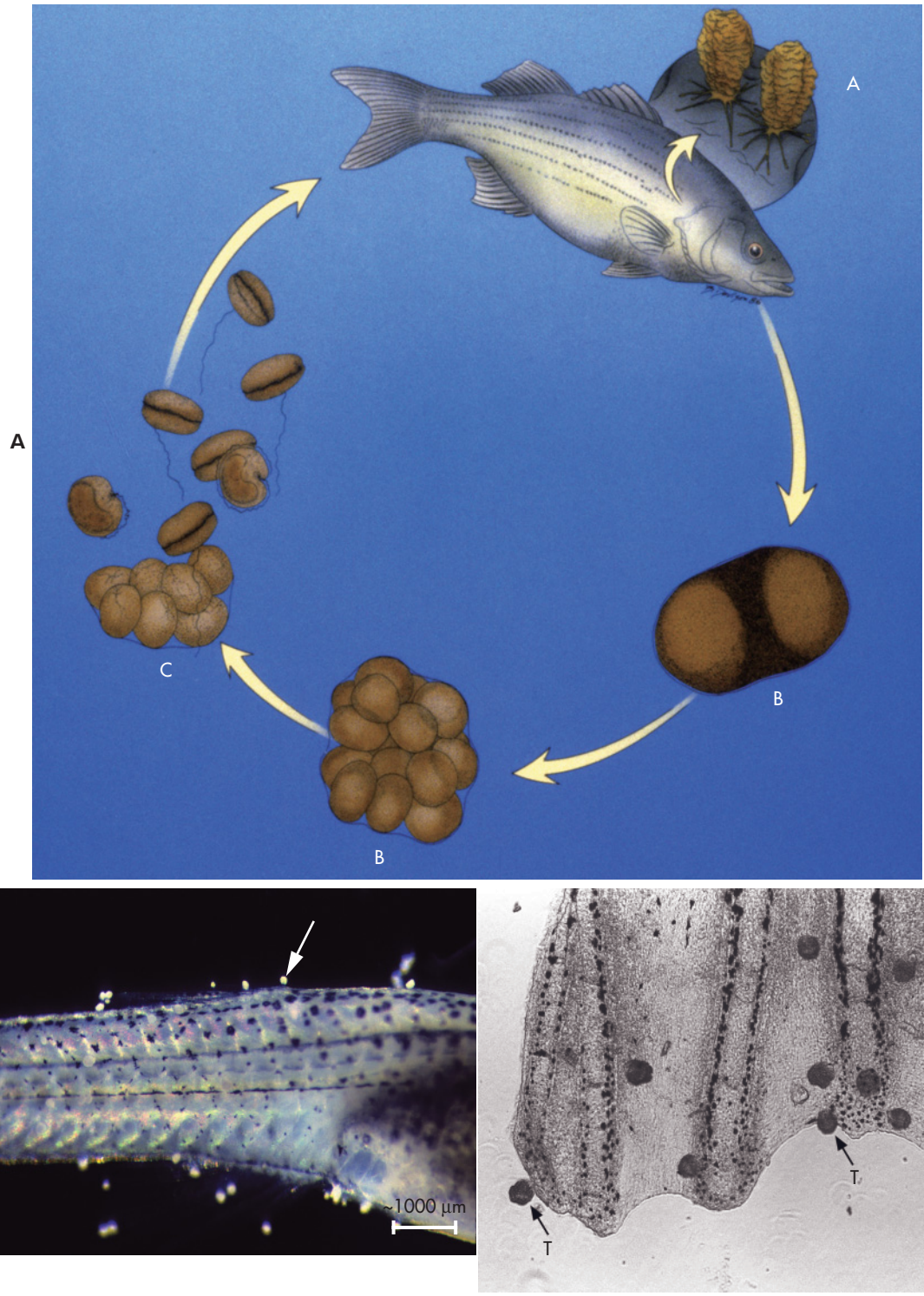

Fig. II-27. A. *Amyloodinium ocellatum* life cycle. *A* = trophont; *B* = tomont; *C* = dinospore. B. *A. ocellatum*. Trophonts on small fish. Note that parasites are on the surface of the skin (*arrow*). C. Fin clip of a fish infested with *A. ocellatum*. Note that the irregularly shaped trophonts (*T*) are attached to the surface of the skin. Trophonts can range from 50 to 350 µm. The root-like rhizoids illustrated in Fig. II-27, *A*, are not usually visible in attached trophonts.

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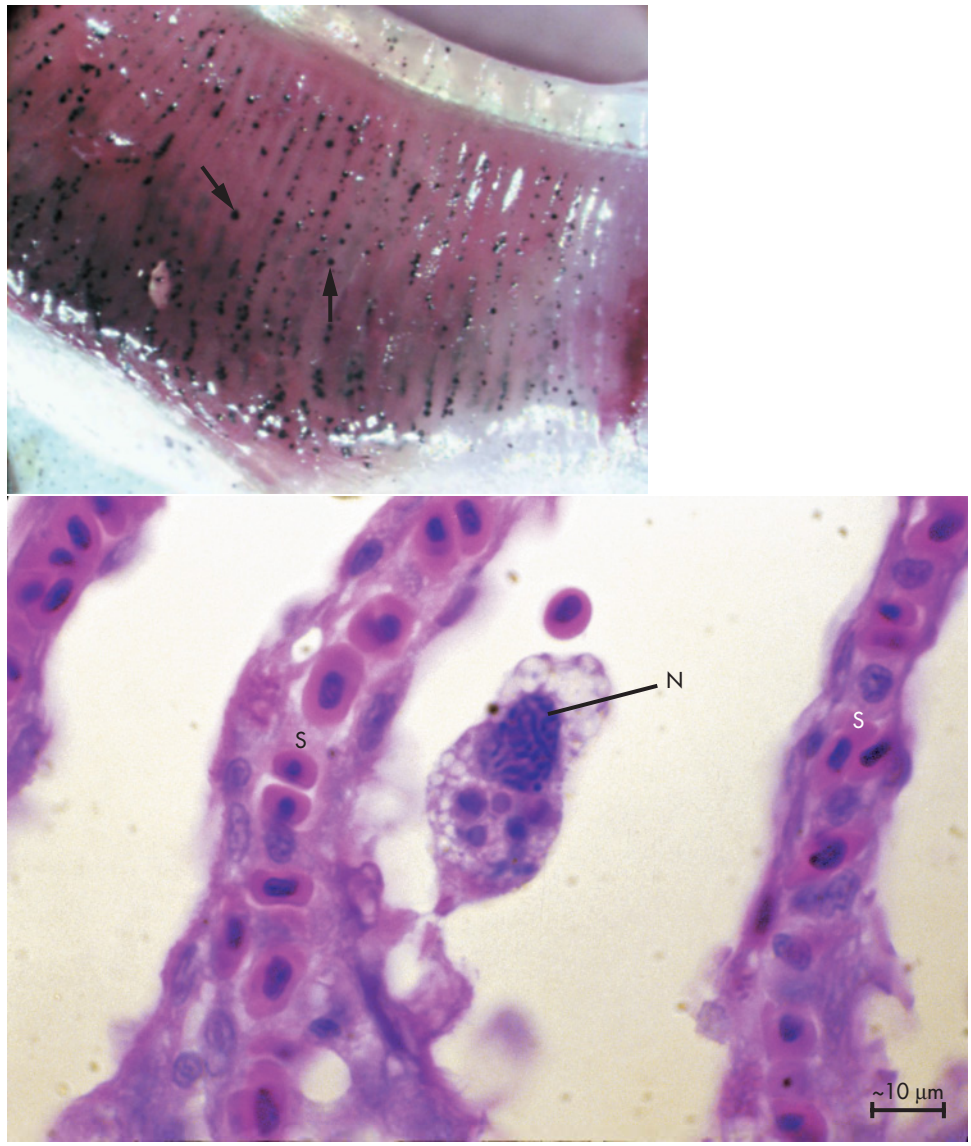

Fig. II-27.—cont'd. D. Low magnification of an entire gill arch from a heavily infested fish stained with Lugol's iodine. Note that the trophonts (*arrows*) stain dark brown. E. Histological section of gill with trophont. Key features: size; irregular shape; attachment to surface of epithelium; nucleus (*N*) with permanently condensed chromosomes. *S* = secondary lamellae. (*A* figure by B. Davison-Degraves and E. Noga; *D* photograph courtesy of J. Burke; *E* photograph by L. Khoo and E. Noga.)

free-living dinoflagellates. Typical dinoflagellate morphology is apparent only during the disseminative (dinospore) stage (Fig. II-27, A).

The life cycle is virtually identical to that of *I. multifiliis* (see PROBLEM 20). The trophont (Fig. II-27, A through E) attaches to and feeds on the host's epithelium. After the trophont feeds for several days, it detaches from the host, retracts its rhizoids (root-like structures used to attach to the epithelium), and becomes a tomont. The tomont divides, producing up to 256 (usually 64 or less) motile, infective dinospores (Brown 1934; Nigrelli 1936). Dinospores are 8–13.5 µm long by 10–12.5 µm wide. The dinospores attach to a host, differentiate into a trophont, and continue the cycle.

Environmental Requirements

Optimal temperature for most isolates is 23–27°C (73–81°F). Tomont division is limited to 16–30°C (61–86°F; Paperna 1984). Infestations do not occur at less than 17°C (63°F). Tomonts stop dividing at low temperatures, but some isolates can produce dinospores when

returned to 25°C (77°F), even after 4 months at 15°C (59°F) (C.E. Bower, personal communication).

Amyloodiniosis has caused disease in salinities ranging from 3 to 45 ppt. Isolates vary in salinity tolerance. For example, Red Sea isolates do not divide below 12 ppt salinity (Paperna 1984), while epidemics commonly occur at 3 ppt salinity in the Gulf of Mexico (Lawler 1977a) and at 5 ppt in Australia (Fielder and Bardsley 1999). Salinity tolerance decreases at suboptimal temperatures.

Pathogenesis

The gills are usually the primary site of infestation. Heavy infestations may also involve the skin and eyes. Tomonts that are occasionally seen in the gastrointestinal tract (Hojgaard 1962; Brown 1934) were probably swallowed by the host.

Rhizoids (Fig. II-27, A) anchor the parasite to the host cells (Lom and Lawler 1973). A single trophont can damage and kill several host cells (Lom and Lawler 1973; Noga 1987), which probably accounts for the severe injury inflicted on the host by trophonts.

Mild infestations (e.g., 1–2 trophonts per gill filament) cause little pathology. However, heavy infestations can cause serious gill hyperplasia, inflammation, hemorrhage, and necrosis. Death can occur within 12 hours (Lawler 1980). Some acute mortalities are associated with apparently mild infestations, suggesting that hypoxia may not always be the cause of death in all primary gill infestations. Osmoregulatory impairment and secondary microbial infections caused by severe epithelial damage may also be important.

Diagnosis

Gross skin infestation by *Amyloodinium* is most easily seen on dark-colored fish as is also true for freshwater velvet (Fig. II-27, A), using indirect illumination, such as by shining a flashlight on top of the fish in a darkened room. Observing fish against a dark background also helps. Heavily infested skin may have a dusty appearance (velvet disease); however, this is not a common finding, and fish often die without obvious gross skin lesions.

Definitive diagnosis is easily made by identification of trophonts in biopsies (see Fig. II-27, C) or histological sections (see Fig. II-27, E). Trophonts can also be visualized by staining with Lugol's iodine (Fig. II-27, D). Parasites can also be brushed off the surface of heavily infested skin. Trophonts can be dislodged by placing fish in a small (as small as possible) container of freshwater for 1–3 minutes (Bower et al. 1987). Trophonts settle to the bottom of the container after 15–20 minutes. The parasites can be aspirated from the sediment and identified microscopically. Note that tomonts will also be present, since dislodgement stimulates the trophonts to form tomonts.

A gene test can detect trophonts on infected tissue and as little as one dinospore per ml in seawater (Levy et al. 2007a). However, the test is not yet commercially available.

Related Nonpathogenic Dinoflagellates

The closely related *Crepidoodinium virginicum* (Lom and Lawler 1973) only infests estuarine topminnows (*Fundulus*, *Lucania*, and *Cyprinodon*) in the Gulf of Mexico and the Atlantic Ocean near Virginia, while *C. australe* (Lom et al. 1993) infests sand whiting, an estuarine fish in New South Wales, Australia. *Crepidoodinium* is not pathogenic but only uses the fish as an attachment site. Trophonts can be up to 820 × 235 µm and are green because of the presence of chloroplasts (Lom and Lawler 1973).

Treatment

Amyloodinium ocellatum is highly virulent and must be treated as soon as it is detected to prevent a catastrophe. The free-swimming dinospore is susceptible to drugs (Lawler 1980; Paperna 1984), but trophonts and tomonts are resistant, making eradication difficult. For example, tomonts tolerate copper concentrations that are over 10 times the levels that are toxic to dinospores (Paperna 1984). Even tomonts inhibited from dividing can often resume dividing when returned to untreated water (Paperna et al. 1981). Treatment with 100–200 mg/l formalin for 6–9 hours detaches trophonts from fish, but they resume division after removal of formalin (Paperna 1984). Thus, treatments must be long enough to allow all trophonts and tomonts to form dinospores or fish must be moved to an uncontaminated system. Periodic examination for reinfestation after treatment is advisable.

The most widely used treatment is copper (Bower 1983; Cardeilhac and Whitaker 1988), which will control outbreaks, but some parasites may remain latent on the fish (C.E. Bower, unpublished data). Bower (personal communication) discovered that the antimalarial chloroquine diphosphate is safe and effective. It is less toxic than copper and may also eliminate latent infestations, but it is expensive. Many other agents have been tested with little succcess against amyloodiniosis (Noga and Levy 2006).

Lowering the temperature to 15°C (59°F) arrests the disease (Paperna 1984), but this is almost never feasible. Lowering salinity delays but does not prevent infestations (Barbaro and Francescon 1985), unless fish are placed in freshwater. A 5-minute freshwater bath dislodges most but not all trophonts (Kingsford 1975; Lawler 1977a). However, there is evidence that fish given two treatments of hydrogen peroxide may be cured of the infestation (Montgomery-Brock et al. 2001); however, this requires that the dislodged tomonts are removed, not allowing them to continue their life cycle or that the fish be moved to an uncontaminated system.

Dinospores can be killed with ultraviolet radiation (Lawler 1977a). Quarantine of new fish for at least 20 days may reduce but not eliminate the risk of parasite

introduction. Dinospores remain infective for at least 6 days at 26°C (79°F; Bower et al. 1987). Fish produce an immune response after natural (Smith et al. 1994) and experimental (Cobb et al. 1998) challenge. Vaccines are being explored.

PROBLEM 28

Freshwater Velvet Disease (Freshwater Velvet, Rust Disease, Gold Dust Disease, Pillularis Disease, Freshwater *Oodinium*)

Prevalence Index

WF - 2

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, golden, dust-like sheen (velvet) on skin

Treatment

1. Salt prolonged immersion

COMMENTS

Epidemiology

Piscinoodinium is the freshwater analogue of *Amyloodinium* (see PROBLEM 27). Most reports of the parasite have been on aquarium fish in North America (*P. limneticum*) and Europe (*P. pillulare*), as well as food fish in Asia (Shaharom-Harrison et al. 1990; Lom and Schubert 1983; Ramesh et al. 2000) and South America (Carneiro et al. 2002).

Many tropical fish are susceptible to *Piscinoodinium*, with anabantids, cyprinids, and cyprinodontids frequently affected. Temperate species (e.g., common carp, tench) and larval amphibians (*Amblystoma mexicanum*, *Rana temporaria*, and *R. arvalis*) are also susceptible (Geus 1960). Mass mortalities in tank-reared tilapia have been associated with decreasing temperatures (dropping from 30° to 21°C; Ramesh et al. 2000), but epidemics in pond-cultured fish have been observed with both increasing and decreasing temperatures (Shaharom-Harrison et al. 1990).

Despite its very close similarities to *Amyloodinium*, recent genetic studies have shown that it is actually not a close relative of *Amyloodinium* but rather has undergone convergent evolution (Levy et al. 2007b).

Life Cycle

The life cycle is the same as for *Amyloodinium* (see Fig. II-27, A). Trophonts are yellow-green, pyriform or sac-like, up to 12 × 96 µm. They are almost round when mature (Lom and Schubert 1983) and somewhat less irregular in texture, compared with *Amyloodinium*. Up to 256 dinospores (10–19 µm long × 8–15 µm wide in *P. limneticum*) are produced from each tomont. The life cycle may be completed in 10–14 days under optimal conditions. Optimal temperature for *P. pillulare* is 23–25°C (73–77°F), with sporulation requiring 50–70 hours for an average-sized tomont. At 15–17°C (59–63°F), sporulation requires 11 days (van Duijn 1973). Optimal conditions are probably similar for *P. limneticum*. Under crowded conditions or in stagnant water, sporulation is inhibited and smaller dinospores are produced. Lower temperature slows the life cycle (Jacobs 1946).

Pathogenesis

Clinical signs are similar to amyloodiniosis, except that fish can withstand much heavier infestations. The parasite is most pathogenic in young fish that may die within 1–2 weeks; older fish may live for months. Heavy infestations (Fig. II-28, A, B, and C) produce a yellow or rusty sheen to the skin when viewed under direct light. There may also be excess mucus, darkening of the skin, dyspnea, anorexia, and/or depression (Shaharom-Harrison et al. 1990). Skin ulcers (Shaharom-Harrison 1990) and tattered, sloughing epithelium (Schäperclaus 1951) have been seen in some cases.

Histopathology ranges from separation of the respiratory epithelium to severe hyperplasia of the entire gill filament. Filament degeneration and necrosis may occur. Some parasites may become almost entirely covered by hyperplastic epithelium (Shaharom-Harrison et al. 1990; van Duijn 1973), probably because of the chronic irritation caused by infestation. Some of these parasites may even sporulate (Geus 1960).

Diagnosis

Definitive diagnosis is easily made by identification of trophonts in biopsies. Trophonts look almost identical to *Amyloodinium* (see Fig. II-27, A through C).

Treatment

Both species of *Piscinoodinium* are treated the same. The relatively mild pathogenicity of *Piscinoodinium* usually allows ample time to control outbreaks. It is often advisable to raise the temperature to 24–27°C (75–81°F) to speed up the life cycle during treatment. Leaving aquaria without fish for 2 weeks at this temperature will eliminate the parasites. Dinospores remain infective for only up to 48 hours (Jacobs 1946; van Duijn 1973), but ample time must be allowed for delayed emergence of dinospores from tomonts. Reducing lighting to inhibit autotrophy has also been advocated during treatment (van Duijn 1973).

The safest and most effective treatment for piscinoodiniosis is prolonged immersion salt (about 1 teaspoon per 5 gallons of water). This is also an effective prophylactic (R. Goldstein personal communication). For heavy, life-threatening infestations, a 35 ppt, 1- to 3-minute salt bath dislodges trophonts. Exposure of *Piscinoodinium*-infested matrinxa to 6 ppt NaCl for 96 hours significantly reduced the parasite load on the transported fish and was apparently well tolerated by this stenohaline freshwater fish (Carneiro et al. 2002).

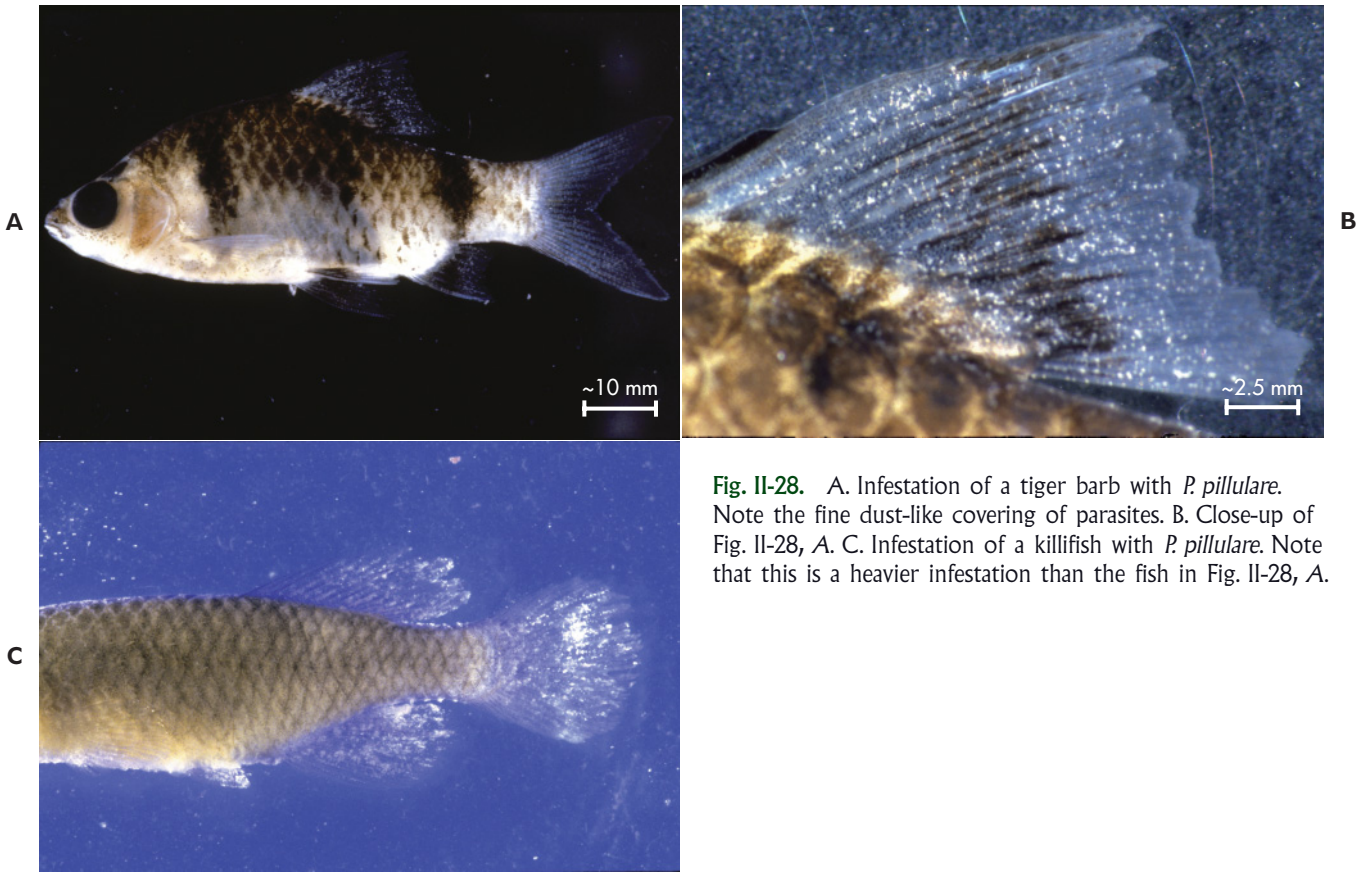

Fig. II-28. A. Infestation of a tiger barb with *P. pillulare*. Note the fine dust-like covering of parasites. B. Close-up of Fig. II-28, *A*. C. Infestation of a killifish with *P. pillulare*. Note that this is a heavier infestation than the fish in Fig. II-28, *A*.

Copper has been advocated as a treatment (van Duijn 1973), but its unpredictable toxicity in soft, acid water often makes it dangerous to use, especially since many commonly affected aquarium fish are maintained under those conditions. Heating water to 33–34°C (91–93°F) reportedly controls infestations (Untergasser 1989), but some aquarium fish cannot tolerate such high temperatures (see PROBLEM 2). Chloroquine diphosphate has not been tested against piscinoodiniosis, but its success with amyloodiniosis suggests that it may be useful.

Piscinoodinium has been observed asymptomatically on a number of fish, including goldfish, common carp, Siamese fighting fish and colisa gourami (Thilakaratne et al. 2003), suggesting that strict quarantine of susceptible fish is needed to prevent inadvertent spread.

PROBLEM 29
Ichthyobodosis (Costiosis, *Ichthyobodo necator* Complex)

Prevalence Index
WF - 2, CF - 1, CM - 4
Method of Diagnosis
1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination
Typical signs of protozoan ectoparasite; especially, drop in temperature; bluish or whitish film on body
Treatment
1. Formalin bath
2. Formalin prolonged immersion
3. Potassium permanganate prolonged immersion
4. Raise temperature >30°C (86°F)
5. Salt bath (freshwater only)
6. Secnidazole oral
7. Triclabendazole
8. Metronidazole oral

COMMENTS
Epidemiology
Ichthyobodo necator (previously known as *Costia necatrix*) is one of the smallest ectoparasites that infest fish (about the size of a red blood cell). *Ichthyobodo* is especially dangerous to young fish and can attack healthy fry and even eggs. In older fish it is associated with some type of predisposing stress.

Ichthyobodo necator causes disease over a wide temperature range (2–30°C [36–86°F]). In warm water fish, it is usually a problem in cooler temperatures (<25°C [77°F]) and is reported to die above 30°C (86°F; Langdon 1990). Parasites from cold water fish (e.g.,

salmonids) have concomitantly lower temperature optima.

While classically a disease of freshwater fish, *Ichthyobodo* can survive transfer to seawater and cause mortality in marine-adapted salmonids (Urawa and Kusakari 1990). *Ichthyobodo* also occurs in purely marine fish (Cone and Wiles 1984; Diamant 1987; Bokeny et al. 1994; Morrison and Cone 1986; Todal et al. 2004; Callahan et al. 2005). While *Ichthyobodo* has previously been considered to constitute mainly a single species (*I. necator*), transmission experiments suggested that marine isolates from flatfish may be a different species of *Ichthyobodo* (Urawa and Kusakari 1990). Most recently, molecular genetic analysis has confirmed that this taxon is a multispecies complex (*Ichthyobodo necator* complex) and contains at least 9 different species with varying host preferences (Todal et al. 2004; Callahan et al. 2005). Many of these species infest multiple hosts, indicating that movement of infested fish from one region to another has a high potential for spreading exotic isolates. In one instance, the same species was obtained from both marine and freshwater fish, further suggesting that, unlike virtually all other protozoan ectoparasites, certain *Ichthyobodo* species may not be limited by salinity. Recent morphological and molecular studies have resulted in the description of a new species, *Ichthyobodo hippoglossi*, which infests Atlantic halibut (Isaksen et al. 2007). *Ichthyobodo*-like flagellates also occur on octopus (Forsythe et al. 1991); it is not known if these can infest fish.

Pathogenesis

Ichthyobodo exists in two forms (Joyon and Lom 1969). The detached, mobile form (Fig. II-29, A_1 and B) has two or, if predivisional, four flagella, all of which are difficult to see in actively moving parasites. While the parasite feeds on the fish, it is curled into a pyriform shape and is attached to and penetrates the epithelium

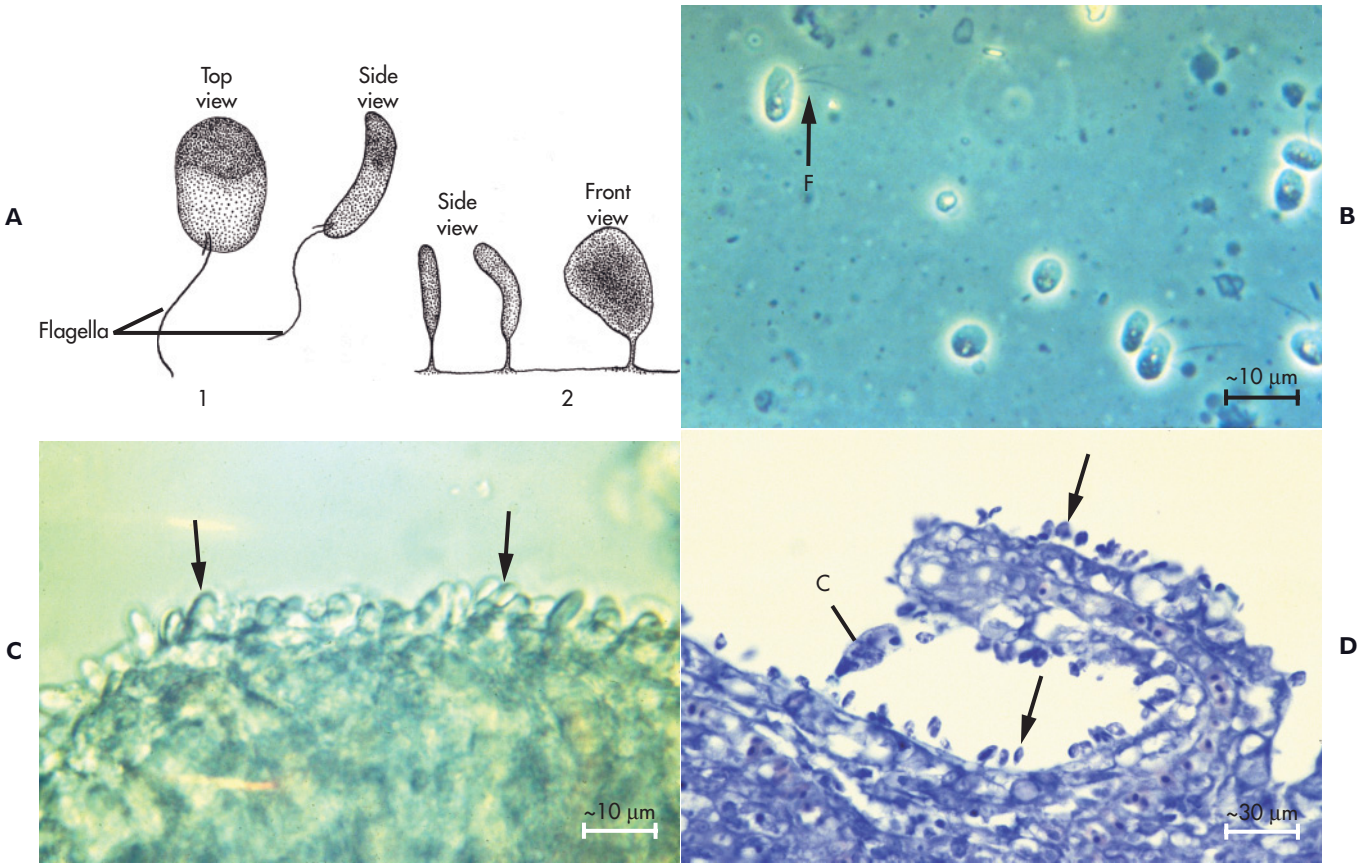

Fig. II-29. A. *Ichthyobodo*. Diagrams with key characteristics: (*1*) Free-swimming stage: size (~5–8 × 10–15 µm); slightly asymmetrical; oval body on top view; flattened, crescent shape on side view; single or paired flagella directed posterolaterally. (*2*) Attached stage: pyriform shape; flagella are not easily seen when attached. B. Wet mount of the free-swimming stage of *I. necator*. *F* = flagellum. C. Wet mount of many *Ichthyobodo* (*arrows*) attached to the gill epithelium. D. Histological section of gill with a heavy *I. necator* infestation (*arrows*). Note the pyriform, dorsoventrally flattened shape on side view. A larger, unrelated ciliate (*C*) is also present. Giemsa. (*B* and *C* photographs courtesy of G. Hoffman.).

(Fig. II-29, A_2, C, and D). The transition between forms occurs within a few minutes.

Ichthyobodo can cause considerable mortalities—sometimes with little obvious pathology (Fig. II-29, D), but other times with spongiosis and epithelial sloughing. Tissue irritation also leads to epithelial hyperplasia and increased mucus production, giving fish a bluish cast (slime).

Diagnosis

Diagnosis of the genus *Ichthyobodo* is easily made from skin or gill biopsies (Fig. II-29, B and C). The free-swimming form exhibits a characteristic flickering motion when it moves, which is caused by the change of refractility when it turns its crescent-shaped body. Attached parasites are more difficult to detect, but, in heavy infestations, they can be located by focusing up and down at high magnification on the edge of the gill epithelium, where they form palisades. They may also be seen slowly swaying while attached.

Small numbers of parasites (e.g., <~2 per high power field on a gill biopsy) usually do not cause clinical signs. *Ichthyobodo* may quickly leave a dead host, making estimations of parasite numbers in histological sections difficult. Note that cryptobids (see PROBLEM 30) and nonpathogenic, ectocommensal bodonid flagellates may also be found on fish skin and gills; these should not be confused with *Ichthyobodo*.

Regarding identification of a particular *Ichthyobodo* species, recent studies have demonstrated that morphological differences among certain species are evident in stained smears (Isaksen et al. 2007). However, such morphological features are yet to be determined for the majority of presumptive species.

Treatment

One application of an appropriate treatment usually controls ichthyobodosis, but infestations on euryhaline species may be resistant to salt treatment. *Ichthyobodo* appears to be an obligate parasite.

Secnidazole, triclabendazole and metronidazole all appear to be highly effective as oral medications but are all too expensive to be economically feasible on commercial food fish farms (Tojo and Santamarina 1998b). Whether different *Ichthyobodo* species vary in drug susceptibility is unknown. With the realization that multiple species of this parasite exist, care should be taken to avoid introducing *Ichthyobodo*-infested fish into new environments.

PROBLEM 30

Gill *Cryptobia* Infestation (Cryptobiosis)

Prevalence Index

WF - 3, WM - 3, CF - 3, CM - 4

Method of Diagnosis

1. Wet mount of gills with parasite
2. Histopathology of gills with parasite

History/Physical Examination

Typical signs of protozoan gill ectoparasite; especially emaciation, anorexia

Treatment

1. Formalin bath
2. Formalin prolonged immersion

COMMENTS

Epidemiology/Pathogenesis

Cryptobia is a widely distributed group of 10 species of kinetoplastid flagellates that can colonize many freshwater or marine fish. They are weak pathogens. One of the most common species is *Cryptobia branchialis*, a drop-shaped ($12–22 \times 3.5–4.5\ \mu m$) bacteriovore common in polluted fresh or marine waters.

Diagnosis

Cryptobia is distinguished from the morphologically similar *Trypanoplasma* (see PROBLEM 44) by its less developed undulating membrane and its tissue predilection (gill or gastrointestinal tract; see PROBLEMS 44 and 75). Taxonomically related nonpathogenic, ectocommensal, bodonid flagellates occasionally inhabit the gills. *Cryptobia* is differentiated from *Ichthyobodo* (see PROBLEM 29) by its morphology, flowing, amoeboid motility; and relatively superficial attachment to gill tissue via its recurrent flagellum (Fig. II-30).

Treatment

Gill cryptobids are easily treated with formalin, but eliminating the culpable stress will often allow spontaneous recovery.

PROBLEM 31

Gill Amoebic Infestation (Amoebic Gill Disease, AGD, Neoparamoebosis)

Prevalence Index

CF - 4, CM - 2

Method of Diagnosis

1. Wet mount of gills with parasite
2. Histopathology of gills with parasite

History/Physical Examination

Typical signs of protozoan gill ectoparasite

Treatment

1. Freshwater bath for 2–6 hours (*Neoparamoeba* only)
2. Formalin bath (cochliopodid only)

COMMENTS: NEOPARAMOEBA

Epidemiology/Pathogenesis

Neoparamoeba (= *Paramoeba*) *perurans* is a major impediment to cage-cultured Atlantic salmon production in Tasmania, where it is estimated to account for 20% of production costs (Morrison et al. 2006b). It has also caused intermittent, serious epidemics in Ireland, France, Norway, and the United States (Washington). Marine-cultured rainbow trout are also affected. It is an emerg-

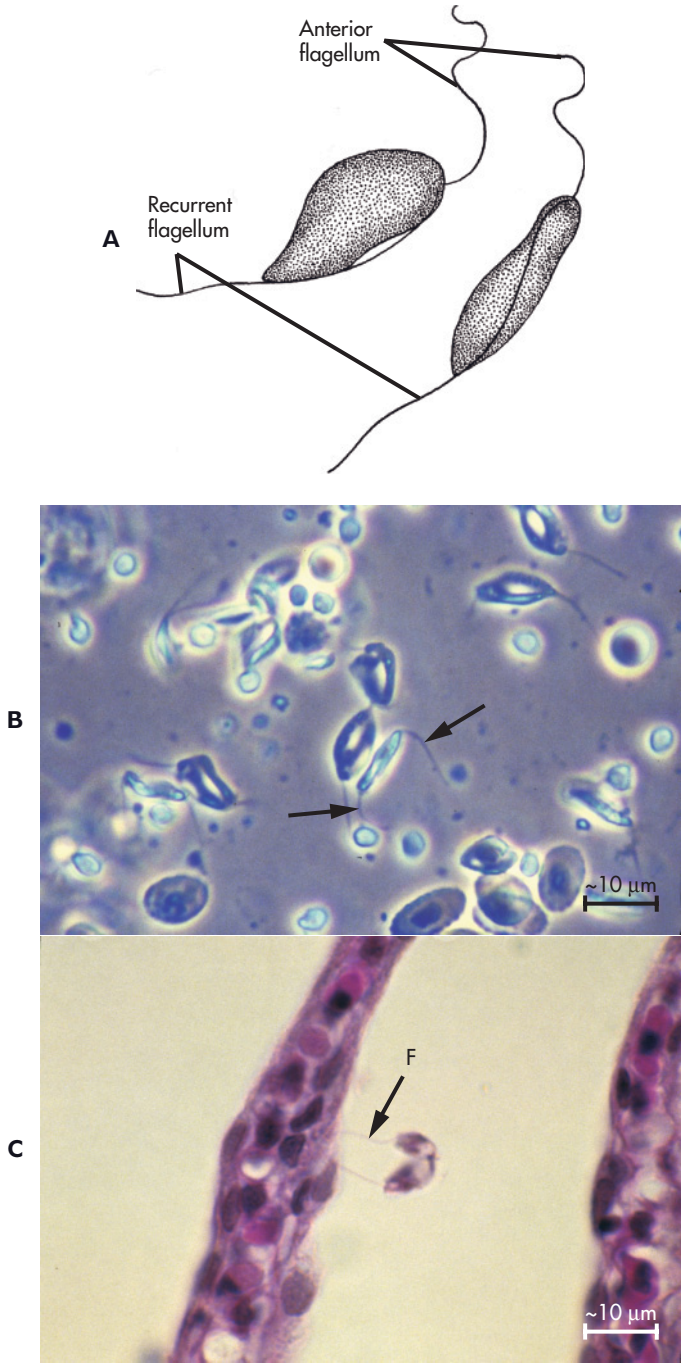

Fig. II-30. A. *Cryptobia*. Diagram with key characteristics: size (~10–20 × ~3–6 µm); pleomorphic shape; two flagella (one directed anteriorly and the other [recurrent flagellum] directed posteriorly). The recurrent flagellum sometimes forms a short, undulating membrane (see *Trypanoplasma*). B. Wet mount of *Cryptobia eilatica* from the gills of European sea bass. Note the two flagella (*arrows*), directed anteriorly and posteriorly. C. Histological section of two cryptobids from striped bass attached to a gill secondary lamella by their recurrent flagellum (*F*). Hematoxylin and eosin. (*B* photograph courtesy of A. Diamant; *C* photograph by L. Khoo and E. Noga.)

ing problem for turbot culture in northwest Spain and has also been reported in European seabass and sharpsnout sea bream (Dyková and Novoa 2001). It has caused chronic mortality (up to 2% per day) in Atlantic salmon and up to 25% losses over 3 months in turbot (Kent 1992; Zilberg and Munday 2006).

The infestation is transmitted in water (Akhlaghi et al. 1996) and amoebae are widespread in the environment (marine sediment, cage surfaces, etc.; Nowak et al. 2000). Long-term infestations in salmonids require high (>32 ppt) salinity, while infestations in turbot can occur at 22 ppt, a salinity that totally cures salmonids of the infestation (Zilberg and Munday 2006). Severity increases with temperature, with disease occurring in salmonids at 12–20°C (54–68°F). Crowding, poor water exchange, cage fouling, and previous gill damage may be risk factors (Kent et al. 1988b). Outbreaks typically occur in the first summer after transfer of fish from freshwater to sea cages. Clinically affected fish develop elevated serum sodium levels before the onset of behavioral signs (Munday 1988). Grossly, the gills display excess mucus and multiple whitish-grey, swollen foci due to the lamellar hyperplasia. Gross lesions are more diffuse in rainbow trout. In turbot, there are clubbed filaments and excess mucus with patches of grayish discoloration on the periphery. The main histopathological lesion in salmonids and turbot is multifocal lamellar hypertrophy with epithelial hyperplasia (Fig. II-31, A and B), eventually resulting in severe lamellar fusion. There is a primary focal neutrophilic, then mononuclear infiltrate. There may also be mucus cell hyperplasia. Recovery, especially in rainbow trout, is characterized by focal lymphoid nodules at the base of the secondary lamellae.

Diagnosis

Strong presumptive diagnosis can be made by observing typical gross lesions with characteristic histopathology. Definitive diagnosis requires specific identification of *Neoparamoeba perurans*. Like all amoebae, *Neoparamoeba* is best diagnosed by examining it in wet mounts. Wet mounts reveal free-floating amoebae (15–40 µm diameter) with up to 50 digitiform pseudopodia. Amoebae will attach to the slide within an hour, allowing observation of one or more parasomes. The parasome is a symbiotic organism (*Perkinsiella amoeba*) that lives within the cytoplasm. Fixation and Fuelgen-staining also reveals the parasome (Fig. II-31, C, D, and E). Amoebae remain best attached with Bouin's or Davidson's fixative and have a hyaline ectoplasm fringing the granuloplasm. In histological sections, the amoeba is vacuolated and adherent to the gill epithelium (Figs. II-31, B and E).

Neoparamoeba pemaquidensis was previously considered the presumptive cause of AGD. Two other species, *N. branchiphila* (Dyková et al. 2005) and *N. perurans* (Young et al. 2007), were later implicated in AGD lesions in Tasmania. But, more recent data indicates that

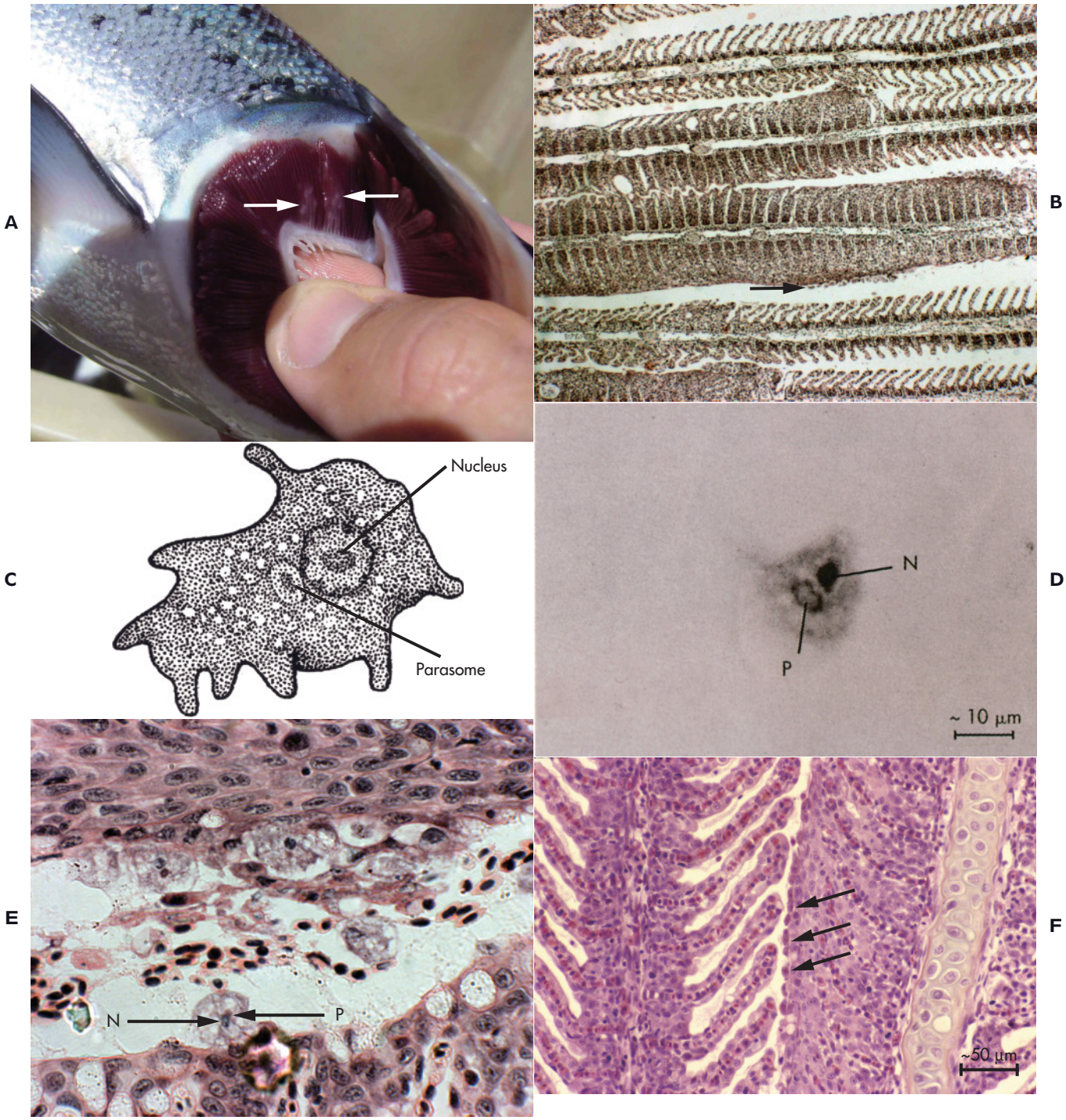

Fig. II-31. A. Gill of Atlantic salmon with amoebic gill disease. Note the multiple, whitish-grey, swollen foci (*arrows*) due to lamellar hyperplasia. B. Histological section of *Neoparamoeba perurans* (*arrow*) on the gills of an Atlantic salmon. Note the focal, severe lamellar hyperplasia and branchitis on parasitized lamaellae. Hematoxylin and eosin. C. *Neoparamoeba perurans*. Diagram with key characteristics: size (~25 µm); nucleus; parasome. D. Stained smear of *Neoparamoeba*. Note the nucleus (*N*) and parasome (*P*). Feulgen stain. E. Histological section of *Neoparamoeba perurans* on gill showing the nucleus (*N*) and parasome (*P*). Hematoxylin and eosin. F. Histological section of cochliopodid amoeba (*arrows*) on the gills of a rainbow trout. Hematoxylin and eosin. (*A*, *B*, and *E* photographs courtesy of M. Adams; *D* photograph courtesy of M. Kent; *F* photograph by L. Khoo and E. Noga.)

Neoparamroeba perurans is the cause of AGD not only in Tasmania, but worldwide in both salmonids and other species (Steinum et al. 2008; Young et al. 2008). Gene probes have been developed to detect infested fish and differentiate the different species of *Neoparamoeba* (Young et al. 2007). Antibody probes can identify some species (Zilberg and Munday 2006b), but cross-reactivity with other amoebae makes them less reliable.

Treatment

Although stressful, the most effective and commonly used treatment is a 2- to 6-hour, on-farm, freshwater bath in a treatment cage having freshwater that is towed to the culture cages. The need to have ready access to freshwater limits the locations where culture cages can be sited (Morrison et al. 2006b). Hyposalinity not only kills the amoebae, but also reduces the osmotic stress from gill damage. Most importantly, it also removes excess mucus. Using soft water is best, probably because it enhances mucus sloughing (Roberts and Powell 2002). However, there is some evidence that the parasite is developing resistance to freshwater baths (Parsons et al. 2001). Alternatively, fish can be transferred to brackish water if feasible; salinity must be <4 ppt to be effective. *Neoparamoeba* is resistant to most other ectoparasiticides. Bithionol has shown efficacy in experimental trials (Florent et al. 2007). It is more difficult to control at higher temperatures (16–18°C [61–64°F]).

Salmonids develop resistance to reinfection after a single exposure but no vaccine is available. Levamisole has experimentally induced increased resistance to reinfection (Findlay et al. 2000) but results are inconsistent. There is experimental evidence that isolates become more virulent with increased passage on fish, so an all in–all out strategy might be preferable to continued culture of multiple age classes (Zilberg and Munday 2006). An experimental vaccine is under development.

OTHER GILL AMOEBIC INFESTATIONS

Cochliopodid Amoeba

Cochlipodid amoebae (Fig. II-31, C) have incited proliferative responses in rainbow trout that may appear as grossly visible nodular gill masses (nodular gill disease) in the United States, Canada, and Germany (Daoust and Ferguson 1985; J. Lom, personal communication); in some cases, cochliopodid infestations have developed after fish were treated for bacterial gill disease (A. Noble, personal communication; Bullock et al. 1994).

Other amoebae have been rarely reported as infestations of the gills of various fish (Dyková and Lom 2004). All amoebae can be definitively diagnosed by examining them in wet mounts. In many cases, this is mandatory for identification since no antibody or gene probes are available.

PROBLEM 32

Sessile, Solitary, Ectocommensal Ciliate Infestation

Prevalence Index

WF - 1, CF - 2

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, organically polluted water

Treatment

1. Formalin bath
2. Formalin prolonged immersion
3. Copper prolonged immersion

COMMENTS

Epidemiology/Pathogenesis

The sessile, solitary, ectocommensal ciliates *Apiosoma*, *Riboscyphidia*, and *Ambiphrya* attach to the skin or gills with a holdfast (scopula) (Fig. II-32, A_1, A_2, A_3, B, C, D, and E) (Lom 1973b). Attachment apparently causes only superficial damage to the epithelium (Lom and Corliss 1968), which belies the ectocommensal nature of these organisms. Like the sessile, colonial ectommensals (see PROBLEM 33), they reproduce by binary fission and use the host primarily for attachment. They derive little, if any, nutrition directly from the fish. They feed on bacteria and suspended organic debris, which is prevalent in nutrient-rich (i.e., polluted) water. Thus, they are good indicators of poor water quality. Many of these ciliates can probably be free-living. They are only moderately pathogenic, but high numbers on the gills can physically impede gas exchange. They may also act as a nidus for bacterial colonization.

Capriniana (formerly *Trichophrya*) *piscium* is a common suctorian ciliate that commonly infests gills of channel catfish, Eurasian perch and northern pike, among other species (Fig. II-32, A_4). It has no cilia when attached to fish; instead, it has characteristic tentacles that emanate from an amorphous body (Fig. II-32, F and G). Attachment probably causes little damage to the epithelium, but heavy infestations can cause mechanical blockage of respiration. *Capriniana* feeds on ciliates and suspended organic debris. *Capriniana* is not taxonomically related to *Apiosoma*, *Riboscyphidia*, or *Ambiphrya* and reproduces by budding, forming motile, ciliated stages that can colonize a new host (Lee et al. 1985).

Diagnosis

Diagnosis is easily made from wet mounts or histology. Identification to species is not needed, since all members of the same genus are treated similarly.

Treatment

Like the sessile, colonial, ectocommensal ciliates (see PROBLEM 33), medical treatment should always be

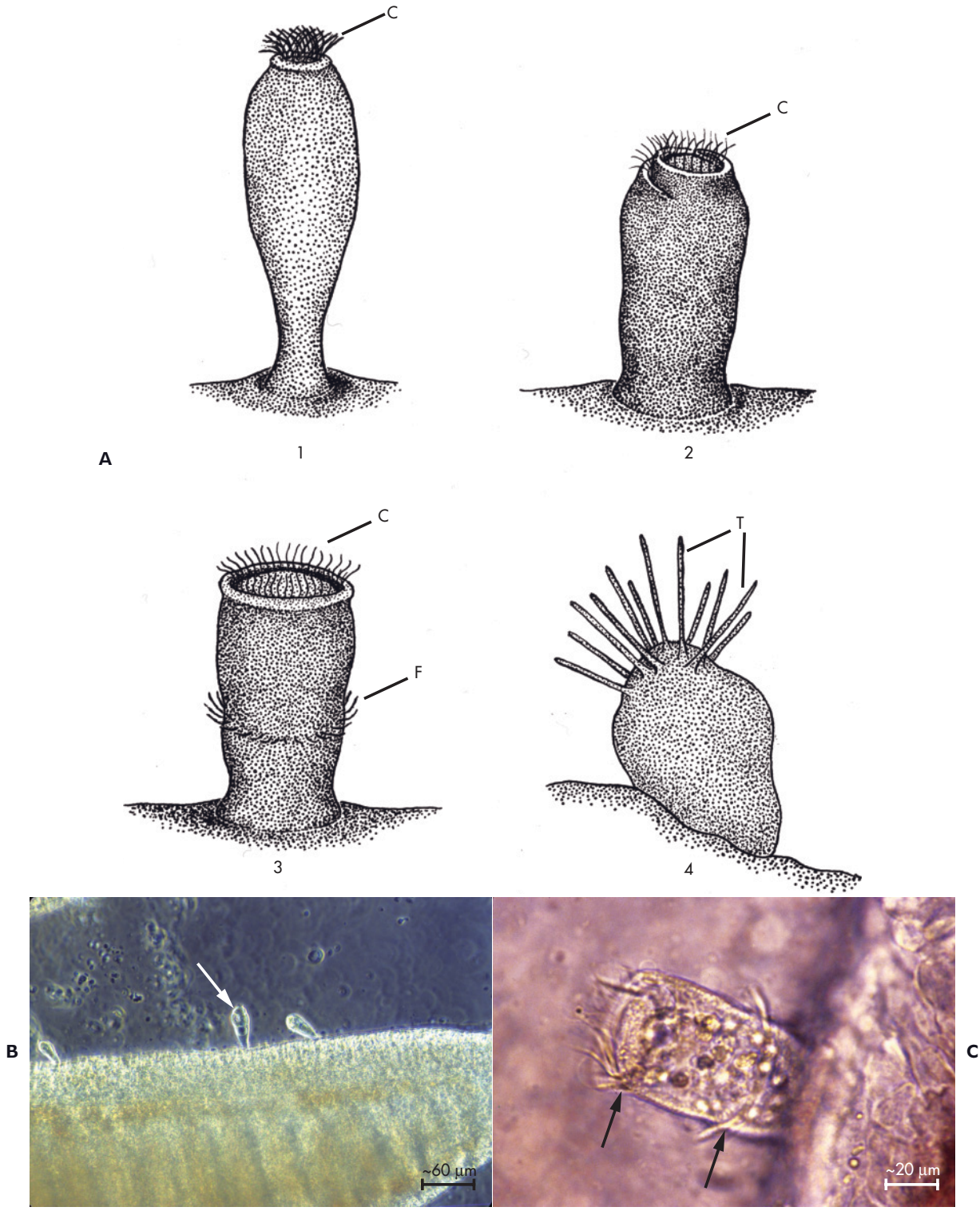

Fig. II-32. A. Sessile, solitary, ectocommensal ciliates. Diagrams with key characteristics (*C* = cilia). Most range from ~40 to 100 µm. All except *Capriniana* may occur on skin or gills: (*1*) *Apiosoma* (*66* species); elongated body; only oral cilia; freshwater; (*2*) *Riboscyphidia* (~18 species): cylindrical to conical body; only oral cilia; freshwater or marine; (*3*) *Ambiphrya* (4 species): cylindrical to conical body; oral cilia; permanent, motionless, equatorial, ciliary fringe (*F*); freshwater; (*4*) *Capriniana piscium:* variable size (usually 40–110 × 25–70 µm); pleomorphic shape; feeding tubes (*T*); body adhered to secondary lamella of gill. B. Wet mount of *Apiosoma* (formerly *Glossatella*) infestation (*arrow*). Note the vase shape. C. Wet mount of *Ambiphrya* (formerly *Scyphidia*). Note the oral and aboral cilia (*arrows*).

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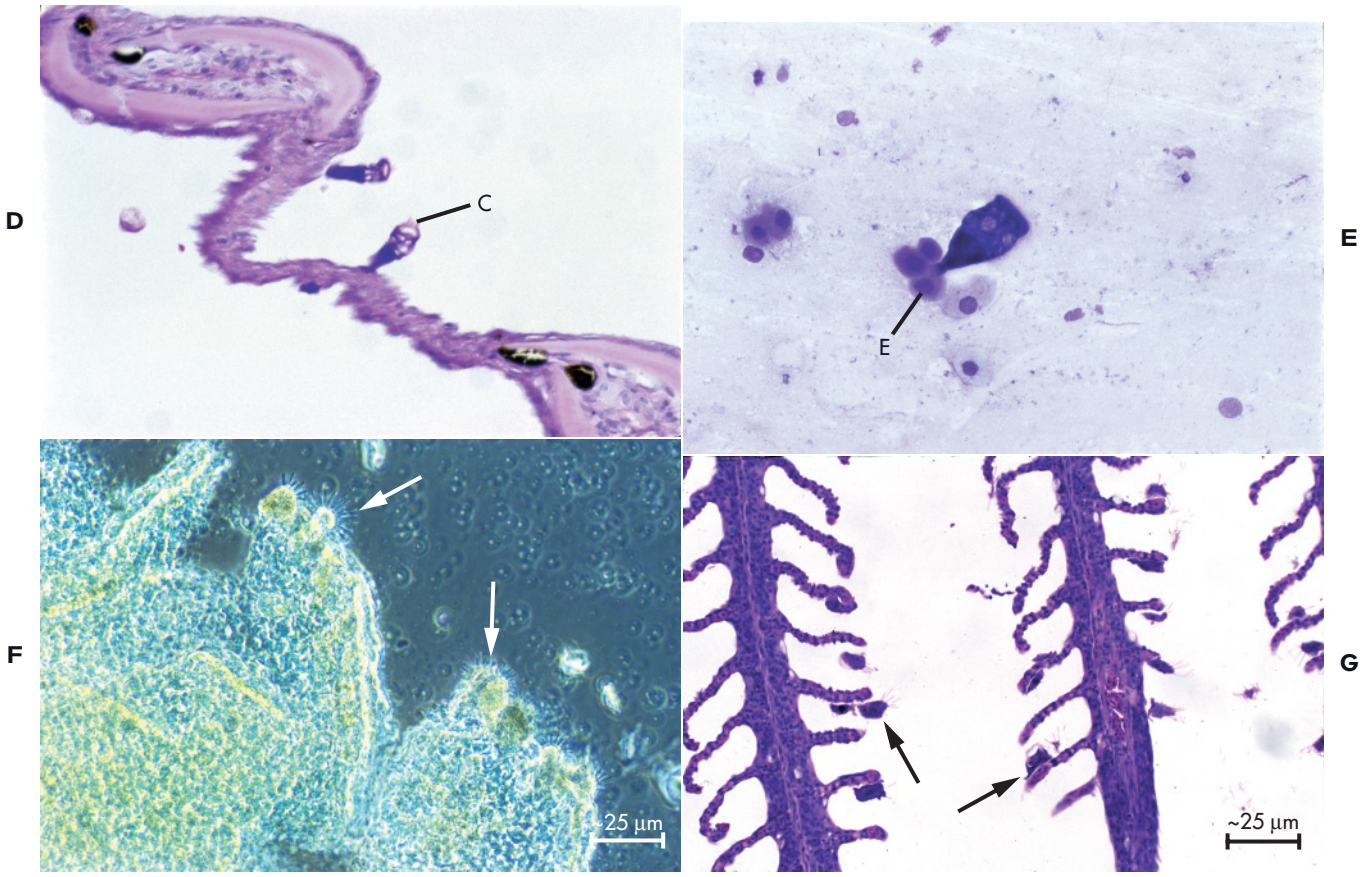

Fig. II-32.—cont'd. D. Histological section of *Apiosoma* attached to skin. *C* = cilia. Hematoxylin and eosin. E. Stained smear of a *Apiosoma*. Note that the parasite is still attached to epithelial cells (*E*) of the skin. Modified Wright's. F. Wet mount of *Capriniana piscium* (*arrows*). Note the feeding tubes. G. Histological section of *Capriniana piscium* (*arrows*). Note the feeding tubes protruding from parasites that have adhered to base of secondary lamella. Hematoxylin and eosin. (*C* photograph courtesy of A. Colorni.)

accompanied by an improved environment. Treatment with formalin is usually effective for freshwater species of *Apiosoma*, *Riboscyphidia*, and *Ambiphrya*. Treatments for marine pathogens have not been established, but they probably respond to similar remedies. *Capriniana* can be resistant to formalin and should be treated with copper.

PROBLEM 33

Sessile, Colonial, Ectocommensal Ciliate Infestation (Red-Sore Disease)

Prevalence Index

WF - 1, CF - 4

Method of Diagnosis

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite

History/Physical Examination

Typical signs of protozoan ectoparasite; also, various-sized amorphous masses on the skin, in mouth, or on gill arches; organically polluted water

Treatment

1. Formalin bath
2. Formalin prolonged immersion
3. Potassium permanganate prolonged immersion
4. Salt bath weekly × 3
5. Salt prolonged immersion

COMMENTS

Epidemiology/Life Cycle

Epistylis is the most common and pathogenic type of sessile, colonial ectocommensal ciliate. It is commonly associated with a mixed infection of Gram-negative bacteria known as red-sore disease (Esch et al. 1976). This bacteria-parasite complex is common in pond-raised fish

in the southern United States and elsewhere, especially during warmer months. Centrarchids, ictalurids, *Morone* spp., and many other fish are susceptible.

Stalked ectocommensal ciliates reproduce by binary fission along the longitudinal axis. To move to another site, the zooid in the colony transforms into a disc-shaped telotroch with equatorial cilia for locomotion. Like other ectocommensal protozoa (see PROBLEM 32), these organisms feed on bacteria and other small food items present in the water. They use the fish as a surface for attaching. Thus, their presence is indicative of organically polluted water that would tend to have a high concentration of bacteria. *Epistylis* can be free-living (W. Rogers, personal communication), but such species can only colonize severely debilitated fish (J. Lom, personal communication). Most *Epistylis* infestations are caused by species that are more adapted to feeding on fish. One species of *Epistylis* was transferred by some investigators into the genus *Heteropolaria* (Foissner et al. 1985).

Pathogenesis

Epistylis produces white or hemorrhagic lesions (red-sores) on the flanks or on the tips of bony prominences, such as the fins (Figs. II-33, A and B), jaws, or gill cover. They may also infect the oral cavity or gills. They must attach to a hard surface and thus anchor to some calcified tissue (e.g., fin ray, scale). The skin is ulcerated wherever they are attached and lesions always have bacterial infections, especially aeromonads and other Gram-negative rods (Esch et al. 1976; see PROBLEM 46). Red-sore lesions may become secondarily invaded by water molds. Gross lesions may also look similar to water mold infections (see PROBLEM 34). Lesions tend to be chronic, but acute mortalities can occur, usually caused by systemic bacterial infection.

Diagnosis

Typical stalked, noncontractile zooids are diagnostic (Fig. II-33, C, D, and E). *Epistylis* zooids have a C-shaped macronucleus and should not be mistaken for ich trophonts (see PROBLEM 20) or trichodinids (see PROBLEM 22) when they are detached from their colonies. The colony stalk without zooids should not be mistaken for fungal hyphae. Several other colonial peritrichs rarely colonize debilitated fish: the stalks of *Vorticella*, *Zoothamnium*, and *Carchesium* are contractile, and either unbranched with a single zooid or branched, bearing many zooids (Lom and Dyková 1992).

Treatment

Epistylis infestations are occasionally resistant to formalin; salt baths (Foissner et al. 1985) or prolonged salt exposure is usually effective, if tolerated by the fish. Advanced cases may need to be treated for systemic bacterial infections. Other stalked ectocommensals from freshwater fish probably respond to the treatments listed. Marine ectocommensals are probably susceptible to formalin or a freshwater bath.

PROBLEM 34

Typical Water Mold Infection (Saprolegniosis, Oomycete Infection, Winter Kill)

Prevalence Index

WF - 1, CF - 1

Method of Diagnosis

Wet mount of skin or gills having broad (7–30 μm), nonseptate hyphae

History/Physical Examination

White, brown, red, or green cottony mass on skin or gills (slimy glistening mass when fish is out of water); acute stress, especially temperature drop, recent transport, or trauma

Treatment

1. Salt prolonged immersion
2. Copper prolonged immersion
3. Bronopol bath
4. Hydrogen peroxide bath (eggs only)
5. Formalin bath (eggs only)
6. Malachite green bath
7. Malachite green prolonged immersion
8. Malachite green flush
9. Malachite green constant flow
10. Malachite green swab
11. Methylene blue prolonged immersion (eggs only)

COMMENTS

Epidemiology

Water molds (class Oomycetes) are one of the most common infections of freshwater fish and are far more common than true fungal infections (see PROBLEM 72). Water molds are also important pathogens of some estuarine fish in warm temperate and tropical waters (see PROBLEM 35); they are distributed worldwide. Virtually every freshwater fish is probably susceptible to at least one species. The class Oomycetes is divided into four orders, three of which have species that can infect fish (Saprolegniales, Leptomitales, and Peronosporales). The great majority of fish pathogens are in the family Saprolegniaceae (Saprolegniales). Some Oomycetes can infect amphibians; others are important pathogens of aquatic invertebrates.

Water molds are classical opportunists that normally feed saprophytically on dead organic matter. There is increasing evidence that these infections in fish are associated with immunosuppression. Outbreaks often occur after a drop in temperature or when temperatures are near the physiological low end for a particular fish species (Roberts 1989c). This may be due not only to lower immunity, but also to the fact that many Oomycetes are

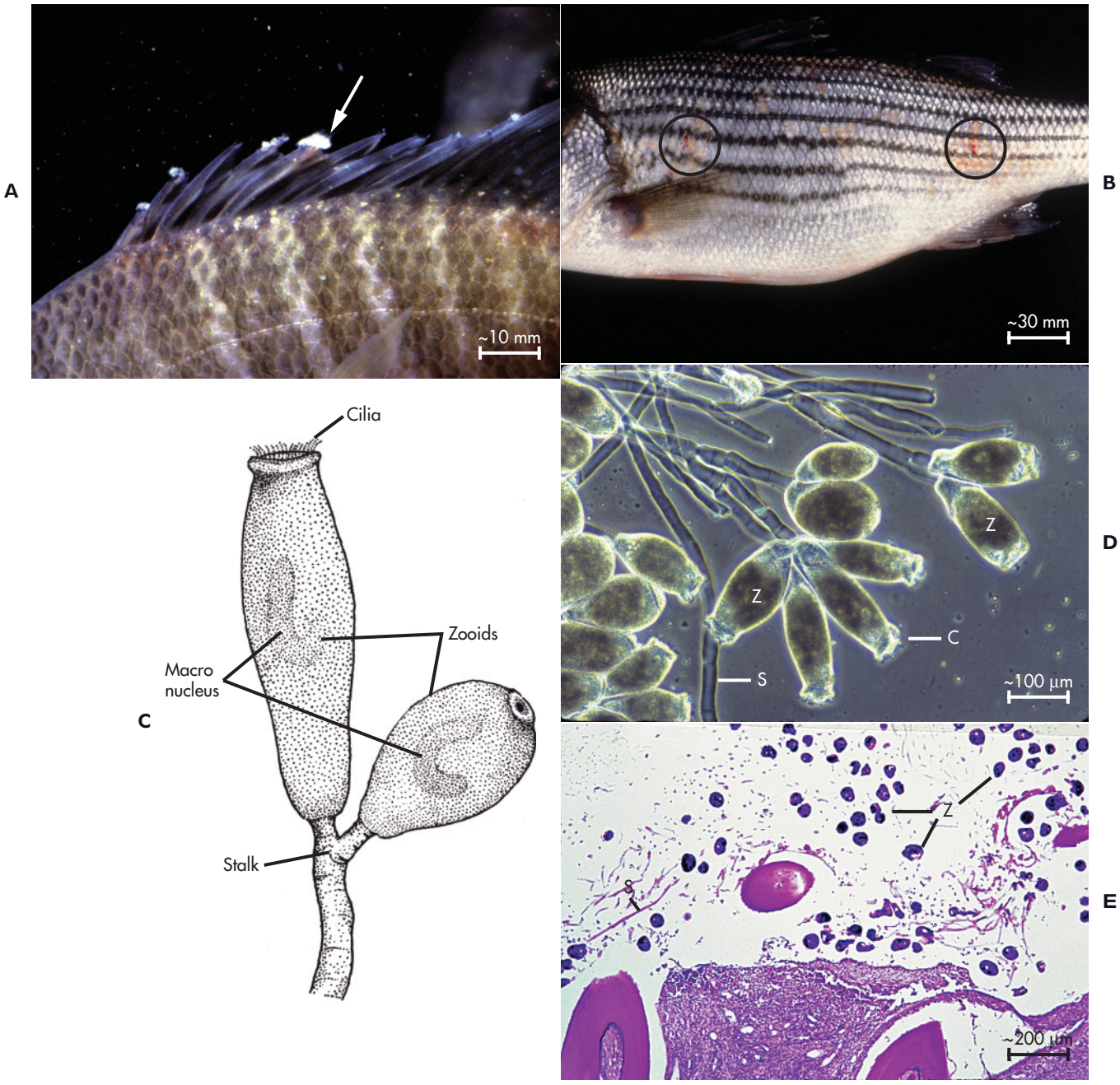

Fig. II-33. A. *Epistylis* infestation on the dorsal fin of a bluegill (*arrow*). B. *Epistylis* infestation on the skin of a striped bass (*circles*). C. *Epistylis*. Diagram with the following key characteristics: size of individual zooids (150–300 × 40–60 µm or 50–80 × 20–30 µm); cilia surrounding the mouth; stalk connecting zooids. D. *Epistylis*. Wet mount of a skin scraping: *Z* = zooids; *C* = cilia; *S* = stalk. E. *Epistylis*. Skin lesion with zooids (*Z*). *S* = stalk. Hematoxylin and eosin. (*A* photograph by S. Smith and E. Noga.)

more active in the cooler months of the year (Hughes 1962). Skin wounds caused by mechanical trauma or other pathogens provide a portal of entry for water molds (Tiffney 1939a, 1939b; Scott and O'Bier 1962). Handling, crowding, heavy feeding rates, and high organic loads also appear to increase the risk of saprolegniosis. When fish are exposed to acute confinement stress, their skin can actually slough off (Udomkusonsri et al. 2004) and this severe skin loss makes them much more susceptible to water mold infection (Udomkusonsri and Noga 2005).

Most lesions are caused by *Saprolegnia* (which is why the disease is called saprolegniosis), but other Oomycetes cause a clinically identical disease. There may be primarily parasitic strains of water molds. For example, *Saprolegnia parasitica* appears to be highly pathogenic, while *Pythium* and *Leptomitus* are only weakly pathogenic (Scott and O'Bier 1962). Of all the Oomycetes, *Saprolegnia parasitica* and *S. diclina* are probably the two species most commonly isolated from fish; they are closely related to each other and are often referred to as the *S. diclina-S. parasitica* complex (Neish and Hughes 1980; Noga 1993b). Most studies of this complex have involved salmonids. More than one pathogen may occur in lesions (Pickering and Willoughby 1977).

When saprolegniosis affects fish during very cold temperatures, it is often called winter kill. The disease occurs when pond temperatures drop below 15°C (59°F) and often after a cold front has rapidly dropped the temperature. The disease may be caused by immunosuppression because of the rapid temperature drop, possibly in combination with chronically high ammonia levels or exposure to some environmental stress in the prior summer/fall. A similar clinical pattern has been observed in channel catfish and hybrid striped bass (S. Gabel, personal communication).

Transmission

Water molds are ubiquitous saprophytes in soil and freshwater. They are appropriately named, requiring water for growth and sporulation; this differentiates them from most terrestrial (true) fungi that can produce aerial spores. Most transmission is probably by motile zoospores (Fig. II-34, A) produced by the vegetative hyphae, although other reproductive stages (e.g., gemma) may also be important. The zoospore allows dissemination to distant sites. It is important to realize that most fish infections are probably acquired from inanimate sources (i.e., water molds sporulating on dead organic matter).

Pathogenesis

Typical water mold infection presents as a relatively superficial, cottony growth on the skin or gills (Fig. II-34, B). Such lesions usually begin as small, focal infections that can rapidly spread over the surface of the body. It is not unusual for large lesions to suddenly appear within 24 hours. Newly formed lesions are white due to the presence of the mycelia; with time, the lesions often become colored red, brown, or green because of the trapping of sediment, algae, or debris in the mycelial mat (Fig. II-34, C). If the water mold is observed on a fish removed from the water, the mycelium appears as a slimy, matted mass on the body (Fig. II-34, D).

Although they grow rapidly over the skin's surface, typical water mold infections rarely penetrate beyond the superficial muscle layers (Fig. II-34, F). Superficial damage to the skin or gills can be fatal. Loss of serum electrolytes and protein is proportional to the percentage of skin affected (Richards and Pickering 1979). Thus, morbidity and mortality increase as the amount of affected skin or gill tissue increases. With acute lesions, fish usually die within several days or recover within several weeks.

Oomycetes are important pathogens of fish eggs (see PROBLEM 103). Infections most often begin in unfertilized or otherwise nonviable eggs. Once established, they can rapidly spread to healthy eggs, eventually resulting in complete loss of the brood. Oomycetes rarely infect the gastrointestinal tract of small fry and may penetrate into the viscera.

Diagnosis

Although water molds have traditionally been referred to as aquatic fungi, recent molecular studies have indicated that they are more closely related to algae than to true fungi (Dick et al. 1999). However, oomycetes and true fungi share some morphological characteristics, such as production of hyphae and formation of spores for reproduction. However, hyphae of true fungi are septate while those of oomycetes are aseptate (Latijnhouwers et al. 2003).

Observation of a cottony, proliferative growth on the skin or gills should alert the clinician to a possible diagnosis of typical water mold infection. Some other pathogens (e.g., *Flavobacterium*, *Epistylis*) can cause grossly similar lesions but are easily differentiated microscopically.

Clinical diagnosis of typical water mold infection is easily made from wet mounts, which have broad, aseptate, hyphae of variable width (~7–30 μm; Fig. II-34, E). Histologically, presumptive diagnosis of saprolegniosis is based upon the presence of relatively shallow lesions that have broad, aseptate hyphae. Hyphae are usually visible with hematoxylin and eosin stain (H and E) and stain strongly with silver (e.g., Gomori methenamine silver, Fig. II-35, D). There is little inflammation, and the hyphae usually do not extend past the superficial muscle layers.

Diagnosis requires that affected fish be alive when examined, because water molds are ubiquitous saprophytes in soil, freshwater and, to some extent, estuarine environments; dead fish are fertile substrates for colonization. Oomycetes are also common secondary invaders

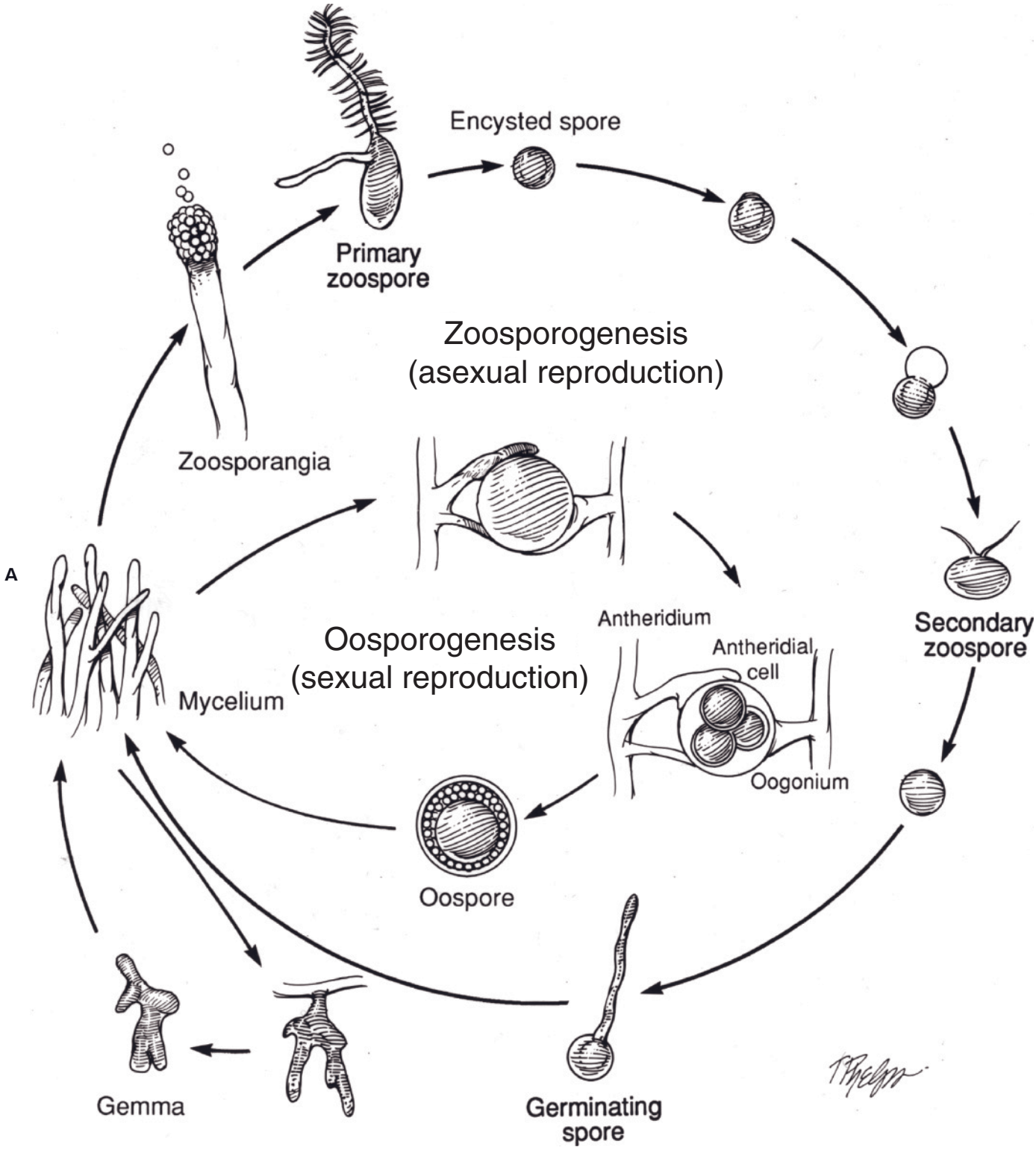

Fig. II-34. A. Life cycle of water molds (Noga 1993b).

Continued.

Fig. II-34.—cont'd. B. Water mold infection of a channel catfish. Note the large, white, cottony mass of hyphae (evident when the fish is in the water) and the loss of normal black pigment over the infected skin. C. Water mold infection of a channel catfish with winter kill. The water mold mycelium (*arrows*) is brown because of trapping of debris. D. Water mold infection (*arrows*) of a hybrid striped bass. Note the glistening, matted appearance compared to Fig. II-33, *B*. The mycelia are darker because of the trapped debris. E. Wet mount from a water mold infection. Broad, nonseptate hyphae (*H*). Zoosporangia (*Z*) are not always present in wet mounts of lesions. F. Histological section of a water mold infection of skin. Note the absence of epithelium, the superficial nature of the lesion, and the lack of inflammation. *H* = hypha; *S* = scale; *D* = dermis. Hematoxylin and eosin.

Continued.

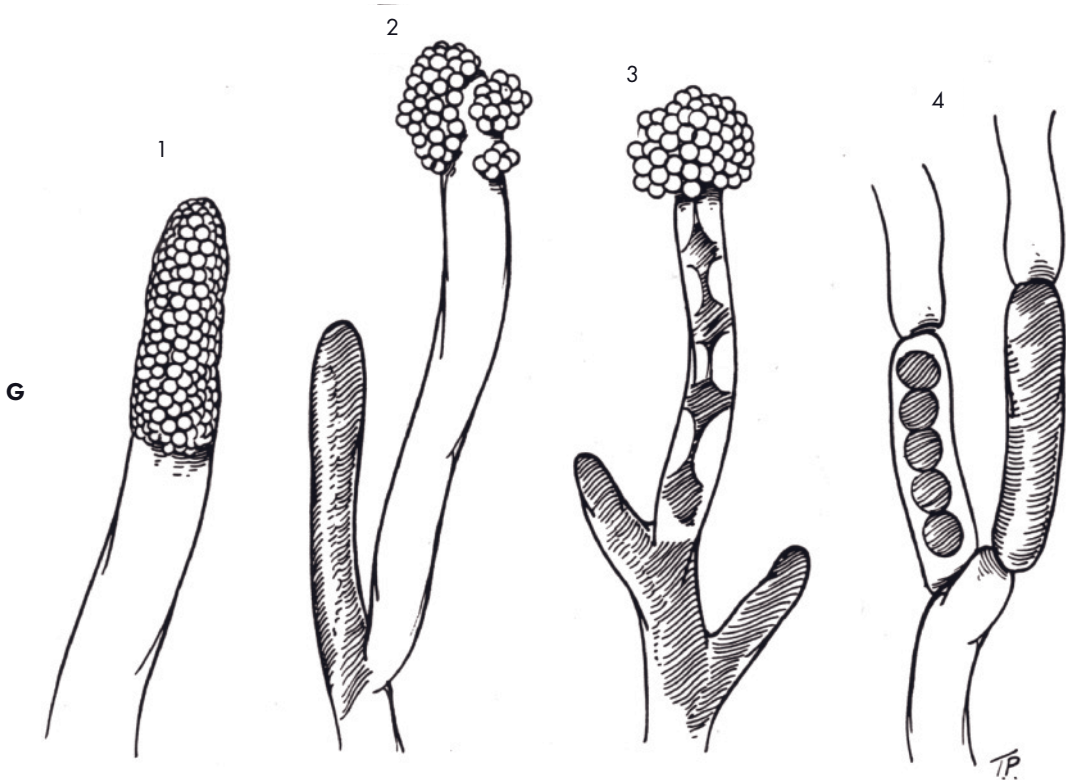

Fig. II-34.—cont'd. G. Zoosporangia of some fish-pathogenic Oomycetes: (*1*) *Saprolagnia;* (*2*) *Achlya;* (*3*) *Aphanomyces;* (*4*) *Leptolegnia* (Noga 1993a). (*B* photograph by R. Bullis and E. Noga; *E* photograph courtesy of A. Colorni.)

of wounds initiated by other pathogens (e.g., bacteria, parasites). In summary, the clinician should always look for other initiating causes when water molds are identified in a lesion.

When winter kill occurs in channel catfish, endophthalmia and a dry, mucus-depleted skin often precedes the appearance of focal water mold infections (Durborow and Crosby 1988). However, in most cases of saprolegnosis, such lesions are not readily apparent.

Identification of Specific Water Molds

Presumptive diagnosis (i.e., identification of broad, aseptate, hyphae in skin or gill lesions) is sufficient for clinical treatment decisions. Oomycetes vary in drug susceptibility in vitro, but the lack of similar data on clinical response to various drugs has made these differences academic. However, determining the type of oomycete involved will become a more important consideration as various therapies are compared in clinical situations.

Definitively determining that a pathogen is an oomycete requires the observation of asexual sporangia. Asexual sporangia also allow classification to genus (Fig. II-34, G). While sporangia are seen occasionally on infected fish (Fig. II-34, E), a culture is usually required to elicit these structures. Identification to species is based on sexual stages (Fig. II-34, A). Many isolates will not produce sexual stages in culture. See **p. 55** for details about isolation of water molds from lesions. Details of culture methodology and induction of reproductive stages are provided in Fuller and Jaworski (1987). Immunological methods also hold promise as future diagnostic tools (Bullis et al. 1990; Fregeneda-Grandes et al. 2007).

Importance of Water Molds in the Case

A diagnosis of saprolegniosis should always include a thorough search for underlying predisposing factors.

Treatment

PROGNOSIS

The chance of recovery from saprolegniosis is directly related to the amount of skin or gill infected by the agent. While mildly infected fish have a good chance of recovery with proper management, fish with large areas covered by hyphae (e.g., Fig. II-34, B through D) usually die. Prophylactic antibiotics may be needed to combat secondary bacterial infections.

TREATMENT OPTIONS

Water molds are among the most difficult diseases to treat. Except for salt, most agents legally approved for food fish (Schnick et al. 1986) are of limited effective-

ness. Malachite green is highly effective for treating water mold infections, but it is not approved for food fish use in most countries because of its teratogenic and mutagenic properties. Hundreds of agents have been tested against Oomycetes, with none being as efficacious as malachite green (Scott and Warren 1964; Olah and Farkas 1978; Alderman and Polglase 1984; Bailey 1984; Bailey and Jeffrey 1989). However, a very promising agent is bronopol, an enzyme inhibitor that has recently been shown to have efficacy similar to malachite green. It is also safer to use on eggs than malachite green. It is currently only approved for food fish in Europe.

Most fish-pathogenic water molds are inhibited by even low prolonged immersion salt concentrations (>3 ppt), which is probably why they do not affect marine fish in high salinities (see PROBLEM 35). Prolonged immersion salt also helps to counteract osmotic stress caused by skin damage and subsequent ion loss. Unfortunately, prolonged immersion salt is impractical in most commercial production situations. While there are species differences in the tolerance to various treatments (e.g., *Saprolegnia* is usually more resistant to most drugs than *Aphanomyces*), whether these are clinically relevant differences is unknown.

PROPHYLAXIS

Because of the acute, fulminating nature of many oomycete infections and their resistance to chemotherapy, prophylaxis is the best strategy. Avoid skin damage and predisposing stresses. Prolonged immersion salt is an effective prophylactic when transporting fish or acclimating them to a new environment. Water molds cannot be eliminated from any culture systems.

With winter kill, copper sulfate appears to be very effective at preventing water mold infections in channel catfish that do not yet have the disease. If channel catfish are treated with copper sulfate before the water mold infection is visible on the fish, copper treatment can prevent winter kill from occurring in over 90% of the fish (Bly et al. 1996; Li et al. 1996). This works because copper sulfate is much more lethal to the zoospore than it is to the water mold hyphae (after it has infected the fish). Thus, it is theoretically possible to prevent winter kill by killing the zoospores. However, on fish farms, winter kill does not always develop in a pond after passage of a cold front, and thus it is not possible to know for certain if winter kill will develop after a cold front passes through. This is probably because other factors, including how the fish responds to the cold stress, determine if winter kill will occur. Thus, there is no way of knowing when to use copper sulfate to prevent the infection because fish respond differently to the cold stress at different times. Furthermore, it would be irresponsible to use copper sulfate after every cold front, and such indiscriminate use might jeopardize its legal use in aquaculture.

PROBLEM 35

Epizootic Ulcerative Syndrome (EUS; *Aphanomyces invadans* Infection, *Aphanomyces piscicida* Infection, Atypical Water Mold Infection, Mycotic Granulomatosis, MG, Ulcerative Mycosis, UM, Red-Spot Disease [RSD])

Notifiable to OIE

Prevalence Index

WF - 2

Method of Diagnosis

1. Culture of *Aphanomyces invadans* from typical ulcers
2. Histology of skin or gills having broad (7–25 µm), nonseptate hyphae with typical ulcers
3. Wet mount of skin or gills having broad (7–25 µm), nonseptate hyphae with typical ulcers

History/Physical Examination

Shallow to deep skin ulcers

Treatment

None proven

COMMENTS

Epidemiology

Epizootic ulcerative syndrome differs from typical water mold infection (see PROBLEM 34) in being an extremely deep, penetrating lesion (Fig. II-35, A and B; compare with Fig. II-34, B). While atypical water mold infection is less common than typical water mold infection, it has become a serious disease, especially in the tropics (Frerichs et al. 1986; Kanchanakhan 2006).

EUS occurs in numerous freshwater and estuarine fish populations worldwide (Table II-35). The disease is a problem in wild, estuarine fish populations of the western Atlantic Ocean (Noga 1990). In the Australo-Pacific and in southern Asia, it is one of the most important diseases affecting cultured fish (Frerichs et al. 1986; OIE 2006). Atypical water mold infections have also been rarely seen in some freshwater aquarium fish (Wada et al. 1994). While these epidemics have been given a number of different names, the concensus is that they should all be termed EUS (Kanchanakhan 2006).

Morbidity and mortality can be high, and epidemics can develop rapidly. Interestingly, once an epidemic has occurred in an area, the prevalence and severity of future outbreaks often subside (Roberts 1989b). Some important cultured species, including tilapia, milkfish, and Chinese carp, are resistant.

Clinical Signs/Pathogenesis

The two most characteristic features of EUS, which make it an atypical water mold infection, are first, the frequently deep, extremely aggressive ulcers that often penetrate into the body cavity (Fig. II-35, A and B) and second, the severe chronic inflammation that is largely directed at the water mold component (Fig. II-35, C).

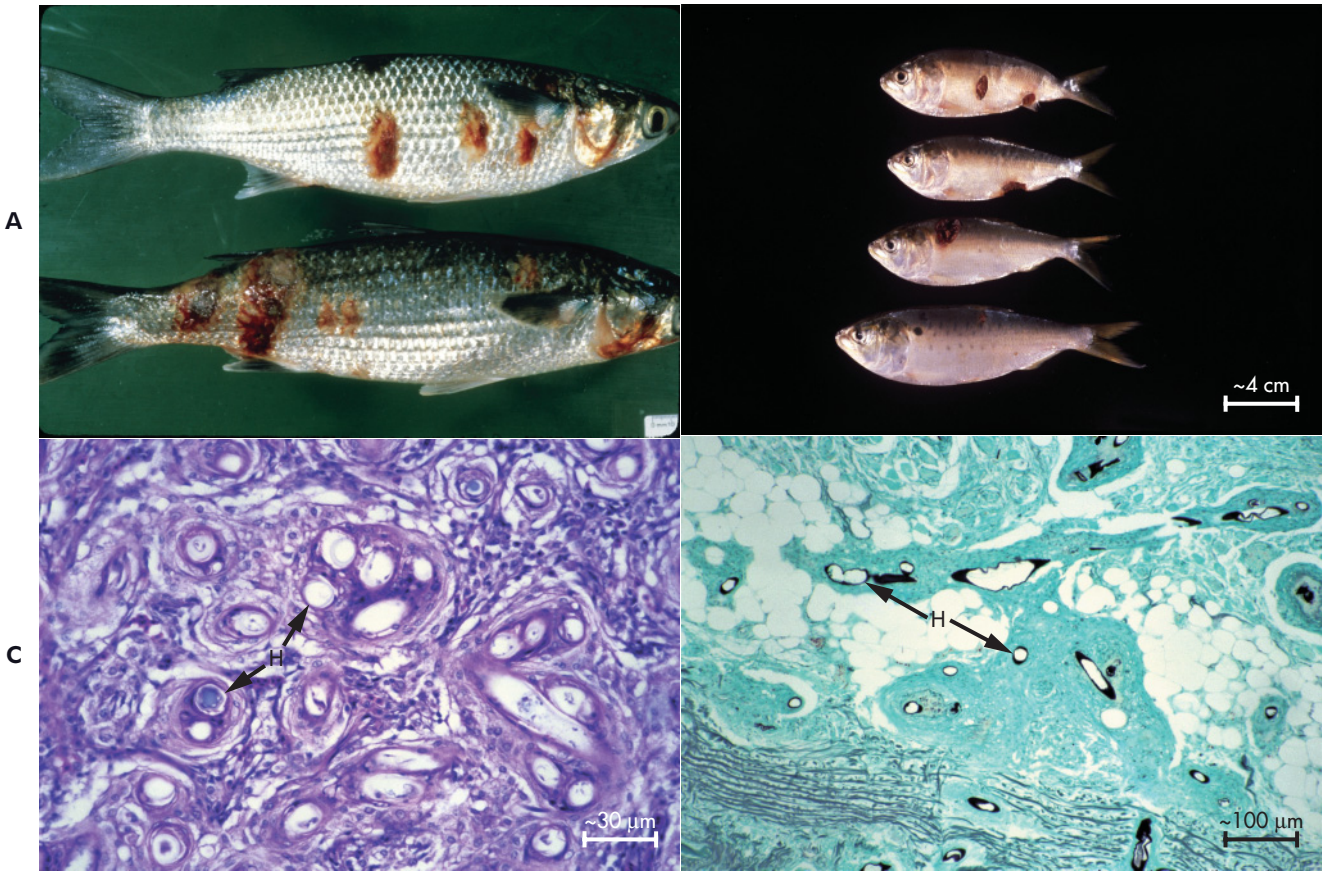

Fig. II-35. A. Relatively early, atypical water mold infection on grey mullet from the Clarence River, Australia. B. Advanced atypical water mold infection on Atlantic menhaden from Pamlico River, United States. C. Histological section of an atypical water mold infection showing chronic inflammatory response to broad, aseptate hyphae (*H*). Hematoxylin and eosin. D. Silver stain of atypical water mold lesion. *H* = hyphae. Gomori methenamine silver. (*A* photograph courtesy of R. Callinan.)

Table II-35. Fish species commonly affected by epizootic ulcerative syndrome. Over 50 species of fish have been confirmed by histological diagnosis to be naturally affected by EUS. A complete list is available in Lilley et al. (1998).

Local name	Reported locations	Primary host range	References
Red spot (Bundaberg disease; Australian epizootic ulcerative syndrome)	Australia New Guinea	Barramundi; grey mullet; yellowfin bream; luderick; grunters (Teraponidae); rainbowfish (Melanotaenidae)	McKenzie and Hall 1976 Callinan 1988 Pearce (undated) Callinan et al. 1995
Epizootic ulcerative syndrome (EUS)	Malaysia Indonesia Thailand Philippines Burma Laos Sri Lanka India Pakistan	Snakeheads; clariid catfish; bagrid catfish; gouramies; barbs	Roberts et al. 1986 Chinabut and Limsuwan 1983 Roberts et al. 1993
EUS	Africa	Churchill; dashtail barb; *Labeo* sp.	Andrews et al. 2008
Ulcerative mycosis (UM)	Western Atlantic (U.S.)	Atlantic menhaden; southern flounder; striped bass; sciaenids (sea trout, silver perch, spot, others); gizzard shad	Noga 1993b Blazer et al. 1999
Mycotic granulomatosis (MG)	Japan	Goldfish; ayu; bluegill	Miyazaki and Egusa 1972 Hatai et al. 1984

Neither of these features is characteristic of typical water mold infections (see PROBLEM 34).

Some lesions are small (~5 mm) foci of reddening on the skin, but many are large, deep, necrotic ulcers up to 25 mm in diameter. When the lesions are examined early in an epidemic, they often contain white, friable material that usually has numerous hyphae interspersed within necrotic muscle. Eventually, the necrotic, water mold-infected tissue sloughs, leaving a crater-shaped cavity that is surrounded by dark red-to-white colored muscle. While a number of water molds have been cultured from lesions (Noga 1993b), *Aphanomyces invadans* (= *A. piscicida*) appears to be the only one that is capable of eliciting the highly characteristic lesions. Numerous bacteria are also usually present, especially aeromonads or vibrios (Noga and Dykstra 1986; Roberts et al. 1986). These are most likely secondary invaders, colonizing an open ulcer.

Diagnosis

PRESUMPTIVE DIAGNOSIS

A presumptive diagnosis of atypical water mold infection is based on the presence of deep skin ulcers that contain broad (at least 7 µm in diameter), aseptate hyphae that usually incite severe, chronic inflammation (Fig. II-35, C). Inflammatory cells are often seen surrounding the hyphae in wet mounts. In histological sections, hyphae may be difficult to see with hematoxylin and eosin but can be seen easily with silver stains (e.g., Gomori's methenamine silver; Fig. II-35, D).

True fungi can also cause chronic ulcers in fish, and this type of response is common in other deep mycoses (see PROBLEM 72). True fungal infections can be differentiated from atypical water mold infections on the basis of hyphal size and color and on the presence of septa. *Ichthyophonus* hyphae (see PROBLEM 71) have similar morphology, but other developmental stages (e.g., cysts) are usually also present. Oomycetes can also be identified ultrastructurally because their tubular mitochondrial cristae differentiate them from all broad, aseptate true fungi, which have plate-like cristae (Dykstra et al. 1986). These other diseases are also very rare compared to EUS.

Definitive Diagnosis

Definitive diagnosis of EUS in EUS-endemic areas is based on either observation of typical histopathology or culture of *Aphanomyces invadans* from lesions (Noga et al. 1988a; Kanchanakhan 2006). Culture is best accomplished by using a nutrient-poor medium, such as glucose-yeast (G-Y) agar, corn meal agar or YpSs agar (Seymour and Fuller 1987), which tends to reduce the growth of bacterial contaminants. Culturing *A. invadans* from EUS lesions is especially difficult because of the many bacteria also present in lesions (Willoughby and Roberts 1994). In heavily contaminated lesions, adding penicillin (about 500 U/ml) and/or streptomycin (about 0.2 µg/ml) may improve yields; however, *Aphanomyces* can be inhibited by antibiotics.

A gene test for detecting *A. invadans* in tissue or water has also recently been developed (Vandersea et al. 2006), but is not yet commercially available.

Causes of Infection

While EUS can be diagnosed as a disease by confirming that the typical lesion with the water mold is present, it is likely that certain environmental factors are essential to development of EUS (Baldock et al. 2005). Like other water molds, *A. invadans* does not appear to be able to infect undamaged skin; thus, some cause of skin damage, possibly associated with immunosuppression, may be needed to initiate an outbreak. EUS occurs mostly during periods of low temperature (18–22°C [64–72°F] in the tropics) and after periods of heavy rainfall, which favor *A. invadans* sporulation. Low temperature is also immunosuppressive, delaying the inflammatory response to oomycete infection (Catap and Munday 1998). Skin damage caused by dinoflagellates of the *Pfiesteria* complex (see PROBLEM 96), were previously suspected to be linked to atypical water mold infection in Atlantic coast estuarine fish (Noga et al. 1996); however, their importance in causing these ulcers has now been questioned. While the initiating cause(s) of EUS epidemics is(are) unknown, there is strong evidence for *A. invadans* being spread from a relatively localized site in Japan or Australia; subsequently, this disease complex has spread from the Australo-Pacific region to now encompass most of southern Asia, as far west as Pakistan and southern Africa (Noga 1993b; Kanchanakhan 2006; Andrew et al. 2008). There is evidence for a single clone being spread in this pandemic (Lilley et al. 1997).

Treatment

There is no known treatment for EUS. While various medications (antibiotics to antiseptics) have been used, there is no evidence for their efficacy. In Thailand, EUS is empirically treated by "improving water quality" by either (1) adding 60–100 kg of lime/1,600 m^2 and repeating this treatment after 3 weeks or (2) adding 200–300 kg of salt/1,600 m^2 (K. Tonguthai, personal communication).

PROBLEM 36
Branchiomycosis (*Branchiomyces* Infection, Gill Rot)

Prevalence Index

WF - 4, CF - 4

Method of Diagnosis

1. Histology of gills with *Branchiomyces*
2. Wet mount of gills with *Branchiomyces*

History/Physical Examination

Necrotic gill lesions

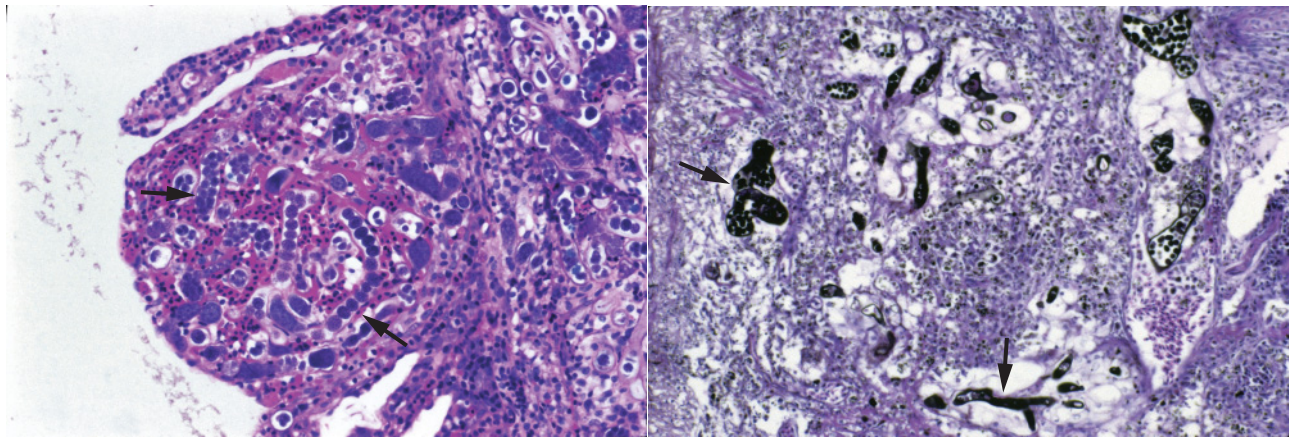

Fig. II-36. A. Histological section through *Branchiomyces*-infected gill. The key diagnostic feature is sporulating hyphae (*arrows*). Hematoxylin and eosin. B. Histological section through *Branchiomyces*-infected gill. The hyphae are black with silver-staining (*arrows*). Gomori methenamine silver.

Treatment
No known treatment

COMMENTS

Epidemiology
Branchiomycosis is a fungus-like disease that has caused acute, often high, mortality in several freshwater fish, including members of the families Anguillidae, Salmonidae, Atherinidae, Ictaluridae, Siluridae, Loricariidae, Cobitidae, Cyprinidae, Esocidae, Centrarchidae, Gadidae, Mugilidae, Cichlidae, Moronidae, Channidae, Gasterosteidae and Percidae. Notable affected species include American eel, European eel, boyeri atherinid, largemouth bass, smallmouth bass, striped bass, pumpkinseed, bluegill, northern pike, three-spined stickleback, and European perch (Neish and Hughes 1980). It has been reported primarily from Europe and Taiwan, but isolated cases have also occurred in the southeast United States (Meyer and Robinson 1973). Other fish reported to be infected by branchiomycosis include bleak, crucian carp, tench, and rainbow trout in Europe, striped mullet and grey mullet in Egypt, Japanese eel in Taiwan, bullseye snakehead in India, tilapia in Israel, and golden nugget loricarid catfish in Brazil (Chien et al. 1978; Paperna and Di Cave 2001). Recently, it has also been reported in fry and small fingerling channel catfish stocked in nursery ponds at warm temperatures (>20°C [68°F]) (Khoo et al. 1998).

There are two species. *Branchiomyces sanguinis* affects common carp, tench, and three-spined stickleback in Europe, while *Branchiomyces demigrans* infects largemouth bass, northern pike, tench, and striped bass in Europe, Taiwan, or the United States (Neish and Hughes 1980). Some have speculated that branchiomycosis is a type of water mold infection (Alderman 1982), but there are too little published morphological data to assign a classification to *Branchiomyces*.

Clinical Signs/Pathogenesis
Gills may be mottled in appearance because of areas of thrombosis and ischemia, which cause alternating areas of dark and light regions in the tissue. Histologically, there are branched, aseptate hyphae with intrahyphal, round bodies ("aplanospores") (Fig. II-36), which look similar to *Saprolegnia* sporangia (see Fig. II-34, A). Both *Branchiomyces* species cause similar pathology, except that *B. demigrans* affects the entire gill, with hyphae penetrating though blood vessel walls into the lumen, while *B. sanguinis* is restricted to gill blood vessels (Wolke 1975). When hyphae penetrate vessels, there can be a granulomatous reaction. In channel catfish, concurrent protozoan infection exacerbates the disease (Khoo et al. 1998).

Diagnosis
Diagnosis of branchiomycosis can be made by examining wet mounts or histopathology of lesions. Characteristic hyphae (Fig. II-36), causing deep branchial infection, are diagnostic. Because hyphae may primarily infect proximal gill lamellae (i.e., near the base of the primary lamellae), these should also be examined, especially if gill damage is grossly visible (Yanong 2003).

Treatment
There is no known treatment. Reducing organic loading and reducing the temperature below 20°C (68°F) have been suggested.

PROBLEM 37

Columnaris Infection (Myxobacterial Disease, Saddleback, Fin Rot, Cotton Wool Disease, Black Patch Necrosis)

Prevalence Index

WF - 1, CF - 1, CM - 3

Method of Diagnosis

1. Culture of bacteria from lesions
2. Wet mount of skin or gills with typical bacteria
3. Histopathology of skin or gills with typical bacteria

History

High temperatures; dyspnea; recent acute stress; late spring to early fall; acute morbidity/mortality

Physical Examination

Ulcers (usually shallow), reddening, erosion, and necrosis of skin; gill necrosis; yellow mucoid material on skin or gills

Treatment

Surface infection only:

1. Potassium permanganate prolonged immersion
2. Copper sulfate prolonged immersion
3. Quaternary ammonium bath

Systemic infection:

Appropriate antibiotic

COMMENTS: FRESHWATER PATHOGENS

Epidemiology

Columnaris, previously referred to as myxobacterial infection, is a common bacterial disease that affects the skin or gills of freshwater fish. *Flavobacterium columnare* (formerly *Flexibacter columnaris;* Bernardet et al. 1996) is the most prevalent member of this group, which has a worldwide distribution and can probably infect most freshwater fish. *Flavobacterium columnare* is an important fish pathogen. It can rapidly infect a population and cause large mortalities (Fijan 1968; Becker and Fujihara 1978; Chen et al. 1982; Michel et al. 2002). Economic costs to the U.S. channel catfish industry are estimated at $50 million annually (Shoemaker et al. 2007). Water temperature and strain virulence are the most important factors determining disease severity.

Risk Factors/Virulence Mechanisms

Flavobacterium columnare is usually pathogenic at higher than ~15°C (59°F). Both mortality and acuteness of disease increase with temperature. For example, experimental infections can kill oriental weatherfish within ~7 days at 15°C (59°F) and in only 1 day at 35°C (95°F; Wakabayashi 1993). While disease may occur at less than 15°C (59°F), it is less severe. In addition, some isolates from tropical fish will not grow at low (16°C [61°F]) temperatures (Decostere et al. 1998). Virulence mechanisms are unclear, but mineral content of the water is important. *F. columnare* is less pathogenic in soft water (Fijan 1968). In one study, optimum hardness was ~70 mg/l and some isolates were virtually nonpathogenic in distilled water (Chowdhury and Wakabayashi 1988b). Pathogenicity paralleled bacterial survival in various media (Chowdhury and Wakabayashi 1988a). However, salinity has the opposite effect; 3 ppt salinity prevents infection and disease in channel catfish (Altinok and Grizzle 2001a). There is no apparent relationship between serotype and virulence.

Other risk factors include physical injury (e.g., net damage), low oxygen (Chen et al. 1982), organic pollution (Fijan 1968), and high nitrite (Hanson and Grizzle 1985). Exposure to high arsenic levels increased the susceptibility of striped bass to columnaris (MacFarlane et al. 1986). Uneaten feed supports growth of *Flavobacterium columnare* and thus is a source of infection (Sugimoto et al. 1981).

Source of Inoculum

It is likely that many of the flavobacteria and related bacteria infecting fish may occur naturally on healthy fish and in aquatic ecosystems, since many can be routinely isolated from such sources (Austin and Austin 2007).

Clinical Signs/Pathogenesis

Columnaris is primarily an epithelial disease (Fig. II-37, A and B). It causes erosive/necrotic skin and gill lesions that may become systemic. It often presents as whitish plaques that may have a red periphery on the head, back (saddleback lesion), and/or fins (fin rot), especially the caudal fin. Fragments of the fin rays may remain after the epithelium has sloughed, leaving a ragged appearance. Lesions rapidly (often within 24 hours) progress to ulcers, which may be yellow or orange due to masses of pigmented bacteria. Ulcerations spread by radial expansion and may penetrate into deeper tissues, producing a bacteremia.

Gill infections are less common but more serious. Columnaris begins at the tips of the lamellae and causes a progressive necrosis that may extend to the base of the gill arch. A less common peracute syndrome presents as sudden death with systemic infection.

Channel catfish may have systemic *F. columnare* infections without external lesions; internally, there may be swelling of the posterior kidney (Hawke and Thune 1992). The clinical significance of the latter infections is unknown.

Flavobacterium johnsoniae (= *Cytophaga johnsonae*) has been associated with similar disease (superficial skin ulcers) in barramundi and salmonids cultured in freshwater (Carson et al. 1993; Rintamäki-Kinnunen et al. 1997).

Diagnosis

Rapid, presumptive identification of *Flavobacterium columnare* can be made by examining wet mounts of lesions, which have long, thin rods (~0.50–1.0 × 4–10 µm; Fig. II-37, C) with a characteristic flexing or gliding motion. If wet mounts are allowed to stand for a few minutes, the bacteria often aggregate into a writh-

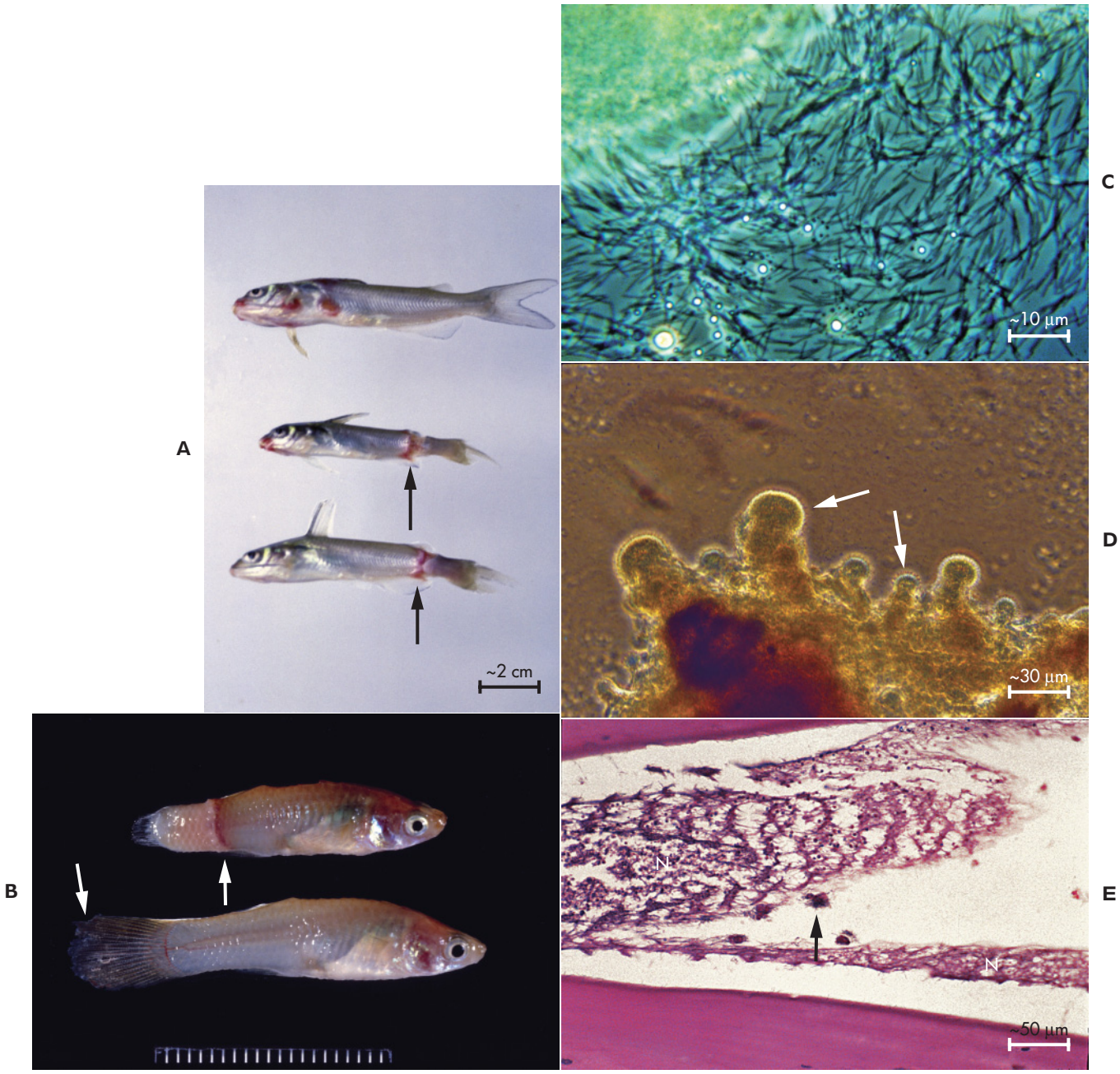

Fig. II-37. A. Columnaris in fingerling channel catfish. The lesion covers the entire posterior portion of the body of the bottom two fish. The leading (anterior) edge of the lesion is much deeper; this area (*arrow*) was secondarily invaded by water molds and other bacteria. B. Severe columnaris in a guppy (*top*). The entire tail has sloughed, and the infection extends halfway up the flank (*arrow*). There is little chance that this fish will survive, even if it is treated. The lower fish has a mild infection on the tail (*arrow*); bar = 2 cm. C. Wet mount of a columnaris lesion showing the characteristic long, thin rods. D. Wet mount of a columnaris lesion showing the typical haystack appearance (*arrows*) produced by aggregations of bacteria. Phase contrast. E. Histological section through a columnaris lesion of the caudal fin that is entirely infected with long, thin rods (*arrow*) associated with tissue necrosis (*N*). Hematoxylin and eosin.

ing mass that appears like a column or haystack (Fig. II-37, D). Other shorter rods (mostly other *Flavobacterium* spp. that do not have a flexing motility) have also been associated with similar gross lesions. Lesions may be secondarily infected by water molds (see PROBLEM 34) or other opportunists. Related bacteria cause epithelial lesions at low temperatures (see PROBLEM 38); these diseases are often proliferative, as well as necrotic.

Presumptive diagosis is sufficient in routine clinical cases. Culture is not usually warranted because most columnaris infections are predictably susceptible to either antiseptics or certain antibiotics. *Flavobacterium columnare* does not grow well on standard bacteriological media; it requires specialized media for both isolation and antibiotic sensitivity testing.

If culture and sensitivity is desired (such information may be useful in case of treatment failure), one must use medium with low nutritional content and high moisture content. Cytophaga agar (Anaker and Ordal 1959) or a 1:10 dilution of nutrient broth in 1% agar has been used, but selective media containing antibiotics greatly enhance isolation, especially from mixed bacterial infections (Bullock et al. 1986; Hawke and Thune 1992). Shieh medium is best for isolation of all pathogenic flavobacteria. Modified Shieh medium (with 1 μg/ml tobramycin) is more effective than standard Shieh medium, which is supplemented instead with polymyxin B (10 U/ml) and neomycin (5 μg/ml) (Decostere et al. 1997). After colonies appear, isolates must be passaged frequently (often every day) or the culture may be lost. Media should be fresh so that there is enough moisture.

Taxonomy

Flavobacterium columnare is considered the cause of columnaris disease in freshwater fish. However, not all gliding bacteria associated with disease in warm waters may be *F. columnare*. For example, Pyle and Shotts (1980, 1981) and Starliper et al. (1988) isolated a number of gliding bacteria from various fish that varied considerably both phenotypically and genotypically. Nonetheless, *F. columnare* appears to be by far the most significant species involved in columnaris disease.

In addition, a large number of similar, Gram-negative bacterial rods have been isolated from fish epithelial lesions and cause other clinically distinct diseases. Most notable among these are *Flavobacterium psychrophilum* (= *Flexibacter psychrophila* = *Cytophaga psychrophila* = *Flexibacter aurianticus*), the cause of bacterial cold water disease in salmonids (see PROBLEM 38) and *Flavobacterium branchiophilum*, a cause of proliferative gill disease (see PROBLEM 39). All these Gram-negative bacteria form colonies that are orange or yellow pigmented, rhizoid, and spreading. The fish-pathogenic *Flavobacterium* species often exhibit gliding motility. Key differentiating features of the presently known pathogens are summarized by Austin and Austin (2007).

Similar bacteria have at times been placed in various other genera. Some of these genera are invalid (e.g., *Myxobacterium*) and others are of uncertain significance as disease-causing agents (e.g., *Cytophaga*, *Sporocytophaga*).

Definitive diagnosis of *Flavobacterium columnare* is based upon biochemical tests or agglutination. *Flavobacterium columnare* is a homogeneous species, although potential cross-reactivity with related organisms has not been fully determined.

Treatment

Early cases of columnaris may be successfully treated with antiseptic baths or prolonged immersion in potassium permanganate or copper sulfate. However, advanced cases (i.e., lesions with exposed muscle or over 5% of body surface area being affected) warrant systemic antibiotics. Isolates are usually susceptible to oxytetracycline and/or nifurpirinol, but many if not most are resistant to ormetoprim-sulfadimethoxine (Anonymous 1986; Hawke and Thune 1992) and other sulfas (Decostere et al. 1998).

If fish with advanced lesions are anorexic, a potassium permanganate treatment may stimulate enough appetite to begin oral medication. Medical therapy must always be accompanied by an improvement in environment. Avoid exposing cultured salmonids to feral fish, which often carry the infection. Lowering the temperature (e.g., adding cold water) will reduce disease severity (Wood 1974). When feasible, raising the salinity may be highly effective (Altinok and Grizzle 2001a), but is not yet validated in clinical cases. Experimental vaccines have been investigated (Moore et al. 1990; Shoemaker et al. 2007), and a live, attenuated vaccine is commercially available (Aquavac-Col™ [Intervet]).

COMMENTS: MARINE PATHOGENS

Columnaris-type infections caused by *Tenacibaculum maritimum* (= *Flexibacter maritimus*) or related bacteria have been observed in young marine fish. Juvenile (<6 cm) black sea bream and red sea bream usually develop lesions in spring after being transported to sea cages (Wakabayashi et al. 1986). Dover sole (black patch necrosis, Campbell and Buswell 1982), Japanese flounder (Baxa et al. 1987), Atlantic salmon smolts (Kent et al. 1988a), and turbot (Pazos et al. 1993) develop similar superficial skin lesions. Stomatitis lesions have also been seen in Atlantic salmon. Gill lesions may also be present (Handlinger et al. 1997). Host-specific strains affect gilthead seabream, turbot and Senegalese sole (Avendaño-Herrera et al. 2005). *Tenacibaculum ovolyticum* (= *Flexibacter ovolyticus*) has caused mortality of Atlantic halibut eggs and larvae, with puncturing of the egg leading to death (Hansen et al. 1992a). *Chryseobacterium*

scophthalmum (= *Flavobacterium scophthalmun*) causes skin and gill hemorrhage, gill hyperplasia and visceral hemorrhage in turbot (Mudarris and Austin 1992).

Much less is known about marine columnaris, but the bacteria are microscopically similar to freshwater columnaris lesions. Isolation methods are similar to that for *F. columnare*, except that salt or seawater should be added to media. *Tenacibaculum maritimum* requires medium having at least 15 ppt and some require 30 ppt seawater (not NaCl only) for isolation. Oxytetracycline has been used to treat *T. maritimum* infections in bream, but this treatment has not always been effective (Wakabayashi 1993). Black patch necrosis is resistant to many antibiotics but responds to placing sole on a sand substrate to reduce abrasions (also see PROBLEM 98).

PROBLEM 38

Bacterial Cold Water Disease (BCWD; Peduncle Disease, *Flavobacterium psychrophilum* Infection, Rainbow Trout Fry Syndrome [RTFS])

Prevalence Index

CF - 1, CM - 3

Method of Diagnosis

1. Culture of bacteria from lesions
2. Wet mount of skin with typical bacteria
3. Histopathology of skin with typical bacteria

History

Cold temperatures; early spring; acute to chronic morbidity/mortality

Physical Examination

Erosion and ulceration (usually shallow) of skin

Treatment

1. Quaternary ammonium constant flow
2. Chloramine-T constant flow
3. Copper constant flow
4. Appropriate antibiotic

COMMENTS

Epidemiology

Bacterial cold water disease, caused by *Flavobacterium psychrophilum*, is common in freshwater salmonids and is a serious problem in salmonid hatcheries. It is probably endemic in salmonid culture. Coho salmon are especially vulnerable, but all salmonids are probably susceptible. As with columnaris (see PROBLEM 37), water temperature and strain virulence are the most important factors determining disease severity. BCWD is often associated with erythrocytic inclusion body syndrome (EIBS) (see PROBLEM 44). EIBS anemia may predispose fish to BCWD (Holt et al. 1993). *Flavobacterium psychrophilum* also causes systemic disease in eels and cyprinids in Europe (Austin and Austin 2007) and is responsible for RTFS, a serious disease in young rainbow trout in Europe and Chile (Vatsos et al. 2006).

Risk Factors/Virulence Mechanisms

Flavobacterium psychrophilum is usually pathogenic at less than ~10°C (50°F) but can cause disease at up to 16°C (61°C). The disease usually appears in spring, when temperatures are 4–10°C (39–50°F) (Holt et al. 1993). Mortality is most acute at ~15°C (~59°F); mortality decreases at higher temperatures (Holt et al. 1993). Mortality usually begins within 5–10 days after infection and peaks 20–60 days later. Mortalities typically are 5–10% but may reach 90% in some epidemics. Like *F. columnare*, strains vary widely in pathogenicity (Holt et al. 1993). Extracellular products appear to be the major cause of clinical signs (Otis 1984). The disease may recur after some stress (e.g., handling, during smoltification, etc.) and often co-occurs with other infections, such as viral infections.

Source of Inoculum

It can be isolated from the surface of clinically normal fish (Holt et al. 1993) and can also occur in wild fish (e.g., roach, perch) and in waters adjacent to rainbow trout farms (Madsen et al. 2005). Colonization of fish may be a forerunner to disease and skin damage may be needed to initiate infections. It can also colonize the surface of eggs (Vatsos et al. 2006). Vertical transmission in salmonids is likely because the bacterium is commonly found on eggs and can be isolated from reproductive tissues of a high percentage of fish (up to 76%) (Holt et al. 1993). It might enter eggs during water hardening (Kumagai et al. 2000). However, in ayu, the bacterium is only outside the egg (Kumagai et al. 2004). The bacterium may be very long-lived in the environment (months) and both wild fish and amphibians might serve as reservoirs.

Clinical Signs/Pathogenesis

Bacterial cold water disease causes epithelial erosions and necrotic skin lesions but often becomes systemic (Wood and Yasutake 1956). The most common form is a subacute to acute infection in young fish. In yolk sac fry (alevins), erosions damage the skin covering the yolk. Fish often have a distended abdomen, bilateral exophthalmos and severe anemia. Fish with brain infections may have a soft hemorrhagic swelling on the head, and fish may exhibit spiral swimming.

In older fish the course is more chronic. Typical signs of peduncle disease appear (Fig. II-38), which are similar to columnaris infections. Internally, there may be hemorrhage (Otis, 1984). Bacteria are most common in highly vascularized tissues, including secondary lamellar capillaries, kidney, heart, and spleen (Wood and Yasutake 1956). Inflammation is typically mild or absent. Moribund fish with no external lesions and dark color are seen late in epidemics.

In the most chronic form of the disease, recovered coho salmon often develop spinal deformities (lordosis, scoliosis, vertebral compression) at 3–4 months of age

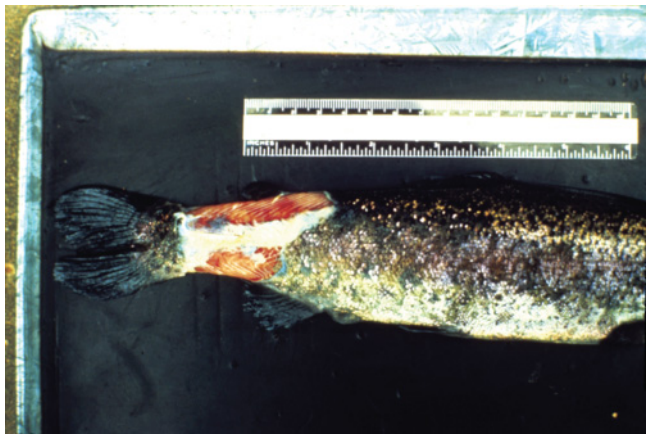

Fig. II-38. Rainbow trout with typical bacterial cold water disease (peduncle disease). (Photograph courtesy of National Fish Health Research Laboratory.)

(Wood 1974). Fish that recover from typical BCWD may also develop neurological disease, presumably from the localization of bacteria in the cranium. Unilateral hyperpigmentation also suggests nervous tissue damage. The bacterium is readily isolated from brain (Kent et al. 1989).

Diagnosis

Rapid, presumptive identification of *Flavobacterium psychrophilum* can be made by examining wet mounts of affected skin or internal organs, which have long, thin rods (~0.50–1.0 × 4–10 µm) with a characteristic flexing or gliding motion, like *F. columnare* (see Fig. II-37, C). Histopathology can also be used for presumptive diagnosis; some bacteria on the skin may be lost during processing.

Presumptive diagnosis is sufficient in routine clinical cases. For confirmatory diagnosis, culture is required, but fish-pathogenic *Flavobacterium* species do not grow well on standard bacteriological media, requiring specialized media for both isolation and antibiotic sensitivity testing (tryptose yeast extract agar with 5% fetal bovine serum). Identity should be confirmed by antibody probe (agglutination). *Janthinobacteriun lividum* (PROBLEM 57) has also been linked to rainbow trout fry syndrome but is rare compared to *F. psychrophilum*.

Treatment

Early, surface (skin) cases of BCWD may be successfully treated with quaternary ammonium, chloramine-T, or copper sulfate constant flow for one hour. However, systemic infections are common, requiring systemic antibiotics. Oxytetracycline, oxolinic acid, sarafloxacin, ormetoprim-sulfadimethoxine, florfenicol and amoxicillin have been used with varying degrees of success. Unfortunately, *F. psychrophilum* seems to rapidly develop resistance and thus the antibiotic sensitivity of the isolate should be determined. Treating alevins is difficult because at this life stage they do not eat until the yolk sac is absorbed. In fish with advanced lesions, combined treatment with an antiseptic flush followed by antibiotic has been effective (Schachte 1983). Keeping alevins in shallow rather than deep troughs, keeping water flows in incubators low (Wood 1974), and inhibiting excessive movement of alevins to prevent abrasions (Leon and Bonney 1979) can reduce infections. Avoid exposing cultured salmonids to feral fish, which often carry the infection. In farms that have no history of BCWD, take appropriate measures to prevent introduction, including restocking with fish or eggs that have been obtained from a supplier that screens for the infection. Eggs, fry, and fingerlings should be raised in water supplies that do not have resident fish or amphibians that might transmit the infection (e.g., well water).

PROBLEM 39

Bacterial Gill Disease (BGD; Proliferative Gill Disease [PGD], *Flavobacterium branchiophilum* Infection, Pigmented Bacteria Gill Disease)

Prevalence Index

CF - 1

Method of Diagnosis

1. Culture of bacteria from lesions
2. Wet mount of gills with typical bacteria
3. Histopathology of gills with typical bacteria

History

Overcrowding; low DO; high ammonia; high turbidity

Physical Examination

Lethargy; flared opercula; coughing; dyspnea; mucus strands trailing from gills

Treatment

1. Salt bath
2. Quaternary ammonium bath
3. Chloramine-T bath
4. Diquat bath

COMMENTS

Epidemiology

Bacterial gill disease is an important disease in cultured freshwater salmonids (Wakabayashi et al. 1989). It has been a problem in Europe and appears to be spreading, having also been found in Korea (Ko and Heo 1997). *Flavobacterium branchiophilum* (previously known as *F. branchiophila*) causes a chronic, proliferative response in gill. No studies have examined the pathophysiological effects of BGD, but it probably causes respiratory and osmoregulatory impairment, which depends on normal epithelium function. Up to 25% mortality can occur (Speare et al. 1991).

Risk Factors/Virulence Mechanisms

While there appear to be differences in pathogenicity among BGD bacteria isolates, bacterial gill disease is

mainly a production management disease. Risk factors include low oxygen, high turbidity, high ammonia, and overcrowding. Water temperature does not appear to affect pathogenicity. Outbreaks have occurred at 5°C (41°F) in cyprinids and almost 20°C (68°F) in salmonids (Turnbull 1993a). Transmission is via water (Ferguson et al. 1991).

Clinical Signs/Pathogenesis

GROSS LESIONS

The gill is the only target organ. There are clinical signs of respiratory impairment, including lethargy, dyspnea, coughing, and flared opercula. Strands of mucus may trail from the gills. In early stages, the gills may be hyperemic, with swollen primary lamellae. Increased mucus may trap debris. Later, secondary water mold infections (see PROBLEM 34) or opercular damage may occur (Ostland et al. 1990).

HISTOPATHOLOGY

Bacterial gill disease is primarily an epithelial disease. Bacteria may initially colonize the tips of the secondary lamellae and then spread inward, inducing a proliferative branchitis (Fig. II-39) that causes epithelial hyperplasia. Fusion of secondary lamellae may occur distally, forming a partially enclosed space with bacteria, sloughed epithelial cells, and mucus (Fig. II-39). Hyperplasia may also cause obliteration of the entire interlamellar space and in severe cases may cause fusion of adjacent primary lamellae.

Diagnosis

Rapid, presumptive identification of *Flavobacterium branchiophilum* can be made by examining wet mounts or histopathology of lesions, which have long, thin rods (~0.50–1.0 × 4–10 µm), similar to *F. columnare* (see Fig. II-37, C). Early lesions of BGD can be hard to detect with histopathology because bacteria are often lost during processing (Turnbull 1993a).

The susceptibility of bacterial gill disease to antiseptic-type therapies makes presumptive diagnosis sufficient for clinical cases. If desired, culture may be performed as described for columnaris (see PROBLEM 37). *Flavobacterium branchiophilum* can be difficult to isolate in the absence of clinical disease (Heo et al. 1990).

Treatment

Bacterial gill disease usually responds to antiseptic baths. Providing adequate oxygen is useful supportive therapy. Reducing stressors is important. It is likely that this organism may occur naturally on healthy fish and possibly in aquatic ecosystems.

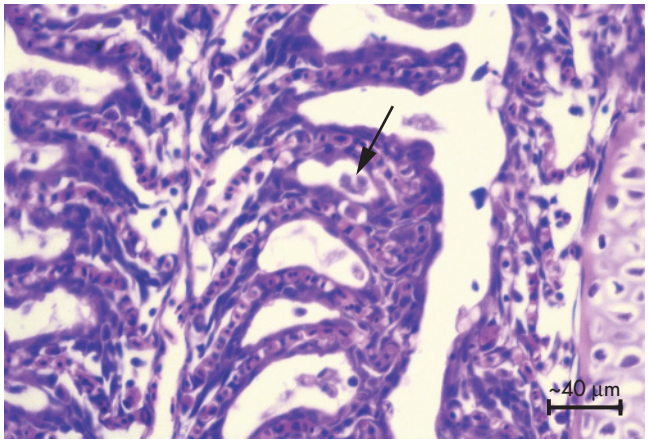

Fig. II-39. Bacterial gill disease. Hyperplasia and fusion of adjacent secondary lamellae. The hyperplastic lesions surround bacteria and cell debris (*arrow*). Hematoxylin and eosin.

PROBLEM 40
Lymphocystis

Prevalence Index

WF - 1, WM - 1

Method of Diagnosis

1. Histology of skin or gills showing massively enlarged dermal fibroblasts.
2. Wet mount of skin or gills showing massively enlarged dermal fibroblasts

History/Physical Examination

Recent stress; various-sized, white to pink, pinpoint to mulberry-size masses, especially on skin, but also in the buccal cavity and on the gills; rarely present on serosal surfaces of internal organs

Treatment

1. Isolate affected individual(s)
2. Prophylactic antibiotics

COMMENTS

Epidemiology

Lymphocystis is a chronic (usually many weeks), self-limiting disease affecting many cultured and wild marine and freshwater fish (Lawler et al. 1977). It has been reported from over 125 species in 34 families (Wolf 1988). Lymphocystis is a disease of higher (i.e., evolutionarily advanced) teleosts and does not affect salmonids, catfish, or cyprinids. Caused by a group of closely related iridoviruses in the genus *Lymphocystivirus*, it is the most common viral infection of aquarium fish. While it causes only low mortality, lymphocystis is disfiguring and can render affected fish unsalable.

Only two lymphocystis viruses are recognized as valid species. Lymphocystis disease virus 1 (LCDV-1) infects flesus flounder and plaice, while Lymphocystis disease virus 2 (LCDV-2) infects dab (Chinchar et al. 2005). The numerous other isolates from other fish species have

not been characterized. The disease is probably caused by several closely related viruses; specific isolates may only be able to infect related fish in the same family or genus. Transmission probably occurs by rupture or sloughing of lesions followed most often by infection of abraded integument (Lawler et al. 1977). Virus is viable in water for about 1 week. The incubation period may be long (weeks to months). Many fish probably carry a latent infection, which may appear after shipping or other stress. Tropical aquarium fish often break with lymphocystis after arrival at a retailer's facility.

Clinical Signs/Pathogenesis

GROSS LESIONS

The lymphocystis virus infects the dermal fibroblasts, producing tremendously hypertrophied cells that are often just visible to the naked eye. Early or mild stages of the disease appear as a salt-like dusting of the body (Fig. II-40, C), which may later coalesce into large neoplastic-like masses of hypertrophied cells (Fig. II-40, A and B). Lesions less commonly affect internal organs or gill (Russell 1974).

Histopathology

Histopathology of infected tissue shows hypertrophied fibroblasts with basophilic, intracytoplasmic inclusions (Pritchard and Malsberger 1968; Fig. II-40, E and F). Viral inclusion material may be lacy with scattered, small condensations of chromatin (plaice type) or large and cord-like, with blebs (mullet type; Ferguson 1989). These lymphocysts are surrounded by a hyaline capsule, which may be responsible for the initially mild inflammatory response. In later stages, as the lymphocysts rupture, numerous inflammatory cells surround the lesions.

Diagnosis

Wet mounts of skin lesions that have typical pathology (Fig. II-40, D) provide strong presumptive evidence for lymphocystis infection and are usually sufficient for clinical diagnoses. Epitheliocystis (see PROBLEM 41) can also produce grossly hypertrophied cells but appears to be much less common than lymphocystis. Epitheliocystis also primarily affects the gills.

If a definitive diagnosis is required, epitheliocystis can be readily distinguished from lymphocystis histopathologically by its presence in epithelial cells that have a hypertrophic host nucleus that is peripheral to a granular basophilic inclusion containing many coccoid or coccobacillary bodies (see Fig. II-41, B; Herman and Wolf 1987). Lymphocysts are dermal fibroblasts, have irregular inclusions, and have an undisplaced nucleus (Fig. II-40, F). Mild gross lesions may be confused with ich (see PROBLEM 20) but are easily differentiated via wet mount.

Lymphocystis may also be confused grossly with some forms of idiopathic epidermal hyperplasia (see PROBLEM 76), but the latter lesions are rare compared with lymphocystis. Lesions such as walleye dermal sarcoma can be differentiated with histopathology.

Treatment

There is no treatment for lymphocystis. Fish should be watched closely for secondary infections and medicated accordingly. Affected fish should be quarantined, preferably for at least 1 month after recovery. Lesions will often regress spontaneously. Stress reduction and avoidance of skin trauma are essential to control. Recovered fish that are stressed will often recrudesce, although some recovered fish appear immune to reinfection.

PROBLEM 41
Epitheliocystis (Mucophilosis)

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

1. Histology of gills or skin with massively enlarged epithelial cells
2. Wet mount of gills or skin with massively enlarged epithelial cells

History/Physical Examination

Small pinpoint masses, mainly on gills but rarely on the skin

Treatment

1. Isolate affected individual(s)
2. Oxytetracycline oral

COMMENTS

Epidemiology

Epitheliocystis is an intracellular, Gram-negative bacterial infection that has been reported from over 50 species of freshwater and marine fish worldwide. It has been associated with mortalities in white sturgeon, common grey mullet, grey liza mullet, striped bass, Australian bass, pacu, largemouth bass, bartail flathead, gilthead sea bream, red sea bream, common carp, Atlantic salmon, rainbow trout, lake trout, yellowtail kingfish, yellowtail, and amberjack (Turnbull 1993b; Herman and Wolf 1987; Paperna et al. 1981b; Nowak and LaPatra 2006). There is one report of infection in an elasmobranch (lemon shark) (Nowak and LaPatra 2006). Transmission between species appears limited and thus epitheliocystis strains may only infect fish within the same family or even species (Hoffman et al. 1969; Nowak and LaPatra 2006).

While documented in a number of wild fish, mortalities have only been observed in cultured fish and are most common in young (larval) fish. When present in small numbers, epitheliocystis may be an incidental finding, but in high concentrations it has been associated with considerable mortalities. Details of its life cycle and pathogenesis are largely unknown. The disease has never been experimentally reproduced.

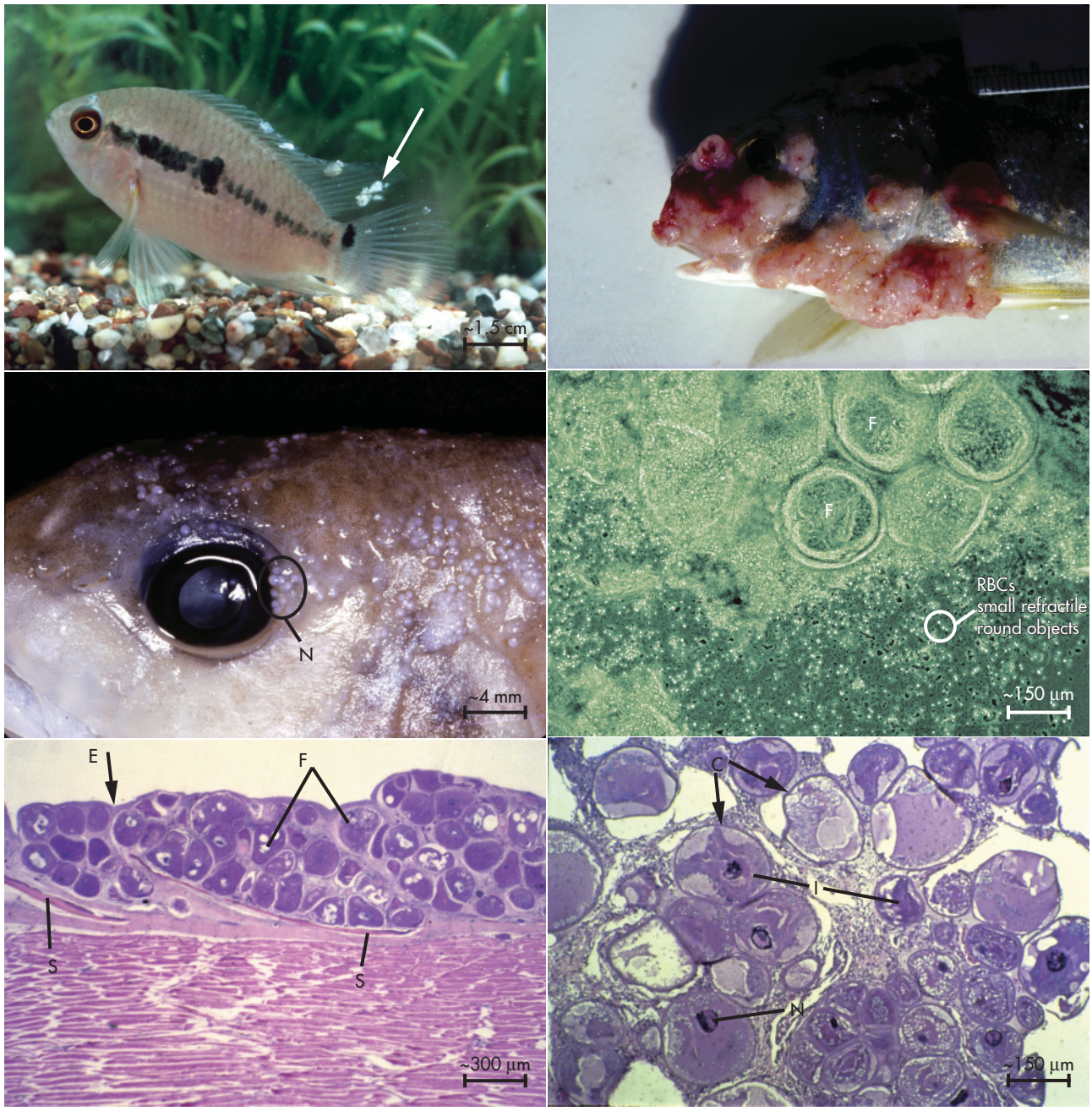

Fig. II-40. A. Severum cichlid infected by lymphocystis on the dorsal fin (*arrow*). B. Atlantic croaker with a severe lymphocystis infection. The reddening (hemorrhage) suggests that this lesion may be secondarily infected by bacteria. C. Atlantic croaker with extensive lymphocystis nodules (*N*). Note the granular, sand grain–like appearance. Preserved specimen. D. Wet mount of lymphocystis lesions showing massively enlarged, virus-infected dermal fibroblasts (*F*) that are over 500 times the size of red blood cells (*RBCs*). E. Histological section through a lymphocystis lesion. Note massively enlarged dermal fibroblasts (*F*) or lymphocysts (*E* = epithelium; *S* = scale). Hematoxylin and eosin. F. Close-up of a lymphocystis lesion that shows diagnostic features, including infected fibroblasts with irregular inclusions (*I*), capsule (*C*), and enlarged, undisplaced nucleus (*N*). Hematoxylin and eosin. (*A* photograph courtesy of T. Wenzel; *C* photograph by M. Jansen and E. Noga; *F* photograph courtesy of L. Khoo.)

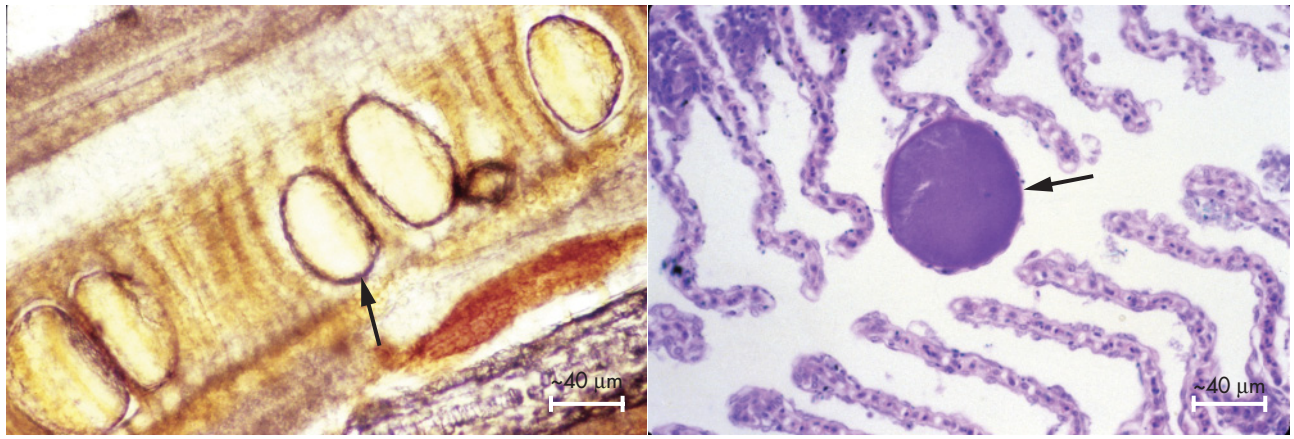

Fig. II-41. A. Wet mount of gill from gilthead sea bream infected by epitheliocystis. Infected host epithelial cells are massively enlarged, each having a smooth, homogeneous inclusion (*arrow*). B. Histological section through epitheliocystis-infected gill cell (*arrow*). The key diagnostic feature is a large, granular, basophilic inclusion, filled with coccoid bodies, which occupies virtually the entire cell. Hematoxylin and eosin. (*A* photograph courtesy of A. Colorni.)

Clinical Signs/Pathogenesis

While not yet proven to be a cause of disease, epitheliocystis has been associated with varying degrees of morbidity and mortality. Gills and rarely skin and pseudobranch are the primary target organs (Turnbull 1993b). Lesions present as white miliary nodules up to ~1 mm in diameter on the skin or gills. Host response varies from no reaction to severe epithelial hyperplasia. Host response is usually most severe with heavy infections, but light infections will occasionally incite inflammation.

Diagnosis

Lesions may grossly resemble *Ichthyophthirius multifiliis* (see PROBLEM 20), lymphocystis (see PROBLEM 40), or other nodular skin lesions but are easily distinguished with histopathology. Epitheliocystis infects skin and gill epithelial cells, resulting in the cells enlarging to 20–400 µm in diameter. Presumptive diagnosis can be made from wet mounts, showing nodular masses in the tissue (Fig. II-41, A).

All major types of epithelial cells can be infected, including chloride and goblet cells (Ferguson 2006). The hypertrophic cytoplasm is peripheral to a fine, granular, basophilic, inclusion containing large numbers of coccoid or coccobacillary bodies (Fig. II-41, B). When the nucleus is seen in sections, it is on the periphery of the cell. Histologically, the major differential is lymphocystis virus infection (see PROBLEM 40), which can be distinguished based on its infection of dermal fibroblasts, presence of irregular inclusions, and undisplaced nucleus. Unlike lymphocystis, epitheliocystis can also infect salmonids, catfish, or cyprinids.

Morphological, immunological and genetic data indicate that epitheliocystis is caused by a group of chlamydia-like organisms (Nowak and LaPatra 2006).

Treatment

There is very little known about effective treatment. Largemouth bass were successfully treated with oral oxytetracycline (Goodwin et al. 2005). Ultraviolet irradiation of the water supply controlled outbreaks in amberjack and leopard coral grouper (Miyaki et al. 1998).

PROBLEM 42
Miscellaneous Skin and Gill Diseases

The following agents are primarily systemic pathogens but may occasionally cause skin or gill lesions:

Myxozoans: Diagnostic spores or developmental stages are easily identified via wet mounts or histopathology (see PROBLEM 63).

Microsporidians: Diagnostic spores are easily identified via wet mounts or histopathology (see PROBLEM 70).

Helminths: Digeneans (see PROBLEM 58) and nematodes (see PROBLEM 60) are easily identified via wet mounts or histopathology.

Trypanoplasms: These are mainly hemoparasites but may occur on the gills as well; they are easily identified in wet mounts or smears (see PROBLEM 44).

Bacterial infections: Some bacterial infections mainly affect the skin or gills, but skin lesions are often a manifestation of systemic disease (see PROBLEM 45).

Ichthyophonus: *Ichthyophonus* (see PROBLEM 71) may cause skin lesions.

Fungal infections: Some systemic fungal infections (see PROBLEM 72) may cause skin lesions.

The following are mainly skin diseases but are diagnosed by rule-out of other problems:

Idiopathic epidermal hyperplasia: (see PROBLEM 76)

The following agents are uncommon to rare diseases that infest/infect the skin or gills:

Dermocystidium: This is a poorly studied group of organisms that typically produces various-sized (usually 0.1–4.0 µm), white, macroscopic nodules on the skin or gills of many fish species (Hatai 1989; Pekkarinen et al. 2003; Feist et al. 2004) (Fig. II-42, A). The cysts contain spherical, 3–10 µm spores. Often an incidental finding, some species have caused mortality in salmonids and other fish. *Dermocystidium* is a member of a newly created group, the class Mesomycetozoea, which is at the boundary between animals and fungi (Perkins 1974; Arkush et al. 2003, see PROBLEM 71). The most diagnostic feature is the presence of a spherical stage (spores), having a large central vacuole or refractile body (Fig. II-42, B). Hyphal-like structures are produced by some species (Lom and Dyková 1992). Various species have been reported from Europe, Japan, China, Russia, and the United States.

X-cells ("Pleuronectid epidermal papilloma"): X-cells are amoeba-like cells of uncertain taxonomy that produce 1 mm nodules to 5 cm polyps in the pseudo-

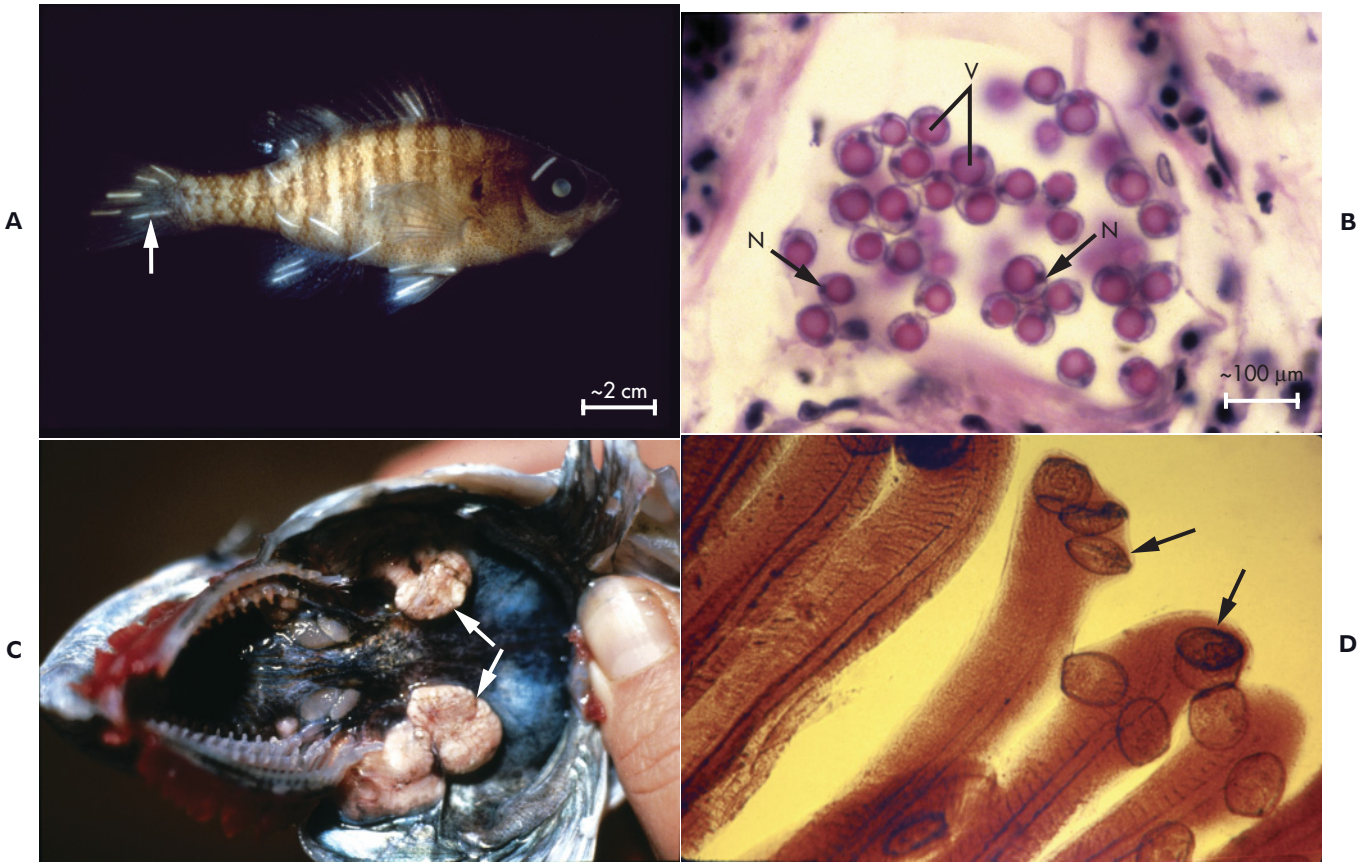

Fig. II-42. A. *Dermocystidium* gross lesion (*arrow*) in the fin of a sunfish. B. *Dermocystidium* spores. The mature spore has a large, PAS (periodic acid–Schiff stain) positive vacuole (*V*) surrounded by a thin rim of host cytoplasm, except where it thickens to make room for the nucleus (*N*). The inclusion is PAS (+) and hematoxylin and eosin (–) (Hatai 1989). C. X-cell lesions (*arrows*) in the gill cavity of a blue whiting. D. Glochidia infestation (*arrows*) of the gills of a fish. (*A* photograph courtesy of D. Demont; *B* photograph courtesy of J. Lom; *C* photograph courtesy of H Möller; *D* photograph courtesy of A. Mitchell.)

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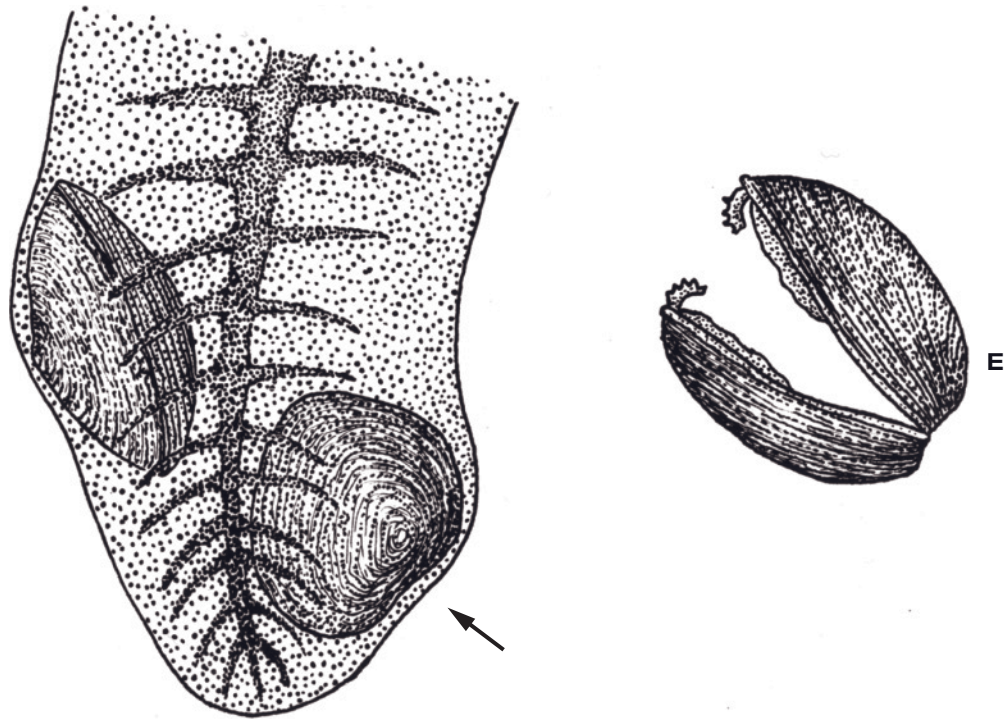


Fig. II-42.—cont'd.

branch or skin of some marine fish, especially Atlantic cod and dab in the North and Baltic Seas in Europe (Waterman and Dethlefsen 1982; Diamant and McVicar 1989) and in North America in the Pacific Ocean (Wellings et al. 1976). Previously speculated to be aberrant host cells (i.e., neoplasia), genetic analysis has shown them to be some type of as yet unidentified protozoan parasite (Miwa et al. 2004). X-cells often form "cysts," which are subivided into compartments (Fig. II-42, C).

Algal infections: Algal infections have been reported from a few fish (Edwards 1978), especially centrarchids. Bony prominences are common infection sites (Vinyard 1953; E. Noga, unpublished data). Infections of the eye (Hoffman et al. 1965), intestinal mucosa (Langdon 1986), and skin (Blasiola and Turnier 1979) have also been reported. No treatments have been reported.

Glochidia-producing freshwater bivalve molluscs: Primarily members of the family Unioidae, this group of freshwater bivalves has an obligatory parasitic stage. The infective larvae (glochidia) are released by the adult clams and are passively dispersed via water currents. They attach to the gills and/or skin of fish, using sharp hooks on each shell valve. This incites a hyperplastic response in the fish's epithelium (Fig. II-42, D, E). Eventually, the parasites are shed when they metamorphose into adult clams. Infestations are usually innocuous unless heavy. No treatments have been reported.

Mycoplasma: *Mycoplasma mobile* is the only mycoplasma that has been isolated from fish. It was cultured from the gills of tench (Stadtlander and Kirchoff 1989). While extensively studied, it has not yet been proven to be pathogenic to fish, although it can damage gill tissues cultured in vitro.

Miscellaneous pathogens: Various other invertebrates have been rarely reported to infest mainly marine fish, including cnidarians, amphipods, cirripeds (barnacles), and ostracods (Kinne 1984). Candiru are small, pencil-thin, South American catish that infest the gill chamber of larger fish (Axelrod et al. 1980).

PROBLEM 43
Incidental Findings

Prevalence Index

WF - 1, WM - 1, CF - 1, CM - 1

Method of Diagnosis

Wet mount of skin or gills with organism/foreign body

COMMENTS

Nonpathogenic protozoans and other organisms are commonly seen in wet mounts from the skin or gills of fish. It is important to recognize these as incidental find-

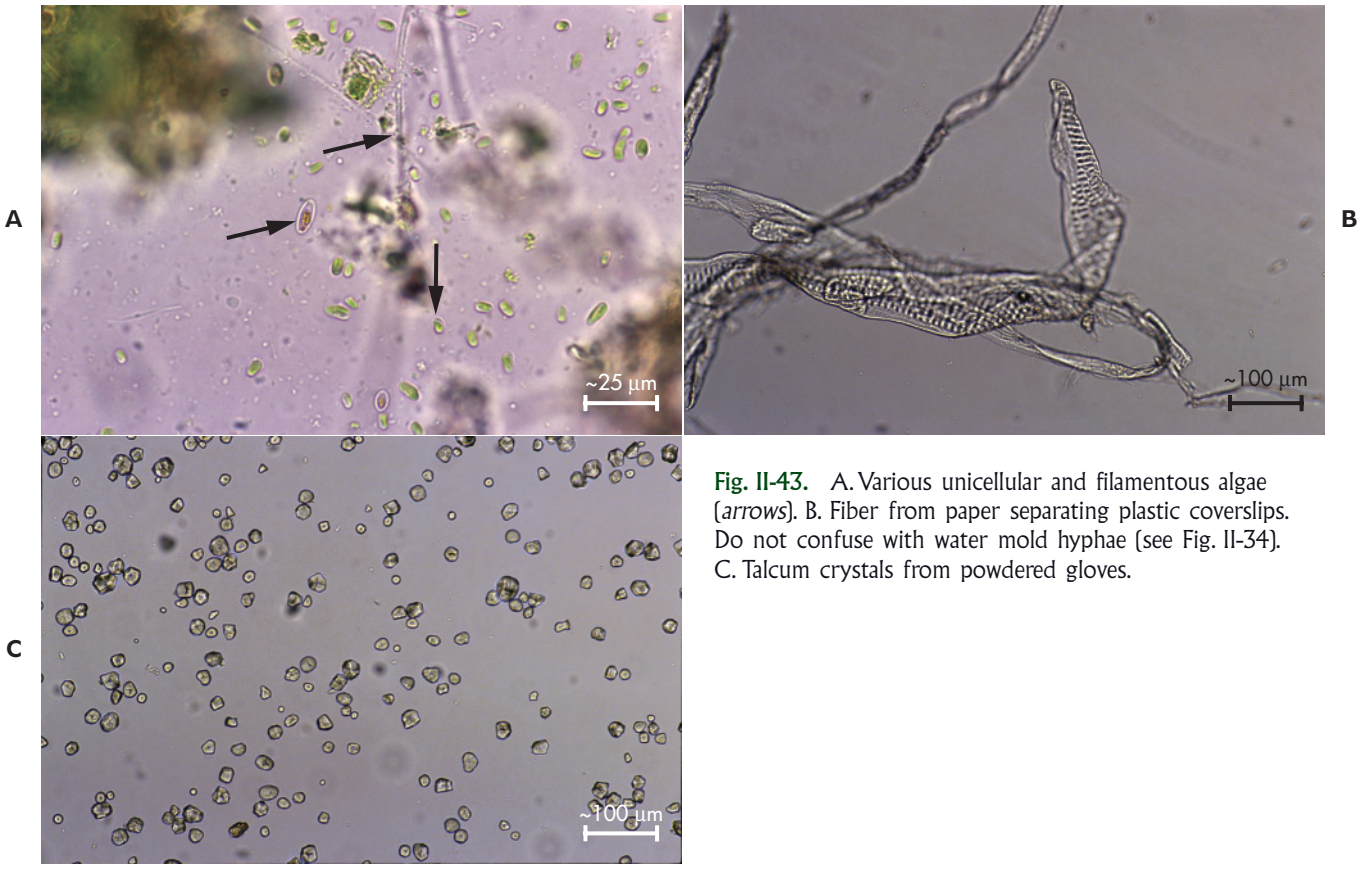


Fig. II-43. A. Various unicellular and filamentous algae (*arrows*). B. Fiber from paper separating plastic coverslips. Do not confuse with water mold hyphae (see Fig. II-34). C. Talcum crystals from powdered gloves.

ings, so that the true cause of the problem is pursued. Fish possess an endogenous skin and gill flora, which probably includes protozoa. It is not unusual to see an occasional ciliate or flagellate on a wet mount of normal skin or gill. Gills commonly trap debris in proportion to the amount of suspended matter in the water and may have various types of algae (Fig. II-43, A), protozoa, and nonpathogenic bacteria. Inanimate objects (Fig. II-43, B and C) may also be seen occasionally.

Gill and especially skin wounds are often secondarily colonized by a wide array of organisms that are simply taking advantage of the nutrient soup provided by this damaged tissue. Some may be present in high numbers. For example, a wound having a large number of bacteria often also has a large complement of phagotrophic protozoa. While these organisms may make some contribution to disease, there is no documented proof of their pathogenicity.

CHAPTER 9

PROBLEM 44

Diagnoses made by examination of a gill clip or a blood smear

44. Primary hemopathies

PROBLEM 44
Primary Hemopathies

Prevalence Index
WF - 4, WM - 4, CF - 4, CM - 4
Method of Diagnosis
1. Wet mount of blood or tissue with pathogen
2. Blood or tissue smear with pathogen
3. Histopathology of tissue with pathogen

History
Lethargy, weight loss, chronic mortality; leech infestation; may be no clinical signs; wild-caught or pond-raised fish
Physical Examination
May be anemia, cachexia, or other clinical signs but is often an incidental finding
Treatment
See specific pathogen; usually best to increase oxygen content of water

COMMENTS
Primary hemopathies are diseases that primarily affect the peripheral blood. Other organs may be affected secondarily. Note that other diseases not mentioned in this chapter may secondarily cause hemopathies (e.g., many viral and bacterial infections, some parasites, toxins).
Blood Flukes
Blood flukes are digenean trematodes that infect the circulatory system. *Sanguinicola* and *Cardicola* infect salmonids and can cause mild-to-heavy mortalities in hatcheries in the western United States that use surface water (Evans and Heckmann 1973). *Sanguinicola* also infects common carp in Europe and mullet in Australia. Sanguinicolids (*Paradeotacylix* spp.) have caused mass mortalities in cultured amberjack in Japan (Ogawa et al. 1993).

The general life cycle is similar to that of other digeneans (see Fig. II-58, A), with fish acting as the final host. Blood flukes need only one intermediate host. Cercariae penetrate the fish and migrate to target organs. Interestingly, some species that infect marine fish use a polychaete as an intermediate host (they are the only digeneans known to not require a mollusk in their life cycle [Lester 1988].)

Adults reside in blood vessels and in the heart or peritoneal cavity. They release fertilized eggs, which lodge in gill blood vessels (intestinal blood vessels in mullet), causing thrombosis, lethargy, and gill irritation, as indicated by flashing. Lamellae are swollen and ischemic. Eggs may incite an inflammatory response. Eggs gradually make their way to the outside. They often embryonate while undergoing the migration and may have fully developed miracidia (Fig. II-44, C).

Infections are usually diagnosed by identifying the ova or miracidia in the gills (or intestine in mullet) (Fig. II-44, C). Avoidance of surface water that contains the intermediate host is the only proven control, although praziquantel might be effective.
Trypanosomes
Trypanosomes (Fig. II-44, A_1) are relatively uncommon blood infections in most cultured fish but are common in wild populations, especially cold water species (e.g., European carp, tench). At times they may also cause heavy infections in some cultured fish (e.g., pond-cultured carp in Europe). They may be encountered on routine examinations, such as when gill clips are examined for other pathogens. They may be found in high concentrations (reportedly up to 1,000,000 organisms/µl blood) and often localize in blood-filtering organs, such as the kidney.

PATHOGENESIS
Trypanosomes can cause anemia, hematopoietic damage, and death. Common pathogenic trypanosomes include *Trypanosoma carassii* (*T. danilewskyi*) (Woo 1987) infecting goldfish, common carp, and some noncyprinids (Woo 1987; Dyková and Lom 1979b), *T. cobitis* infecting weatherfish (cobitids) (Letch 1980), and *T. murmanensis*, a well-known North Atlantic species that infects 13 diverse fish species (e.g., cod, plaice, eels) (Khan 1985).

LIFE CYCLE
Fish trypanosomes are transmitted by leeches—a process that is necessary for the completion of the trypanosome species' life cycle. A single leech species can often

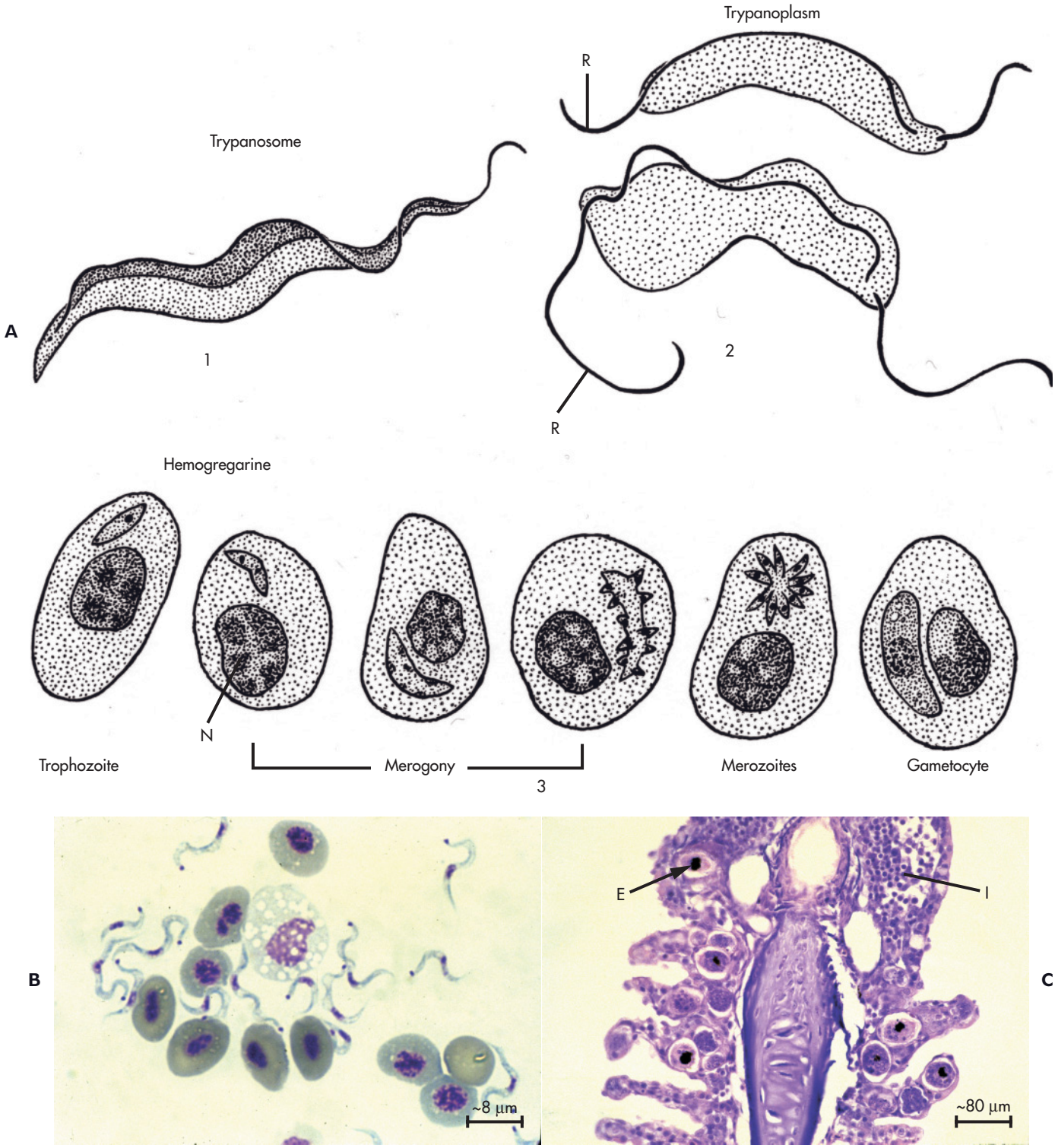


Fig. II-44. Diagram of representative examples of various hemoparasites, with key features: A_1. Trypanosomes: shape; single flagellum directed anteriorly. A_2. Trypanoplasm: pleomorphic shape; two flagella, one directed anteriorly, the other (*R* = recurrent flagellum) directed posteriorly. The recurrent flagellum forms a characteristically wide, wavy, undulating membrane; these organisms are highly similar to *Cryptobia* (see PROBLEM 30). A_3. Hemogregarine (typical development stages). *N* = host cell nucleus. B. Blood smear with trypanosomes. Giemsa. C. Histological section of *Sanguinicola* miracidia lodged in gill vessels of a salmonid. Note the parasite's characteristic, darkly pigmented eyespot (*E*). There is also some branchial inflammation (*I*). Hematoxylin and eosin.

Continued.

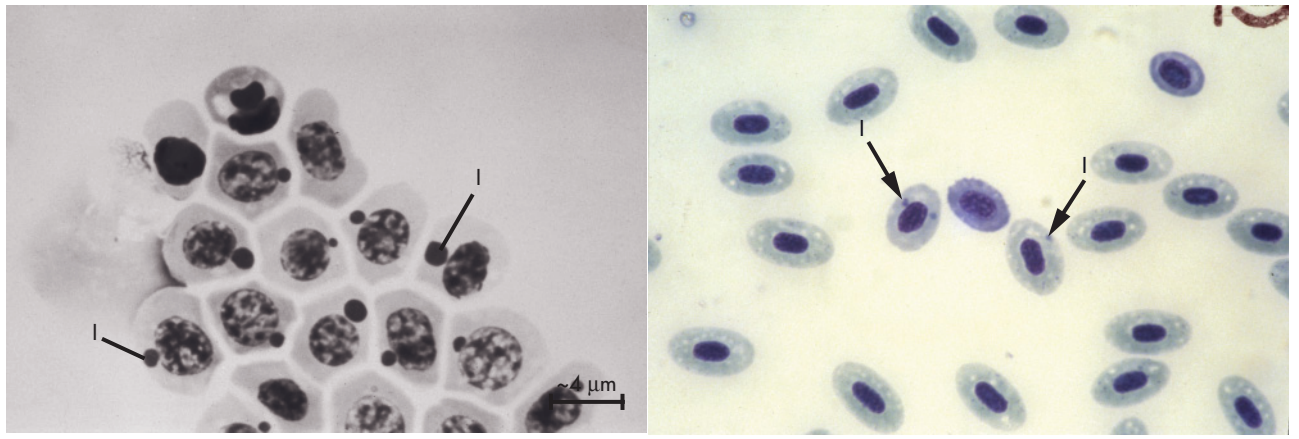


Fig. II-44.—cont'd. D. Blood smears of (*1*) viral erythrocytic necrosis; (*2*) erythrocytic inclusion body syndrome (EIBS). *I* = inclusions. Giemsa. (*A_3* modified from Khan 1972; *B* photograph courtesy of C. Huang; *D_1* photograph courtesy of M. Kent; *D_2* photograph courtesy of C. Smith; *E* photograph courtesy of B. Hjeltnes.)

transmit more than one trypanosome species. They develop in the gut of the leech, producing large numbers of the fish-infective stage (trypomastigotes), which are then transferred to a fish host with the leech's blood meal.

Trypomastigotes in fish blood may be either small forms (acute infection) or large forms (chronic infection), since they increase in size the longer they remain in the blood. Some trypomastigotes of marine fish are up to 100 µm, but most freshwater species are not more than about 50 µm. There may be a mixture of forms in blood because of repetitive infections by leeches. It is not known if all fish forms can start a leech infection. When ingested by a leech, the trypomastigotes form amastigotes, which have no flagellum. The trypanosome then goes through several other developmental stages, eventually producing a trypomastigote, which is infective for fish.

DIAGNOSIS

The major differential is trypanoplasms (Fig. II-44, A_2), which have two flagella. Trypanosomes also wriggle vigorously in one place. Controlling the leech population is the only known method of treatment.

Trypanoplasms

Trypanoplasma (Fig. II-44, A_2) is morphologically similar to *Cryptobia* (see PROBLEM 30), and both genera have been combined by some researchers (Woo 1987, 2006). Thirty-five fish species are infected by trypanoplasms.

LIFE CYCLE

The life cycle is similar to trypanosomes, with a prepatent period immediately after a leech infects the fish, followed by a parasitemia and then either death of the fish or eventual absence of the parasite from the peripheral blood. At this point, there is often a nonsterile immunity (i.e., no parasites in peripheral blood, but the fish is still infected). In some species, there can be several cycles of parasitemia.

PATHOGENESIS

Trypanoplasma borreli causes anemia (sleeping sickness) in goldfish, koi, and other cyprinids (Kruse et al. 1989). In freshwater salmonids, *T. salmositica* causes a virulent systemic disease, with progressive anemia (pale gills), exophthalmos, abdominal distension, and splenomegaly, presumably caused by hypoproteinemia and vascular damage (Woo 2006). Parasites may also cause immunosuppression. *Trypanoplasma bullocki* infects 13 diverse species of marine fish along the western Atlantic and Gulf of Mexico and may contribute to natural mortality in some flatfish (Burreson and Frizzell 1986). Affected fish have a distended abdomen because of edema.

DIAGNOSIS/TREATMENT

Trypanoplasma is distinguished from trypanosomes by its flowing, amoeboid motility and by the presence of two flagella (Fig. II-44, A_2). It is distinguished from the morphologically similar *Cryptobia* (see PROBLEM 30) by its more developed undulating membrane, predilection for blood, and indirect life cycle (leech vector). Note, however, that *T. salmositica* may also occur on the gills and can also be transmitted mechanically (Woo 2006). There is no treatment, except for eliminating the leech vector.

Apicomplexan Hemoparasites

Several types of apicomplexan hemoparasites are uncommonly encountered, mainly in wild fish: haemogregarine coccidia (e.g., *Haemogregarina*, *Cyrilia*, *Desseria*), dactylosomatid coccidia (e.g., *Babesiosoma* and *Dactylosoma*) and those of uncertain taxonomy (e.g., *Haemohormidium* and *Haematractidium;* Fig. II-44, A_3). Davies (1995)

tallied 72 species from marine fish and 31 species from freshwater fish. Diagnosis is based on identification in blood smears. Little is known about their life cycles, but all probably require an intermediate host, such as a leech or parasitic crustacean (Davies and Johnston 2000; Davies and Smit 2001). Most are incidental findings, but *Haemogregarina sachai* infection of cultured turbot in Scotland caused anemia, leucocytosis, and tumor-like granulomas in various tissues (Ferguson and Roberts 1975).

Viral Hemopathies

Viral hemopathies typically present as various types of inclusions, especially in erythrocytes. Some have been associated with disease, but others are only incidental findings. Presumptive diagnosis is usually made from stained blood smears. Definitive diagnosis of specific types of viral hemopathies requires electron microscopic examination of infected cells to identify the specific virus present. The only known treatment is to decrease stress to prevent infection by opportunistic pathogens.

VIRAL ERYTHROCYTIC NECROSIS (VEN; PISCINE ERYTHROCYTIC NECROSIS [PEN])

Viral erythrocytic necrosis (previously known as piscine erythrocytic necrosis) refers to a morphologically heterogenous group of viruses that infect members of 14 families of marine fish, including Atlantic salmon, Atlantic cod, and Atlantic herring. Affected fish include 23 genera in North America, 3 genera in the Pacific Northwest, and 4 genera in Atlantic waters of Europe (MacMillan et al. 1980, 1989a). Some populations have 100% prevalence. The infection can be experimentally transmitted with infected blood within species but not between species. This suggests that a hematophagous vector may be required for transmission. Vertical transmission is also suspected.

Clinically, VEN presents as intracytoplasmic inclusions in the erythrocytes, consisting of masses of viral particles and/or degenerative changes (e.g., karyolysis) of the nucleus (Fig. II-44, D_1). Affected erythrocytes are irregular, more osmotically fragile, with degenerative nuclear and cytoplasmic changes. There are single (rarely multiple), 0.3–4.0 μm inclusions that stain green with acridine orange. Anemia occurs in some infected salmonids, but this has not been experimentally documented.

ERYTHROCYTIC INCLUSION BODY SYNDROME (EIBS)

EIBS causes a progressive, severe anemia in juvenile to yearling chinook and in coho salmon in the Pacific Northwest. It can be transmitted experimentally via water, as well as orally. Recovered fish are resistant. The time-course of infection may be up to 5 months; clinical course and recovery are faster at high temperatures.

Blood smears stained with pinacyanol chloride (best) or Leishman-Giemsa reveal a single, purple-pink, 0.8–3.0 μm inclusion in erythrocytes (Fig. II-44, D_2). Acridine orange staining reveals a red inclusion (Holt and Piacenti 1989). There is also splenic hemosiderosis.

COHO ANEMIA

Coho anemia affects seawater-reared coho salmon in California. By Leishman-Giemsa, there are many 1–2 μm (often rod-shaped) inclusions in erythrocytes. There is also macrophage hemosiderosis in kidney, spleen, and liver (Hedrick et al. 1987b).

INTRAERYTHROCYTIC VIRAL DISEASE OF RAINBOW TROUT

Only one case of this disease has been documented (in Donaldson strain of rainbow trout). It presented as exsanguinating hemorrhage, hypoxia, and sudden death. There were small, basophilic, pleomorphic inclusions in erythrocytes.

Toxicoses

NITRITE POISONING (SEE PROBLEM 5)

Idiopathic Hemopathies

NO BLOOD DISEASE (WHITE LIP DISEASE)

This is a chronic, often severe anemia of channel catfish. Hematocrits can be as low as 1%, with <10 being common (Plumb et al. 1986). Other gross signs include mottled skin, ascites, and pale gills and viscera. The pale flesh may cause rejection by processors. The disease is chronic, but acute mortalities may occur with concurrent environmental hypoxia. Folate deficiency (Plumb et al. 1991), or some other feed-related problem, has been suspected, but now this is not considered to be the likely cause. No blood disease in Mississippi typically affects only 1–2 ponds on a farm, further suggesting that diet is not responsible in these cases (Johnson 1993b).

CHAPTER 10

PROBLEMS 45 through 57

Diagnoses made by bacterial culture of kidney or affected organs

45. Bacterial dermatopathies/systemic bacterial infections: general features
46. Motile aeromonad infection
47. *Aeromonas salmonicida* infection
48. Enteric septicemia of catfish
49. *Edwardsiella tarda* infection
50. Vibriosis
51. Pasteurellosis
52. Enteric redmouth disease
53. Streptococcosis
54. Bacterial kidney disease
55. Mycobacteriosis
56. Piscirickettsiosis
57. Miscellaneous systemic bacterial infections

PROBLEM 45

Bacterial Dermatopathies/Systemic Bacterial Infections: General Features

Prevalence Index

WF - 1, WM - 1, CF - 1, CM - 1

Method of Diagnosis

1. Culture of clinically relevant numbers of bacteria from skin lesions and/or internal organs
2. Clinical signs with histopathological/antibody test/gene test evidence of infection

History

Varies with pathogen; acute to chronic morbidity/mortality

Physical Examination

Red areas on body; skin ulcers; depression; exophthalmos; peritonitis (swollen abdomen)

Treatment

1. Appropriate antibiotic
2. Eliminate responsible stress
3. Disinfect and quarantine if appropriate

COMMENTS

Epidemiology/Pathogenesis

Bacteria are important pathogens in both wild and cultured fish and are responsible for serious economic losses. Some may cause primarily a surface (skin/gill) infection (see PROBLEMS 37, 38, and 39); most can cause systemic disease. A wide array of bacteria cause infections in marine or freshwater fish. Few pathogens infect both freshwater and marine fish. Most pathogens are Gram-negative rods.

Many pathogens can present as only skin infections, especially flexibacteria, aeromonads, and vibrios. Fish may present with fin rot, an imprecise general term for ulcerative, necrotic lesions that affect the fins (see Fig. I-3). Various bacteria are often present in fin rot lesions, but some stress is considered to be the primary cause. The fin rot syndrome includes several diseases and idiopathic responses. Bacterial skin infections can advance to become systemic, leading to much greater and more acute mortality. In at least one instance, typical *Aeromonas salmonicida*, skin ulcers may originate from the hematogenous spread of a systemic infection.

The classical signs associated with systemic bacterial infection are indicative of a bacterial toxemia/septicemia and include diffuse hemorrhage and necrosis of internal organs, especially those involved in filtering blood (spleen, kidney). Kidney and/or spleen is often enlarged. External signs may include skin ulcers, fin necrosis, or hemorrhages on the body (petechiation, ecchymoses) and fins (Fig. II-45). Fish often have unilateral or bilateral exophthalmos (see Fig. I-3) and fluid accumulation in the abdomen (see Fig. I-3). Less commonly, bacteria may be observed in blood smears (Fig. II-45, D)

Not all bacteria that cause systemic disease produce the above clinical signs, but these signs are common. Bacteria are also an important cause of egg mortality (see PROBLEM 103).

Most fish-pathogenic bacteria can reside in the environment or on/in apparently normal fish (latent carriers). Thus, infections are often precipitated by some stress that upsets the natural defenses against these agents (e.g., overcrowding, low DO, high ammonia).

Diagnosis

Definitive diagnosis of bacterial disease requires the culture of the pathogen from skin lesions and/or internal organs, since clinical signs are rarely pathognomonic or even diagnostic for any specific pathogen. Culture is also

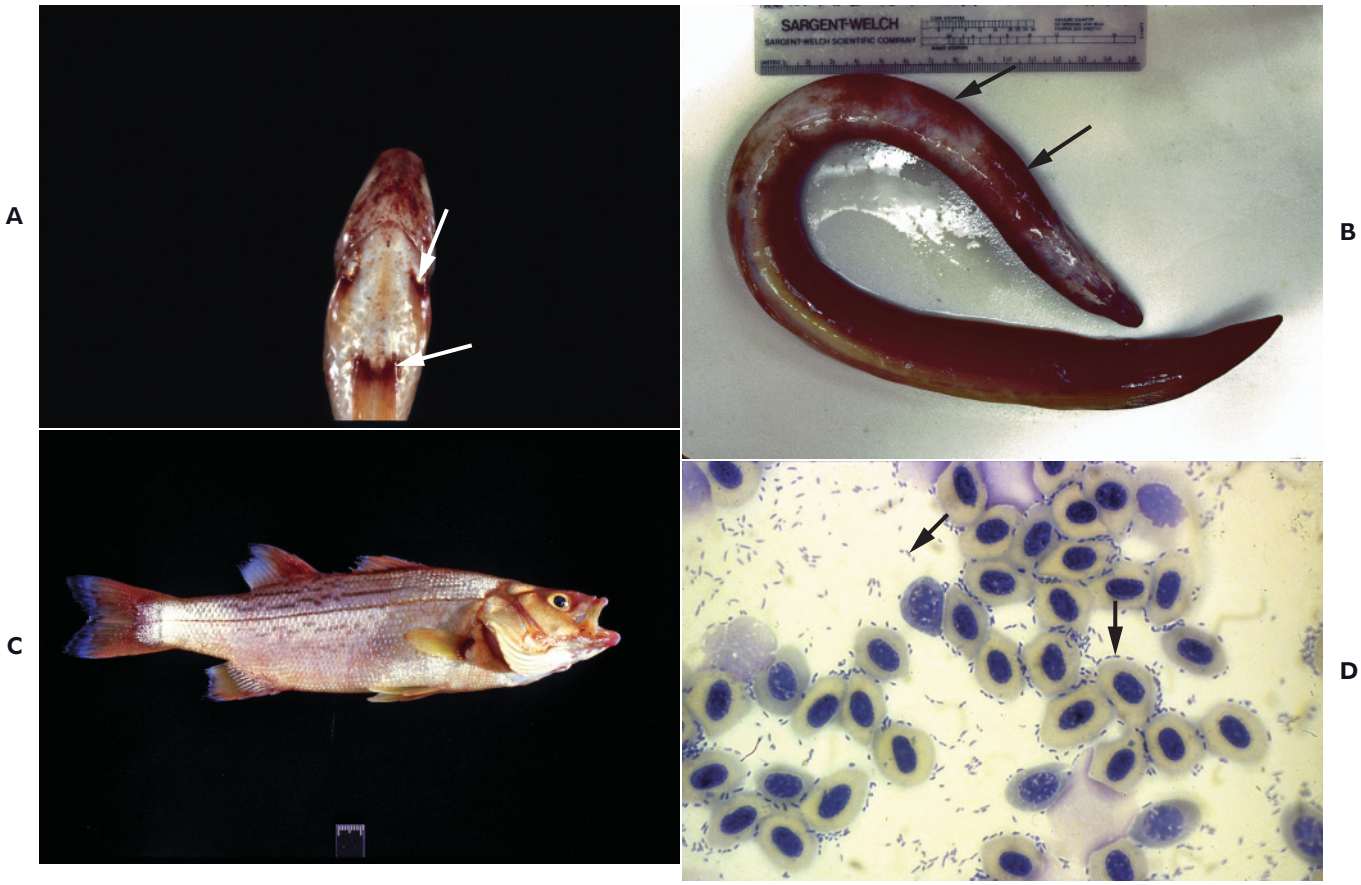


Fig. II-45. Signs of bacterial hemorrhagic septicemia. A. Goldfish (*ventral view*). Petechial hemorrhages and congestion or hemorrhage at the base of the fins (*arrows*). B. American eel (*ventral view*). Ecchymotic hemorrhages (*arrows*). C. Striped bass. Reddening of fins. This could be caused by hemorrhage, hyperemia, or congestion. D. Blood smear of a fish with a systemic bacterial infection. Note the large number of bacteria appearing as short rods (*arrows*), which are typical of Gram-negative bacteria. Giemsa. (D photograph courtesy of B. Hjeltnes.)

essential for determining antibiotic sensitivity, which often varies widely between isolates.

Bacteria may often infect the skin, producing erosions or ulcers. Skin lesions are especially a diagnostic challenge, many times having multiple pathogens. It is often difficult to determine the initiating (i.e., primary) pathogen. It is best to identify the predominant colony type, but this ignores the fact that unrelated organisms may have a similar colonial appearance. Also, the primary pathogen may not be the most common organism at the time of culture, especially if lesions are chronic (see PROBLEM 47).

Culture of internal organs is usually more straightforward although, even here, multiple bacterial species may be present. Kidney is the best tissue for routine isolation of systemic pathogens. However, other tissues may be preferable for certain pathogens or for identifying asymptomatic carriers. Multiple infections are common, and determining all the important pathogens is important. See "**Culturing for Bacteria**" (**p. 49**) for details on sampling for bacteria. Comprehensive coverage of the various methods for identifying bacterial pathogens from fish is provided in Buller (2004) and Austin and Austin (2007). For rapid identification of the pathogen involved, immunodiagnosis is frequently used (Anderson and Barney 1991). Companies selling immunodiagnostic kits and reagents include Aquatic Diagnostics, BIONOR Aqua and Microtek. For genetic identification, specific PCR primers to amplify various bacterial pathogens in gene tests are provided in Buller (2004), but improved primers are constantly being developed so the most current literature should be examined if one intends to perform this test.

Treatment

Medical management is similar for all pathogens and usually requires treatment with antibiotics, although early stages of skin/gill infections may be amenable to antiseptic baths. Treatment options are limited for food fish (see "**Pharmacopoeia**"). Treatment should be initiated as soon as possible, since outbreaks can rapidly move

through a population. Removing the initiating causes of stress is also essential. Feeding antibiotics is the delivery method of choice, but sick fish will often be anorexic. If fish are not eating, treating for other complications, such as external parasites, may help. However, keep in mind that external treatments may also precipitate or enhance the severity of low-grade bacterial infections. There is an increasing prevalence of antibiotic-resistant strains. Antibiotic resistance is highly correlated with prior antibiotic use, so farms that frequently use antibiotics have the most problems. See the "**Antibiotics**" section of the "**Pharmacopoeia**" chapter for discussion of this problem.

PROBLEM 46

Motile Aeromonad Infection (MAI; Motile *Aeromonas* Septicemia [MAS] Red Sore)

Prevalence Index

WF - 1, WM - 4, CF - 1

Method of Diagnosis

Culture of large numbers of motile aeromonad bacteria from typical skin and/or internal lesions

History

Acute to chronic morbidity/mortality

Physical Examination

Red areas on body; skin ulcers; depression; exophthalmos; peritonitis (swollen abdomen)

Treatment

1. Eliminate primary cause
2. Appropriate antibiotic

COMMENTS

Epidemiology

Motile aeromonad infection (MAI) is probably the most common bacterial disease of freshwater fish (Cipriano 2001). All freshwater fish are probably susceptible. Motile aeromonads may also inhabit brackish water (Hazen et al. 1978) but decrease in prevalence with increasing salinity (Kaper et al. 1981), although they are occasionally isolated from diseased marine fish (Larsen and Jensen 1977). MAI has been associated with several members of the genus *Aeromonas*, which are ubiquitous in freshwater environments. By far the most important fish pathogen is *A. hydrophila* (syn. *A. liquefaciens*, *A. formicans*), and members of this group are often referred to as the *A. hydrophila* complex. Many other *Aeromonas* species have been taxonomically identified, but only a few aeromonads have been considered fish pathogens (e.g., *Aeromonas allosaccharophila* [Martinez-Murcia et al. 1992], *A. sobria* [Toranzo et al. 1989], *A. jandaei* [Esteve et al. 1993], *A. bestiarum*, *A. caviae* and *A. veronii* [Carnahan and Altwegg 1996]). Motile aeromonads are also commonly isolated from the mucosal surfaces and internal organs of clinically healthy fish (MacMillan 1985; Harikrishnan and Balasundaram 2005). Highest prevalence is in organically polluted waters (Hazen et al. 1978). Ingestion of contaminated feed may also be a source of infection (King and Shotts 1988).

Predisposing risk factors include high temperatures, overcrowding, organic pollution, and hypoxia. Motile aeromonads often invade skin wounds, commonly with water molds (see PROBLEM 34), or ectoparasites (Noga 1986b). *Aeromonas hydrophila* is often associated with the protozoan *Epistylis* in causing widespread epidemic skin lesions known as red-sore disease (Esch and Hazen 1980) (see PROBLEM 33).

Motile aeromonads are relatively weak pathogens, but isolate vary widely in pathogenicity (Lallier et al. 1980). An S-layer on the cell wall (Chabot and Thune 1991) and more elastase production (Shotts et al. 1985) are present in more pathogenic strains. While both endotoxin and exotoxins (proteases, hemolysins) are produced, their precise relationship with pathogenicity is unclear.

ZOONOTIC CONSIDERATIONS

Motile aeromonads can infect many vertebrates, including frogs, alligators, and man. Reports of gastrointestinal and systemic infection in humans have increased, but the ubiquity of these bacteria, combined with the frequent exposure of humans to these pathogens, suggests that risk of zoonotic infection may be relatively low. Disease can occur via ingestion of infected fish (causing self-limiting diarrhea) or puncture wound (that may cause skin ulcers, cellulitis and/or deep muscle necrosis). Some infections can become septicemic. Immunocompromised individuals are most susceptible but healthy individuals can also become sick (Lehane and Rawlin 2000).

Clinical Signs/Pathogenesis

GROSS LESIONS

Clinical signs of motile aeromonad infection range from superficial to deep skin lesions (Fig. II-46, A and B), to a typical, Gram-negative bacterial septicemia (see Fig. II-45, A), with or without skin lesions (Cipriano 2001).

Skin lesions include variously sized areas of hemorrhage and necrosis on the skin and the base of the fins (Fig. II-46, A). These may progress to reddish or gray ulcers with necrosis extending to the muscle (Fig. II-46, B). Ulcers may progress to hemorrhagic septicemia, with exophthalmos, a distended abdomen that has serosanguinous fluid, visceral petechiation, and a hemorrhagic and swollen lower intestine and vent (Fig. II-46, C). Peracute infections are not associated with skin lesions. Anorexia and dark color are most common with systemic disease.

HISTOPATHOLOGY

Skin lesions include acute-to-chronic dermatitis/myositis. In septicemias, there may be depletion and necrosis of the renal and splenic hematopoietic tissue, necrotic intestinal mucosa, and focal necrosis in the heart, liver, pancreas, and gonad (Bach et al. 1978; Huizinga et al. 1979). The presence of free melanin or lipofuscin from ruptured melanomacrophage centers is characteristic (Roberts 1989b).

Fig. II-46. A. *Aeromonas hydrophila* skin infection. Channel catfish with shallow ulcer. B. Channel catfish with *Aeromonas hydrophila* infection. Note the extensive, relatively deep skin ulcer. C. Rainbow trout with motile *Aeromonas* infection. Note the reddened, swollen vent. [*A* photograph courtesy of A. Mitchell; *C* photograph courtesy of R. Roberts.]

Diagnosis

Definitive diagnosis of motile aeromonad infection requires biochemical identification of clinically significant numbers of the suspect bacterium in target tissues, with attendant clinical signs. It is important to be certain that this is the primary infectious cause of the problem. Motile *Aeromonas* spp. are frequent secondary invaders, following channel catfish virus (PROBLEM 78), *Rhabdovirus carpio* (PROBLEM 83), *Aeromonas salmonicida* (PROBLEM 47), or other infections. Kidney is probably the best organ for isolation; lesions should also be sampled. Proper care should be taken when skin lesions are sampled (see "**Culturing for Bacteria**," **p. 49**). A culture of four to six fish is advisable to confirm the diagnosis. Motile aeromonads often overgrow more fastidious bacteria (e.g., *Edwardsiella ictaluri*, *Aeromonas salmonicida*).

Isolates vary widely in antigenicity, making immunological identification difficult. They are ~0.8–1.0 × 1.0–3.5 µm and motile by a single polar flagellum.

Treatment

Motile aeromonad infection is a classical example of a stress-borne disease. Losses because of MAI are highly dependent on the severity of the environmental stress that precipitated the outbreak. Outbreaks will often resolve themselves without antibiotic intervention if environmental problems are corrected. Also, antibiotic treatment may not be economically justifiable. Thus, whether to medicate should depend on the acuteness of the outbreak (mortality rate, feeding activity), severity of the stress, and speed at which the stress can be eliminated.

Oxytetracycline and nifurpirinol have successfully controlled some outbreaks. Antibiotics have also been used prophylactically in eastern European carp culture just before times of stress (Roberts 1993). However, many isolates are resistant to numerous antibiotics (Shotts et al. 1976; Dixon et al. 1990). Sulfadimethoxine-ormetoprim is often used in the United States for oxytetracycline-resistant isolates.

PROBLEM 47

Aeromonas salmonicida Infection (Furunculosis, Ulcer Disease, Goldfish Ulcer Disease, Carp Erythrodermatitis [CE])

Prevalence Index

CF - 1, CM - 1

Method of Diagnosis

Identification of *Aeromonas salmonicida* from typical skin and/or internal lesions

History

Acute to chronic morbidity/mortality

Physical Examination
Skin ulcers and "furuncles"; red areas on body; depression; exophthalmos; swollen abdomen
Treatment
Appropriate antibiotic

COMMENTS

Epidemiology
Aeromonas salmonicida infection is a common bacterial disease of freshwater fish (Munro and Hastings 1993; Bernoth et al. 1997) and is one of the most important diseases of salmonids. Atlantic salmon are most susceptible; rainbow trout are most resistant. Any age salmonid is susceptible. The organism is also an important pathogen of nonsalmonids. Goldfish, common carp, koi, and American and Japanese eels are most often affected, but bream, roach, dace, chub, tench, pike, bullhead, sculpin, catfish (McCarthy 1978), wrasse (Treasurer and Cox 1991), as well as Artic charr, grayling (Pylkkö et al. 2005), smallmouth bass, northern pike, yellow perch, brook stickleback, sablefish, hybrid striped bass, and lamprey are susceptible.

It has also become a serious problem in marine fish, especially Atlantic salmon culture. Other susceptible marine species include gilthead sea bream, Schlegl's black rockfish, shotted halibut, dab, plaice, flesus flounder, Japanese flounder, turbot, fat greenling, Atlantic cod and common wolf fish (Wiklund and Dalsgaard 1998; Magnadóttir et al. 2002; Austin and Austin 2007). Disease can also occur in wild fish.

TYPICAL VS. ATYPICAL STRAINS
There are three subspecies of *A. salmonicida* (Martin-Carnahan and Joseph 2005): The typical subspecies *A. salmonicida* subspecies *salmonicida* is usually associated with systemic disease (furunculosis), while the "atypical" subspecies *A. salmonicida* subspecies *achromogenes* and *masoucida*, a heterogenous group which does not conform to the typical phenotypic pattern in culture, are usually associated with infections localized to the skin (ulcer disease) and are usually isolated from nonsalmonid fish. However, this distinction is not perfectly demarcated; typical strains have been isolated from ulcer disease lesions (Noga and Berkhoff 1990), and atypical isolates can cause furunculosis (Munro and Hastings 1993).

TRANSMISSION
Skin ulcers are a major source of infection during epidemics, but the mechanism of horizontal transmission is not known. A high percentage of carriers can develop after an epidemic; shedding is via the feces. Vertical transmission via infected ova occurs rarely, if ever (Bullock and Stuckey 1987). In salmonids, outbreaks are typically associated with stress, especially high temperatures. Isolates vary in pathogenicity. *Aeromonas salmonicida* has also been recovered from sea lice (PROBLEM 14) and marine plankton (Nese and Enger 1993), and wild fish may be a source of infection for some salmonids (El Morabit et al. 2004). It is uncertain if the bacterium can survive long-term off of fish.

Clinical Signs/Pathology
Clinical signs of *Aeromonas salmonicida* infection range from superficial or deep skin lesions without systemic involvement (ulcer disease) to a typical, Gram-negative bacterial septicemia (furunculosis) (Fig. II-47, A through D).

FURUNCULOSIS—GROSS LESIONS
Furunculosis, the classical form of *Aeromonas salmonicida* infection, primarily affects salmonids. Clinical signs of furunculosis depend on the time-course of infection, with gross signs more apparent with increasing chronicity. Peracute disease, which is the least common presentation, has been seen in salmonid fry. Fish die rapidly, typically without any gross lesions except darkening (McCarthy and Roberts 1980); there are very high mortalities. The acute form is the most common, especially in growing fish. It presents as a typical bacterial hemorrhagic septicemia, with bacteria disseminated in many tissues; fish often die in 2–3 days; mortalities can be high.

The subacute/chronic form is less common than the acute form. Mostly seen in adults, it presents as a more chronic form of bacterial hemorrhagic septicemia, which may include exophthalmos, bloody discharge from nares and vent, and multifocal hemorrhages in the viscera and muscle (Herman 1968; Roberts 1989b). The gills may be pale from anemia or may have hemorrhages (Bruno et al. 1986). Fibrinous edema and serosanguinous fluid may be present. The gastrointestinal tract may have a necrotic enteritis and catarrhal exudate (Ferguson and McCarthy 1978). The classical but inconsistently present clinical sign of chronic disease is the "furuncle," actually a dark, raised tumefaction, which ulcerates to release serosanguinous fluid (Fig. II-47, A). Furuncles develop from localization of hematogenous bacteria in the muscle or skin, not from an external skin infection. Mortalities are usually low.

FURUNCULOSIS—HISTOPATHOLOGY
Lesions are typical of a bacterial septicemia, with necrosis and hemorrhage, especially of well-vascularized organs (e.g., liver, spleen, kidney) (McCarthy and Roberts 1980). There is often a characteristic lack of immune cell response to infection, probably because of potent leukocidin production (Ellis 1991). Bacterial microcolonies are present in target organs (Fig. II-47, E). Leukopenia is common. Degranulation of eosinophilic granular cells of mainly the intestinal submucosa but also the gills is diagnostic (Vallejo and Ellis 1989).

ULCER DISEASE
Ulcer disease is the most common form of *A. salmonicida* infection in nonsalmonids; salmonids can also be affected. Unlike furunculosis, ulcer disease is typically

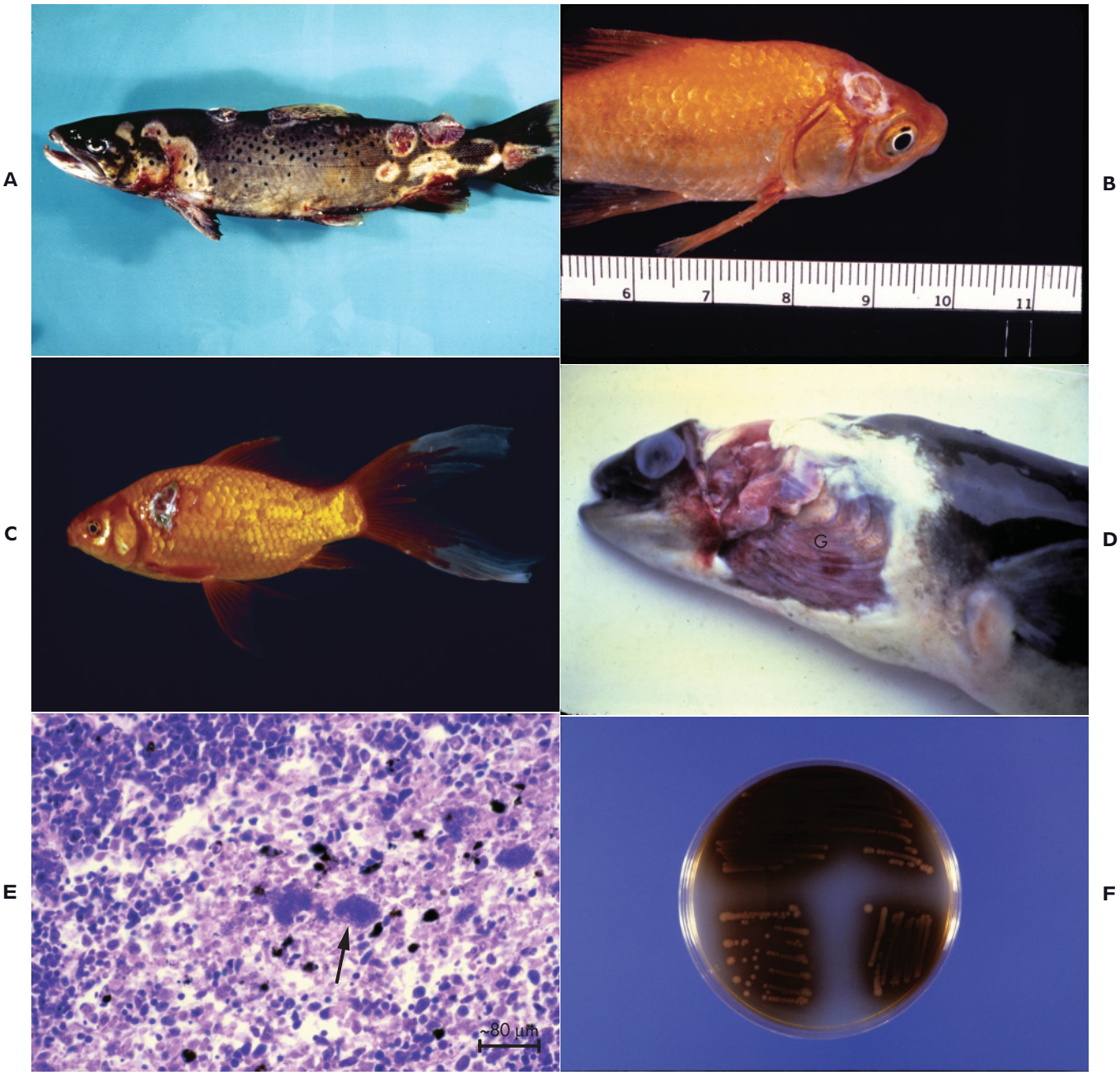


Fig. II-47. A. Salmon with furunculosis. Typical skin ulcers. B. Goldfish with relatively shallow, grey ulcer on head caused by *A. salmonicida*. C. Goldfish with deep, red ulcer caused by *A. salmonicida*. D. American eel with ulceration of head and corneal edema caused by *Aeromonas salmonicida* infection. *G* = exposed gill arches. E. Kidney of a salmonid with several focal *A. salmonicida* microcolonies (*arrow*). Eosinophilic area of necrosis surrounds the colonies. Hematoxylin and eosin. F. Brown, diffusible pigment surrounding *A. salmonicida* colonies grown on trypticase soy agar. (*A* photograph courtesy of R. Roberts.)

localized to the skin and only becomes systemic late in the disease. Carp erythrodermatitis (CE), an important disease in cultured carp in Europe, is caused by a skin infection with atypical *A. salmonicida* (Bootsma et al. 1977). This disease is part of the infectious dropsy of carp (IDC) complex. Infectious dropsy of carp encompasses two types of diseases in cultured carp: the acute form of IDC is now known to be caused by *Rhabdovirus carpio* (PROBLEM 83), while the chronic form of IDC is caused by atypical *Aeromonas salmonicida*.

ULCER DISEASE—GROSS LESIONS

Skin lesions range from whitish discolorations to shallow hemorrhagic ulcers to deep lesions that expose underlying muscle or bone (Shotts et al. 1980; Dror et al. 2006) (Fig. II-47, B and C). Because of their chronicity, lesions are often secondarily infected with water molds, protozoa, and other bacteria. Fish may have hemorrhage on the body and the base of the fins.

In eels, infections begin as depigmented foci that spread to form large patches of necrotic skin up to 16 cm^2 in area. The depigmented patches detach at the dermoepidermal junction, forming large ulcers that expose underlying muscle. The infection commonly affects the head, producing cranial swelling and corneal edema (swollen head disease of Japanese eels, Fig. II-47, D) (Ohtsuka et al. 1984).

ULCER DISEASE—HISTOPATHOLOGY

A mild to severe, primarily mononuclear infiltrate may be present. In eels many lesions have extensive collagen deposition, which contributes to the tissue swelling and belies the chronic nature of the disease (Noga and Berkhoff 1990). Chronic inflammation has also been reported in Atlantic cod skin lesions (Morrison et al. 1984).

Diagnosis

CLINICAL DISEASE—PRESUMPTIVE DIAGNOSIS

Presence of furunculosis is suggested by the presence of necrotic lesions with bacterial microcolonies (Fig. II-47, E). Necrosis of cardiac atrial endothelium may be the only lesion seen in peracute mortality of fry. On tissue smears, cells are bipolar staining, small coccoid to coccobacillary (~1 μm × 2 μm) rods. Other bacteria (e.g., *A. hydrophila*, *Vibrio*) can cause similar lesions, making this a presumptive diagnosis at best. Histopathological lesions of ulcer disease are not diagnostic.

CLINICAL DISEASE—DEFINITIVE DIAGNOSIS

Definitive diagnosis of clinical *A. salmonicida* infection requires identification of the bacterium in target tissues, with attendant clinical signs.

In clinical cases of systemic disease, the bacterium is readily isolated from kidney, spleen, or internal lesions. Isolating the bacterium from ulcer disease lesions can be difficult. Ulcer disease isolates of *A. salmonicida* are often fastidious and difficult to isolate. An enriched medium, such as brain heart infusion agar or 5% blood agar, should be used for primary isolation. Opportunists, such as *Aeromonas hydrophila*, can rapidly outcompete *A. salmonicida*, so primary cultures must be watched carefully daily. It is advisable to sample several fish, especially those with early lesions, where the primary pathogen is more likely to be isolated. Atypical *A. salmonicida* colonies are usually small, circular, grey, and up to 1.5 mm in diameter after 4–7 days at room temperature.

COLONY CHARACTERISTICS

Presumptive identification of typical *A. salmonicida* colonies is indicated by the presence of brown, diffusible pigment around colonies after 24 hours of incubation; this is most easily seen on clear agar (Fig. II-47, F) but is also visible on blood agar. However, both false-positives (e.g., some *Aeromonas hydrophila*, *A. media*, and *Pseudomonas* isolates) and false-negatives can occur. Most atypical strains produce no pigment or take several days to produce pigment.

Characteristically, colonies of most isolates can be pushed along the agar surface with an inoculating loop (Shotts and Teska 1989). The bacterium is nonmotile (differentiating it from motile aeromonads).

Definitive identification of *A. salmonicida* can be accomplished with biochemical tests. However, atypical strains do not conform to the typical biochemical profile for this species (Shotts and Teska 1989), so definitive diagnosis should include immunological confirmation, such as with latex bead agglutination or immunofluorescent antibody, or a gene test (Austin and Austin 2007). Atypical *A. salmonicida* infecting salmonids has previously been misidentified as *Haemophilus piscium*.

CARRIERS

The kidney and lower intestine should be cultured when searching for asymptomatic carriers (Bullock et al. 1983; Rose et al. 1989). Skin and gills also can be cultured, but other bacteria commonly overgrow isolates (Munro and Hastings 1993). False negatives are common. More asymptomatic carriers can be detected by stressing suspect fish with heat shock (raising the temperature to 18°C [64°F] from 12°C [50°F]) or glucocorticoid immunosuppression (0.80 mg triamcinolone acetamide in small brook trout, 8 mg in large fish) (Bullock and Stuckey 1975). Using both heat and corticosteroids increases recovery rate of the bacterium. ELISA is even more sensitive in detecting carriers (Rose et al. 1989).

Treatment

SALMONIDS

During outbreaks, all moribund fish, especially those with skin ulcers, should be promptly removed and disposed of properly (i.e., do not allow contagion to reenter the system). Oral oxytetracycline, furazolidone, oxolinic acid, and potentiated sulfonamides have been used successfully, but many isolates are resistant. In Europe, isolates with multiple resistance are much more prevalent in sea-caged fish, compared with those in freshwater fish (Munro and Hastings 1993). Amoxicillin is most effective against European isolates, although atypical isolates are often resistant (Barnes et al. 1991). Sulfadimethoxine-ormetoprim is used for oxytetracycline-resistant isolates in the United States. Fluoroquinolones (e.g., enrofloxacin, sarafloxacin) are more effective than oxolinic acid (Austin and Austin 2007). Florfenicol is also effective.

Disinfection and quarantine, followed by stocking specific-pathogen-free fish and eggs, can eliminate the infection from facilities, so long as stocks are not re-exposed to water that has infected feral fish. Riverine

waters containing wild salmonids and the presence of infected nonsalmonids around sea cages present important risks to cultured salmonids (Munro and Hastings 1993). Wrasses introduced to control sea lice should have a health exam before introduction; these fish should not be transferred between farms or released into the wild.

Aeromonas salmonicida is probably an obligate pathogen but may survive for long periods off of host fish. Bacteria can survive in water for up to about 3 weeks and may possibly survive for months in sediments (Munro and Hastings 1993). The 6-week period used to fallow sea cages may not be long enough to eliminate the pathogen. It can be transmitted at least short distances via aerosol (Wooster and Bowser 1996). Reducing stress is imperative for long-term management.

Furunculosis vaccines are commercially available; injectable preparations with oil adjuvant provide good protection.

NONSALMONIDS

Because isolation of atypical *A. salmonicida* is often difficult, individual pet goldfish or koi are often treated based on the presence of typical lesions. Enrofloxacin has been successful in some cases (Lewbart 2001). However, other diseases, such as mycobacteriosis, may cause similar lesions, so necropsy and culture is advised when possible. Controlling the secondary bacterial invaders such as *Aeromonas hydrophila* is often key to healing the ulcers, so obtaining culture and sensitivity data on the predominant organism (which is usually not *A. salmonicida*) is important. Wound debridement has also been used for treating ulcers of pet fish (Barker 2001).

Except for goldfish ulcer disease and carp erythrodermatitis, treatment has not been attempted in most cases of nonsalmonid furunculosis. Similar antibiotics should be considered. It is speculated that death from ulcer disease may be due to osmoregulatory damage, rather than toxemia (Munro and Hastings 1993). This is supported by the fact that the adding of estuarine water reduced morbidity in American eels held in freshwater impoundments in North Carolina (N. Marquardt, personal communication). Inhibition of some isolates by seawater cannot be ruled out.

PROBLEM 48

Enteric Septicemia of Catfish (ESC; *Edwardsiella ictaluri* Infection)

Prevalence Index

WF - 1

Method of Diagnosis

Culture of *Edwardsiella ictaluri* from typical lesions

History

Usually acute, but sometimes chronic, mortality; corkscrew spiral swimming

Physical Examination

Raised open ulcer on frontal bone of skull; bloody or clear fluid in peritoneal cavity; swollen abdomen; exophthalmos; red or white areas on body; skin ulcers; depression

Treatment

Appropriate antibiotic

COMMENTS

Epidemiology/Pathogenesis

Enteric septicemia is one of the most important disease that affects channel catfish, causing millions of dollars in losses annually in the United States. It has a high predilection for channel catfish but has been occasionally isolated from other ictalurids (brown bullhead, blue catfish, white catfish [Iwanowicz et al. 2006]), other catfish (walking catfish in Thailand, striped catfish in Indonesia [Yuasa et al. 2003] and Viet Nam [Crumlish et al. 2002]), and unrelated species, such as green knife fish, devario danio and rosy barbs (Kent and Lyons 1982; Waltman et al. 1985; Humphrey et al. 1986). It has recently caused epidemics in wild ayu in Japan (Sakai et al. 2008). It has been associated with neurological disease in poeciliid tropical aquarium fish (R. Francis-Floyd, personal communication). European catfish are experimentally susceptible. Surprisingly, rainbow trout and chinook salmon are very susceptible to experimental challenge (Baxa-Antonio and Hedrick 1992), and it has caused disease in farmed rainbow trout (Keskin et al. 2004). Channel catfish can become asymptomatic carriers but whether these other fish can asymptomatically carry the infection is unknown. Golden shiner, bighead carp, and largemouth bass are totally resistant (Plumb and Sanchez 1983).

TEMPERATURE-DEPENDENT PATHOGENICITY

In channel catfish, ESC is a markedly seasonal disease, with outbreaks occurring when water temperatures hover around 24–28°C (75–82°F) during the day (Francis-Floyd et al. 1987), which is optimum for the bacterium's growth. Thus, it is most prevalent during May and June, as well as September and October in ponds in the southeast United States. Outside this temperature range, mortalities may occur, but they are low and chronic. While an obligate pathogen, the ESC bacterium can survive for over 90 days in pond mud at 25°C (77°F) (Plumb and Quinlan 1986), which may account for recurrent epidemics in ponds. It can probably be carried in the gut of asymptomatic channel catfish.

Clinical Signs/Pathogenesis

GROSS LESIONS

Two forms of ESC that are related to the route of exposure have been described in channel catfish:

Acute (septicemic) form: In the gut route, bacteria are ingested, enter the bloodstream through the intestine, and apparently colonize various organs, causing necro-

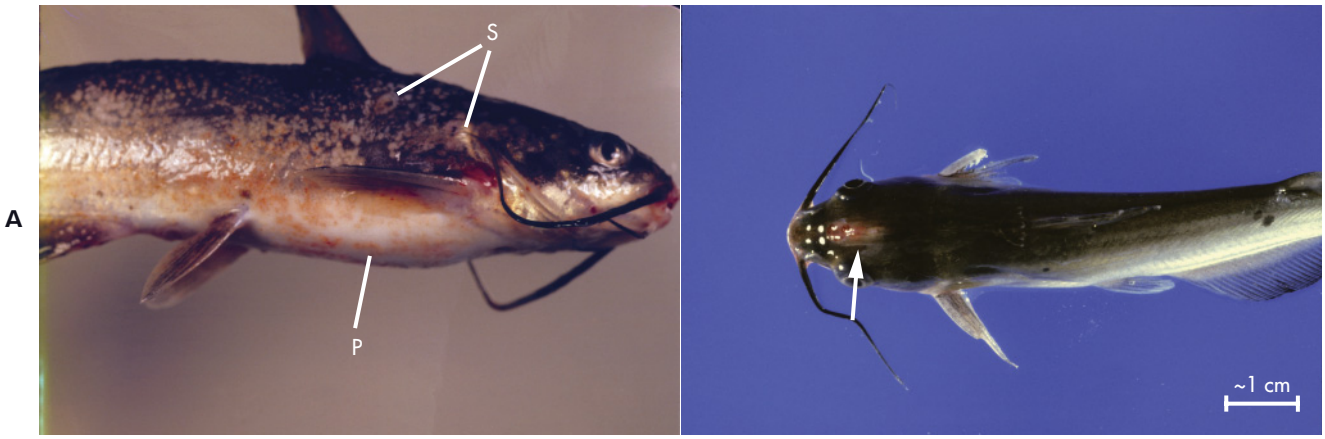


Fig. II-48. A. Channel catfish with ESC. Note skin erosion and ulceration, which appear as false spots (*S*) on the flank and focal petechiation (*P*) on the ventrum. B. Channel catfish with classical hole-in-the-head lesion caused by the erosion of the fontanelle of the skull (*arrow*). (*A* photograph courtesy of M. Beleau.)

sis and ulceration. There is typically acute mortality and in some cases few external signs.

Clinically affected fish may occasionally hang head up in the water and exhibit corkscrew spiral swimming, usually followed by death. Fish may have abdominal distension, exophthalmos, or pale gills. Blood-borne bacteria localizing in the dermis cause necrosis and hemorrhage that result in red-to-tan and slightly raised-to-depressed petechiae on the dorsum, flanks, jaw, and operculum. Petechiae on dark areas of the skin appear as small (1–3 mm), depigmented foci (false spots; Fig. II-48, A).

Internally, the peritoneal cavity contains bloody or clear fluid (which is especially characteristic), hemorrhage and necrosis of the liver, and splenic and renal hypertrophy. There may be petechial hemorrhages in the muscles.

Chronic (encephalitic) form: In the nervous route, bacteria invade the olfactory organ via the nasal opening and migrate up the olfactory nerve to the brain, where the infection spreads from the meninges to the skull and finally to the skin, forming the hole-in-the-head lesion (Fig. II-48, B; Shotts et al. 1986). This is a raised or open ulcer on the frontal bone of the skull. The brain can be entered without cutting the skull. Disease progression is more chronic than via the gut route.

HISTOPATHOLOGY

In channel catfish, enteritis, hepatitis, myositis, and interstitial nephritis begin as acute lesions and develop into chronic-active and then chronic foci. Fish with the nervous form initially develop inflammation in the olfactory sac, which progresses up the olfactory nerve, eventually reaching the olfactory lobe of the brain. The telencephalon (meningo-encephalitis) and overlying bone and skin are primarily affected. Macrophages in lesions often have bacteria (Shotts et al. 1986).

Diagnosis

Definitive diagnosis of clinical ESC requires identification of the Gram-negative bacterium in target tissues, with attendant clinical signs. In the acute form, the kidney is the organ of choice; while in the chronic form, the brain is the best organ for isolation. *Edwardsiella ictaluri* is somewhat fastidious for a fish pathogen and can be overgrown by other bacteria. Colonies are typically pinpoint in size after 24 hours at 30°C (86°F). Identification is usually via standard biochemical tests but immunological tests (fluorescent antibody or enzyme-linked immunosorbent assay [ELISA]) can be used for confirmation or for rapid presumptive diagnosis in tissue smears of infected fish (Anonymous 2003).

In young fish, ESC can be clinically identical to channel catfish virus disease (CCVD; see PROBLEM 78). The major distinguishing feature is the hole-in-the-head lesion (do not confuse with hole-in-the-head of pet fish; see PROBLEM 100), which is considered highly diagnostic for ESC. However, some channel catfish infected with *Edwardsiella tarda* (PROBLEM 49) have recently been observed with hole-in-the-head lesions (L. Khoo, personal communication).

Treatment

Losses because of ESC are highly dependent on the speed with which fish are placed on medication. Fish quickly go off feed after an ESC epidemic begins, making treatment impossible. If ESC is suspected, an attempt should be made to isolate the bacterium for identification and sensitivity testing as soon as possible. The trend is toward using rapid immunodiagnosis (e.g., ELISA) if

ESC is suspected, so that fish can be placed on medication as soon as possible. Medication can later be modified if subsequent culture and sensitivity results warrant it.

Oxytetracycline and ormetoprim-sulfadimethoxine have both been used with varying success, but some *E. ictaluri* isolates are resistant. Also, not all isolates from the same case exhibit the same resistance pattern (Taylor and Johnson 1991). The importance of this finding to choice of medication is not yet known. Some isolates have also been found to be susceptible to kanamycin, streptomycin, neomycin, nitrofurantoin, and/or oxolinic acid in vitro (Waltman and Shotts 1986), but none of these are approved for treating ESC in food fish. Recently, florfenicol has been shown highly efficacious in clinical trials and is approved for treating ESC in the United States. In addition, more judicious use of oxytetracycline and ormetoprim-sulfadimethoxine has resulted in a significant decline in resistant isolates in Mississippi (L. Khoo, personal communication), making them more available for use.

The U.S. catfish industry practice of never completely harvesting a pond, and continually adding new fish, has facilitated the persistence of ESC as a problem. While fish that survive the infection have protective immunity, they also are carriers, probably shedding via the feces. Stress, especially crowding, can precipitate recurrence of an epidemic in a recovered population. Stress also increases the severity of ESC outbreaks but is not always needed to initiate epidemics.

Outbreaks will often spontaneously subside when the water temperature leaves the optimal range. Reducing feeding during epidemics can reduce losses (Wise and Johnson 1998; Lim and Klesius 2003) but slows fish growth, which reduces profitability. Commercial vaccines are available.

PROBLEM 49

Edwardsiella tarda Infection (Edwardsiellosis, Emphysematous Putrefactive Disease, *Edwardsiella* Septicemia)

Prevalence Index

WF - 2, WM - 2

Method of Diagnosis

Culture of *Edwardsiella tarda* from typical skin and/or internal lesions

History

Low, chronic mortality; fish continue to eat

Physical Examination

Deep, malodorous ulcer on flank; red areas on body

Treatment

Appropriate antibiotic

COMMENTS

Epidemiology

Edwardsiella tarda (formerly *Edwardsiella anguillimortifera* and *Paracolobactrum anguillimortiferum*) is one of the most serious threats to Japanese flounder culture (Zheng et al 2004). While it is not reported from American eels, it causes serious losses in Japanese eels in Japan and Taiwan (Waltman et al. 1986). It is also an economically important but relatively uncommon bacterial disease in channel catfish in the United States (Waltman et al. 1986). It can also cause disease in striped bass, goldfish, common carp, grass carp, chinook salmon, largemouth bass, Nile tilapia, striped mullet, red sea bream, and crimson sea bream (Plumb 1993), as well as Siamese fighting fish (Humphrey et al. 1986) and turbot (Padrós et al. 2006). Rainbow trout, yellowtail and oriental weatherfish are experimentally susceptible (Plumb 1999). It has been isolated from the gastrointestinal tract of numerous other cold-blooded animals, from mussels to alligators. Reptiles and amphibians are especially common carriers (Waltman et al. 1986). Wyatt et al. (1979) also found that up to 100% of the crayfish, frogs, and turtles in ponds that contain infected catfish are infected.

RISK FACTORS AND TRANSMISSION

In channel catfish and Japanese eels, the disease is mainly a problem in older individuals, but fingerlings and elvers are susceptible. Most disease seems to occur at high temperature. It is most prevalent in channel catfish at ~30°C (86°F) (Meyer and Bullock 1973) and is most prevalent in Japanese eels during summer. However, it has caused disease in Taiwan-cultured eels at 10–18°C (50–64°F) (Liu and Tsai 1980). It is also associated with organic pollution. In catfish ponds, mortalities are usually low and chronic (<5%), but if fish are stressed, mortalities may be high. Hemolysin and chondroitin sulfate activities may be pathogenic factors.

Transmission and the source of infection during outbreaks in fish are uncertain, although the infection is known to remain dormant in fish tissues. It is present on Japanese flounder farms even when the disease is not occurring, but dies quickly in seawater when not infecting fish, suggesting that terrestrial runoff might be an important source of infection (Mamnur et al. 1994).

Carrion-eating birds may also be an important source of infection (Winsor et al. 1981).

ZOONOTIC ASPECTS

Edwardsiella tarda is an uncommon zoonotic problem, mainly causing enteric disease in humans. It has been isolated from the urine and feces of many mammals (cattle, swine), including man (Clarridge et al. 1980) and marine mammals (seals, sea lions, porpoises) (Coles et al. 1978), although it is typically associated with freshwater environments. In humans, it has been implicated in meningitis, liver abcesses, wound infections, and most commonly gastroenteritis. It can be highly prevalent in catfish fillets from processing plants and may spread to man via the oral route. Puncture wounds can cause skin lesions. There is some evidence that freshwater tropical pet fish might harbor the infection (Humphrey et al. 1986) and

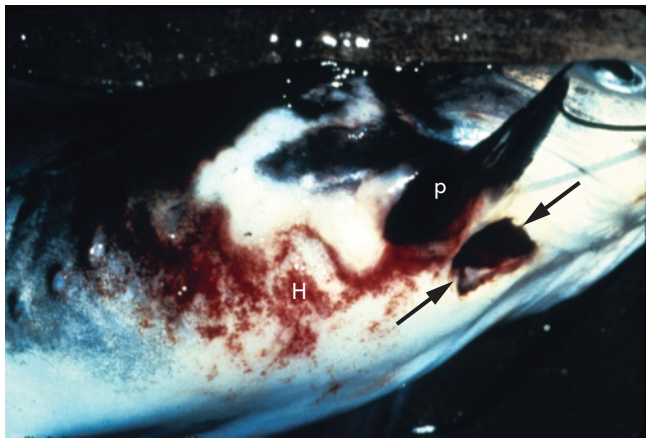


Fig. II-49. Channel catfish with *Edwardsiella tarda* infection. Note the deep fistula on the flank (*arrows*). *P* = pectoral fin; *H* = hemorrhage. (Photograph courtesy of F. Meyer.)

aquarium exposure has been very rarely linked to disease in humans (Vandepitte et al. 1983).

Clinical Signs/Pathogenesis

Clinical signs of edwardsiellosis vary with the species affected but lesions often have masses of bacteria, both surrounded by inflammatory cells and free within tissue.

CHANNEL CATFISH

Lesions are initially seen as 3–5 mm red cutaneous foci on the flanks and caudal peduncle. They are caused by fistulas originating deep in the muscle that extend from malodorous fluctuant subdermal masses (Meyer and Bullock 1973) (Fig. II-49). There is also petechiation and malodorous (hydrogen sulfide production) liquefactive necrosis of the viscera with fibrinous peritonitis. Characteristically, fish may continue to eat even if severely affected. There may be posterior paresis in late stages. Larger fish (>40 cm [16 inches]) are most commonly affected, often broodfish. Some fish may display with hole-in-the-head lesions, making them grossly similar to ESC (PROBLEM 48).

JAPANESE EELS

Japanese eels may exhibit one of two forms (Miyazaki and Egusa 1976a, 1976b): The nephric form (suppurative interstitial nephritis) is more common and is associated with necrotic renal foci that spread to other organs (spleen, liver, gills, stomach, and heart). In the hepatic form (suppurative hepatitis), microabscesses form in the liver and spread to other organs. These lesions appear as light-colored nodules on the viscera. Abscesses may ulcerate through the body musculature.

FLATFISH

In Japanese flounder, in addition to abscessation and granuloma formation in the viscera, hepatocytes can be hypertrophied (Miwa and Mano 2000). Turbot display swollen abdomen, eye tumefaction, hemorrhage, and inflammation in the kidney, liver and spleen (Padrós et al. 2006).

OTHER SPECIES

In striped bass, unusual features include epithelial hyperplasia, which can give the fish a tattered appearance, and necrosis in the lateral line and on the body surface and gills (Herman and Bullock 1986). Anemia and hypoxia also occur. In tilapia, lesions include skin depigmentation, swollen abdomen, and corneal opacity. There are white, bacteria-filled nodules in the gills, kidney, liver, spleen, or intestine (Kubota et al. 1981).

Diagnosis

Definitive diagnosis is based on standard biochemical tests followed by confirmation with an antibody test (agglutination, FAT, or EIA) (Rogers 1981; Amandi et al. 1982). However, since there are many serotypes, a false-negative reaction is possible, especially if using a monovalent antiserum.

The agent can be isolated from affected tissues, especially the kidney, using a simple medium, such as trypticase soy agar (Waltman et al. 1986). Isolates grow best at 37°C (98.6°F) but will appear after 2–4 days at 25°C (77°F) as small, grey, circular, transparent colonies composed of motile Gram-negative rods (Shotts and Teska 1989).

Treatment

As with many fish bacterial pathogens, *E. tarda* is associated with polluted environments. Thus, systemic antibiotic treatment (oxytetracycline) should be accompanied by an improvement in water quality. Some strains of *E. tarda* are resistant to oxytetracycline (Hilton and Wilson 1980). Drug-resistant strains of *E. tarda* that carry transferable R-plasmids have appeared at high frequency in cultured Japanese eels (Aoki et al. 1987). Scarring may occur on surviving fish (Meyer and Bullock 1973).

PROBLEM 50

Vibriosis (Salt Water Furunculosis, *Vibrio* Infection, Hitra Disease)

Prevalence Index

WF - 4, WM - 1, CF - 4, CM - 1

Method of Diagnosis

Culture of large numbers of vibrios from typical skin and/or internal lesions

History

Acute to chronic morbidity/mortality

Physical Examination

Red areas on body; skin ulcers; depression; exophthalmos; corneal ulcers; swollen abdomen

Treatment

Appropriate antibiotic

COMMENTS

Epidemiology/Pathogenesis

Vibriosis is caused by infection with one of several members of the genus *Vibrio*, as well as the related

genera *Moritella* and *Photobacterium* (family Vibrionaceae). Vibrios cause some of the most important diseases of marine fish. All marine fish are probably susceptible to at least one species. Vibrios have been infrequently isolated from freshwater aquarium fish and freshwater salmonids that have been fed marine offal (Hacking and Budd 1971; Kitao et al. 1983; Reddacliff et al. 1993).

Vibrios are typically facultative pathogens that can readily survive and multiply in the environment, although the relative pathogenicity of environmental versus fish isolates is uncertain. Vibrios are commonly isolated from the mucosal surfaces and internal organs of clinically healthy fish, as well as from invertebrates, sediments, and the water column. Highest environmental prevalence is in organically polluted water and high salinity.

A major predisposing risk factor for most types of vibriosis is high temperature, making it a summer disease in most, but not all cases (e.g., cold water vibriosis, winter ulcer). Crowding, organic pollution, and other stressors can also precipitate outbreaks. Strains also vary considerably in virulence, and some strains can cause disease without any predisposing stress. Some vibrios produce hemolysins (which may cause anemia) and proteases (which may cause muscle damage) (Hjeltnes and Roberts 1993).

Some, but not all, vibrios are human pathogens, either as zoonotic agents (see below) or by residing on or in aquatic animals without causing disease.

Clinical Signs/Pathology

VIBRIO ANGUILLARUM (SALT WATER FURUNCULOSIS)

Vibrio anguillarum is the most common fish-pathogenic vibrio. Based upon genetic studies, it was suggested that this organism should belong in a new genus (*Listonella* [MacDonnell and Colwell 1985]); however, this change has not been widely adopted, so the name *Vibrio* will be retained for this discussion. There are 23 serotypes but only three (O1, O2, O3) have been associated with significant fish mortalities (Pedersen et al. 1999).

Clinical signs of systemic *Vibrio anguillarum* infection are similar to *Aeromonas salmonicida* infection (ergo, salt water furunculosis). Both localized skin ulcers and systemic infections can occur. Systemic infections often localize in iron-rich filtering organs, such as spleen and kidney.

In salmonids, three systemic forms of the disease have been described (Hjeltnes and Roberts 1993). The peracute form presents as anorexia, darkening, and sudden death in young fish. Histopathological features include cardiac myopathy with sarcoplasmic vacuolation (which may be the only lesion) and renal and splenic necrosis.

In the acute form, dark, fluctuant, subdermal cavitations ulcerate to release serosanguinous fluid. There is also abdominal distension, anemia, and dermal hemorrhage. Internal signs of typical septicemia include visceral petechiation, splenomegaly, and liquefactive renal necrosis. Histologically, there is necrosis of the liver, spleen, kidney, and heart, as well as depletion of hematopoietic elements. A necrotic enteritis produces a catarrhal, yellow, mucoid exudate.

The chronic form presents as organized, deep, granulomatous muscle lesions on various parts of the body, including the head. Deep muscle lesions may not be apparent until slaughter. Eye lesions are common, including corneal edema, ulceration, and exophthalmos. There is also hemorrhage in the abdominal cavity, contributing to anemia and fibrinous adhesions. Histologically, there is heavy hemosiderin deposition in melanomacrophage centers, presumably because of hemolysins produced by the bacteria.

OTHER VIBRIOS

Other vibrios cause either skin lesions or bacterial hemorrhagic septicemia in various fish species:

***Vibrio ordalii* (= *V. anguillarum* biotype II):** This agent is pathologically and biochemically similar to *V. anguillarum* (formerly known as *V. anguillarum* biotype I), causing a bacterial hemorrhagic septicemia in marine fish in Japan and the Pacific Northwest of the United States (Schiewe et al. 1981). Differentiating features include the tendency of *V. ordalii* to form bacterial microcolonies in muscle, gill, and gastrointestinal tract (Ransom et al. 1984). Bacteremia also typically develops later in the course of the disease.

***Vibrio salmonicida* (Hitra Disease, Cold Water Vibriosis):** *Vibrio salmonicida* is a serious problem in sea-cultured Atlantic salmon in Europe, including Norway, the Shetland Islands and the Faroe Islands (Hjeltnes and Roberts 1993) and has been identified in cultured stocks in the northwest Atlantic, including Canada and the United States (O'Halloran et al. 1992). It also occurs in Atlantic cod. In salmonids, clinical signs are similar to *V. anguillarum*, ranging from peracute mortality with no clinical signs to a chronic, hemorrhagic septicemia. Outbreaks occur in winter, typically when temperatures drop below 5°C (41°F), and continue until the temperature drops below 2°C (35.6°F). Morbidity can resume when temperatures warm to 2–3°C (35.6–37.4°F) in spring and continue until temperatures exceed 8°C (46.4°F). Outbreaks begin with anorexia, depression, and disorientation, proceeding to abdominal distension, rectal prolapse, reddening of the fins and skin, and pale gills. Internally, there may be fluid in the peritoneal cavity and hemorrhages on the swim bladder, abdominal fat, and other viscera (Fig. II-50). Histopathology is similar to *V. anguillarum* but often with severe heart and muscle damage (myonecrosis). The bacterium can survive for over one year in seawater.

***Moritella viscosa* (= *Vibrio viscosus;* Vintersår, winter ulcer):** Winter ulcer affects juvenile and adult trout

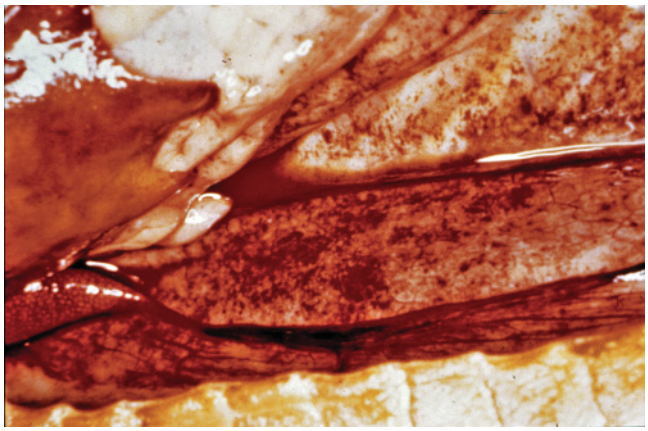


Fig. II-50. Atlantic salmon with vibriosis (Hitra disease). Massive hemorrhages in the viscera. (Photograph courtesy of H. Möller.)

and Atlantic salmon in Norway and Iceland when temperatures are <8°C (<46.4°F; typically February through April) (Benediktsdóttir et al. 1998). It affects marine-cultured fish, as well as freshwater hatcheries when seawater is added to tanks during smoltification. Winter ulcers begin as shallow, scale-covered lesions that progress to deep ulcerations. Skin damage is a risk factor. Mortality is usually low (0–10%), ranging up to 20% in one month. Morbidity can be high (up to 50% of a population affected) and lesions cause downgrading of the carcass at slaughter, resulting in significant economic losses (Lunder et al. 1995). There is evidence that *Vibrio wodanis* might also be involved in lesion development (Benediktsdóttir et al. 2000). *Moritella viscosa* also causes a similar disease in other marine fish, such as Atlantic cod and turbot (Gudmundsdóttir and Björnsdóttira 2007).

***Moritella marina*:** This bacterium was isolated from shallow skin ulcers of Atlantic salmon in Iceland (Benediktsdóttir et al. 1998).

***Photobacterium damselae* subsp. *piscicida*:** see PROBLEM 51.

***Photobacterium damselae* subsp. *damselae* (= *Vibrio damsela*):** This agent causes skin ulcers or systemic disease in a wide range of fish, including blacksmith damselfish, yellowtail, turbot, gilthead sea bream, brown shark (Fouz et al. 1992), and red-banded sea bream (Austin and Austin 2007). It also causes skin ulcers in humans (Love et al. 1981).

Vibrio parahaemolyticus: This agent causes serious disease in tropical species such as grouper and yellow croaker in China (Y. Zhou, personal communication). It has also caused disease in estarine killifish (Alcaide et al. 1999).

***Vibrio harveyi* (= *Vibrio carchariae* = *Vibrio trachuri*):** This agent causes eye lesions, skin ulcers and systemic disease. It was first isolated from a sandbar shark and lemon sharks (Colwell and Grimes 1984). Affected fish developed subdermal necrotic "cysts" and necrosis and inflammation of viscera and brain (Grimes et al. 1985). It also has caused disease in Japanese horse mackerel, summer flounder, Senegalese flounder, red drum, coioides grouper, spotted grouper, silvery black porgy, snook, jack crevalle, milkfish and marine sunfish (Iwamoto et al. 1995; Austin and Austin 2007).

Vibrio alginolyticus: This agent appears to cause disease only in highly stressed individuals, including gilthead sea bream, silver sea bream, brownspotted grouper, mullet, turbot, and other marine species (Colorni et al. 1981; Austin and Austin 2007).

***Vibrio vulnificus* biogroup 2:** This agent is pathologically similar to *V. anguillarum* and has caused bacterial hemorrhagic septicemia in Japanese eels in Japan, ovate pompano in China, and European eels in England, Spain, Denmark, and the Netherlands (Austin and Austin 2007). It appears that biogroup 1, the main human vibrio pathogen derived from the marine environment, is not a fish pathogen (Actis et al. 1999).

***Vibrio cholerae* (non 01):** This agent has rarely been reported as a fish pathogen from ayu in Japan (Muroga et al. 1979) and goldfish in Australia (Reddacliff et al. 1993). Experimental challenges demonstrated the organism to be highly pathogenic to ayu and Japanese eels. The goldfish had been kept in a low salt concentration; even very low (1–2 ppt) salt concentrations can facilitate the growth of *V. cholerae* (Singleton et al. 1982). This is a zoonotic agent.

Vibrio fischeri: This agent was isolated from diseased turbot in Spain that exhibited skin papillomas and visceral neoplasia (Lamas et al. 1990).

Vibrio ichthyoenteri: This agent has been associated with intestinal necrosis causing opacity of the intestine in Japanese flounder (Kim et al. 2004).

Vibrio logei: This agent has been associated with shallow skin lesions in cultured Atlantic salmon in Iceland at low temperatures (~10°C [~50°F]) (Benediktsdóttir et al. 1998).

Vibrio pelagius: This vibrio was associated with an epidemic in larval and juvenile cultured turbot in Spain at 12–15°C (54–59°F). Affected fish had skin lesions and a systemic infection (Villamil et al. 2003).

Vibrio splendidus: This agent has been isolated from cultured turbot in Spain, Atlantic salmon in Scotland, turbot, European sea bass and corkwing wrasse in Norway, and New Zealand turbot and New Zealand brill in New Zealand (Austin and Austin 2007). It causes a typical bacterial hemorrhagic septicemia.

Vibrio tapetis: This agent has been isolated from diseased corkwing wrasse in Norway (Jensen et al. 2003).

Diagnosis

Definitive diagnosis of vibriosis requires identification of the bacterium in target tissues (usually by biochemical tests of a culture), with attendant clinical signs. Isolation in a mixed culture from normal colonization sites (e.g., skin, gastrointestinal tract) on fish does not necessarily indicate that the vibrio is reponsible for the disease. It is important to be certain that this is the primary infectious cause of the problem. Vibrios can be secondary invaders. The kidney is probably the best organ for isolation; lesions should also be sampled.

When possible, the temperature of isolation should approximate that of the host/environment. For example, *V. salmonicida* and *M. viscosa* are psychrophilic and should be incubated at low temperatures (12–16°C [54–61°F]) to achieve isolation; *Vibrio salmonicida* colonies take 3–5 days to appear; the small, translucent colonies may be missed without careful examination.

Vibrios are Gram-negative, short (~0.5–2.0 μm), motile, usually curved rods. Almost all either require or have enhanced growth in the presence of sodium, but fish pathogens are usually readily isolated on a rich nutrient medium (e.g., Columbia blood agar). Tissue-invading pathogens typically have relatively low sodium requirements, which is a factor in their ability to survive in the host.

Treatment

Vibriosis is a classical example of a stress-borne disease. Losses caused by vibriosis are highly dependent on the severity of the environmental stress that precipitated the outbreak, varying from acute to chronic. Salmonids often break with vibriosis after movement from freshwater to seawater. Exposure to copper (>30 μg/ml) or iron (>10 μg/ml) also increases susceptibility to vibriosis (Hetrick et al. 1979; Austin and Austin 1993). Oxytetracycline, nitrofurans, potentiated sulfonamides, and oxolinic acid have been used successfully, but there can be resistance to these drugs, especially in *V. anguillarum* and *V. salmonicida* (Hjeltnes and Roberts 1993). Commercial bacterins, available for certain vibrios (e.g., *V. anguillarum*, *V. ordalii*, and *V. salmonicida*) provide good protection for populations at risk. Reducing stress is imperative for long-term management.

PROBLEM 51

Pasteurellosis (Pseudotuberculosis, *Photobacterium damselae* subsp. *piscicida* Infection, Photobacteriosis)

Prevalence Index

WF - 4, WM - 1

Method of Diagnosis

Culture of *Photobacterium damselae* ssp. *piscicida* from typical skin and/or internal lesions

History

Acute to chronic morbidity/mortality

Physical Examination

Small hemorrhages on operculum or base of fin; abnormal skin color; enlarged spleen, kidney (acute form)

Multiple, white foci on spleen and kidney (chronic form only)

Treatment

Appropriate antibiotic

COMMENTS

Epidemiology

Photobacterium damselae subsp. *piscicida* (formerly known as *Pasteurella piscicida* [Gauthier et al. 1995], hence the term "pasteurellosis" still used as reference to the disease) causes one of the most serious bacterial diseases in warm and temperate marine aquaculture. "Pasteurellosis" is a common disease in cultured marine fish in Japan, including ayu, black sea bream, red sea bream, red grouper, oval fish, and yellowtail (Kitao 1993a) and has recently caused disease in Senegalese sole (Zorilla et al. 1999) and Atlantic bluefin tuna (Mladineo et al. 2006). *Photobacterium damselae* ssp. *piscicida* also affects cultured hybrid striped bass (Hawke et al. 2003) and has caused isolated epidemics in wild white perch, striped bass, and Gulf menhaden in coastal waters of the United States (Chesapeake Bay, Long Island Sound, Galveston Bay) (Snieszko et al. 1964; Lewis et al. 1970; Paperna and Zwerner 1976; Robohm 1983; Hawke et al. 1987). It has also been isolated from snakehead in Taiwan (Tung et al. 1985). More recently, it has been isolated from cultured and wild marine fish (e.g., gilthead sea bream, European sea bass) in the Mediterranean areas of Spain, France, Italy, and Croatia (Margariños et al. 1992; Mladineo et al. 2006), as well as Greece, Turkey, Malta, and Israel. It has also recently caused disease in fish cultured in the Red Sea (Kvitt et al. 2002). Rudd and chub isolates in England (Ajmal and Hobbs 1967) may actually be atypical *Aeromonas salmonicida* (see PROBLEM 47).

Genetic studies (amplified fragment length polymorphism [AFLP]) indicate that there are two main genetic groups/strains: one originating from Japan and the other originating from the Mediterranean Sea/Europe. The latter group can be further subdivided into "European" and "Israeli" strains (Kvitt et al. 2002).

Mode of transmission is unknown, although fish-to-fish contact and an invertebrate vector have both been suggested. Oral transmission is likely. The reservoir of infection is uncertain, although striped bass were believed to be the major source of infection in Chesapeake Bay. The bacterium appears to be short-lived in the environment, but further studies are needed to confirm this (Magariños et al. 1994). Host susceptibility varies significantly, since many unrelated fish species were not clinically affected during a striped bass epidemic (Hawke et al. 1987).

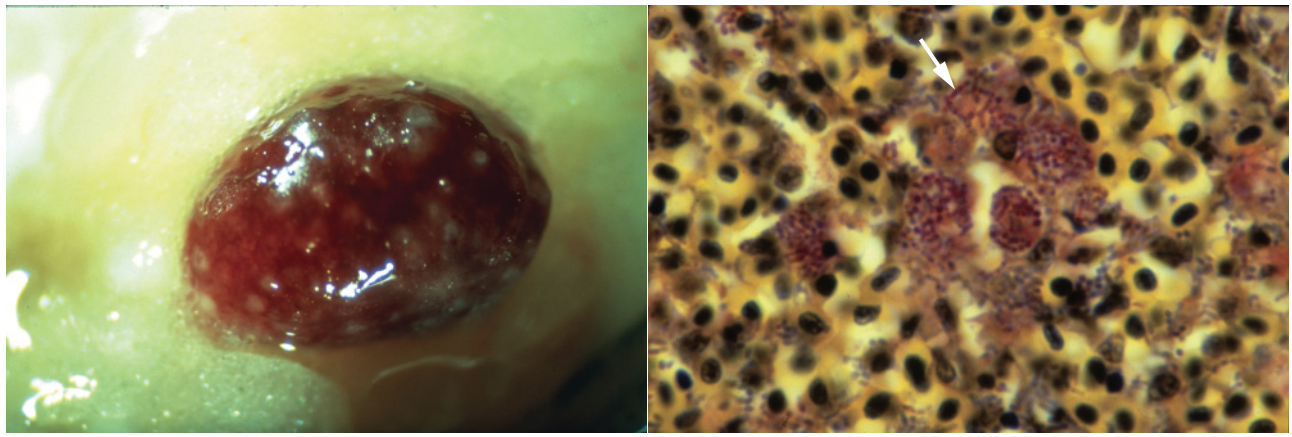


Fig. II-51. A. Spleen of gilthead seabream with multiple white foci caused by *P. damsela* subsp. *piscicida*. B. Histological section of spleen from gilthead seabream showing phagocytes containing numerous bacteria (*arrow*). (*A* and *B* photographs courtesy of A. Colorni.)

In Japan, epidemics occur when salinities drop to <30 ppt after a heavy rain and temperatures rise over 25°C (77°F) (Kitao 1993a). Epidemics do not develop if the temperature remains below 25°C (77°F). High temperature has also been associated with outbreaks in the United States (Hawke et al. 1987).

Clinical Signs /Pathogenesis

Photobacterium damselae subsp. *piscicida* causes a bacteremia/septicemia that takes one of two forms.

ACUTE FORM

In the acute form, few clinical signs are present. There may be small hemorrhages around the gill covers or the bases of the fins (Snieszko et al. 1964), or there may be abnormal skin pigmentation and enlarged spleen and kidney (Hawke et al. 1987). Histologically, there is acute necrosis of spleen, liver, and pancreas with no inflammation.

CHRONIC FORM

In the chronic form, there are 1–2 mm miliary lesions in the kidney and spleen (Fig. II-51, A) that are composed of bacteria that incite a chronic inflammatory response (Fig. II-51, B). The appearance of this latter lesion, grossly resembling mycobacteriosis (PROBLEM 55), has led to its being misleadingly called pseudotuberculosis.

Diagnosis

Presumptive diagnosis of *Photobacterium damselae* subsp. *piscicida* is based on presence of typical gross lesions having Gram-negative, nonpigmented, short (~0.5–0.75 × 1–2 µm long), nonmotile rods that stain bipolarly. Note that *Aeromonas salmonicida* (see PROBLEM 47) is morphologically similar and has been mistaken for *P. damselae* ssp. *piscicida* (Hastein and Bullock 1976). Confirmatory diagnosis can be performed by using slide agglutination, immunofluorescence, or a gene test.

The agent can be isolated from affected organs, especially kidney and spleen, by using nutrient agar at room temperature. Shiny grey-yellow, entire, convex, 1–2 mm colonies develop after 48–72 hours. Culturally, *P. damselae* subsp. *piscicida* most closely resembles non-pigment–forming isolates of *Aeromonas salmonicida*.

Treatment

Infections in Japan have been treated with many different antibiotics, such as ampicillin (Kusuda and Inoue 1977) and potentiated sulfonamides (Fujihara et al. 1984). However, there are serious problems with resistance (Kitao 1993a).

Oxytetracycline was not very effective in controlling an outbreak in striped bass; however, this was believed to be due to inadequate tissue levels attained in target organs and not to resistance of the bacterial isolate (Hawke et al. 1987). A number of experimental vaccines have been studied (e.g., Fukuda and Kusuda 1981; Kusuda et al. 1988; Thune et al. 2003), but none are yet commercially available.

PROBLEM 52

Enteric Redmouth Disease (ERM; Redmouth, Yersiniosis, Blood Spot, *Yersinia ruckeri* Infection)

Prevalence Index

WF - 4, WM - 4, CF - 1, CM - 4

Method of Diagnosis

Culture of *Yersinia ruckeri* from typical skin and/or internal lesions

History

Dark fish that cannot find food (blind); acute to chronic mortalities

Physical Examination

Typical of hemorrhagic septicemia; especially dark coloration, exophthalmos, hemorrhage in mouth and eyes, depression, swollen abdomen

Treatment
Appropriate antibiotic

COMMENTS

Epidemiology
Yersiniosis has been reported in the United States, Canada, Australia, Africa, and Europe. *Yersinia ruckeri* is an important pathogen of salmonids (Tobback et al. 2007). Rainbow trout are especially susceptible, but steelhead, lake, cutthroat, brown and brook trout, and coho, sockeye, chinook, and Atlantic salmon are also affected. While any age salmonid is susceptible, ERM primarily affects fish at or near market-size, making it a potentially devastating disease. The bacterium has also been less frequently isolated from diseased channel catfish, European sea bass, emerald shiners, fathead minnows, cisco, baeri sturgeon, turbot, peled, whitefish, and muskum whitefish. It has also been isolated from asymptomatic goldfish, common carp, European eel, burbot, coalfish, and arctic char (Stevenson et al. 1993; Danley et al. 1999; Bullock 2003). Aquatic invertebrates (crayfish) and even mammals (muskrats) can harbor large numbers of bacteria. The bacterium appears capable of surviving in sediments for months.

Pathogenesis
ERM outbreaks usually begin with chronic, low mortality, which generally escalates. Severity of ERM outbreaks depend mainly on strain virulence and degree of environmental stress. There are six serovars of *Y. ruckeri*, Types I to VI (Austin and Austin 2007). Type I (Hagerman) is the most common, widely distributed, and pathogenic. But, not all serovar I isolates are pathogenic, and other serovars can be highly lethal (Stevenson et al. 1993).

Rainbow trout are most commonly affected at ~7.5 cm (~3"), with more chronic infections occurring in larger (12.5 cm) fish. Peak disease severity is at 15–18°C (59–64°F). The incubation period at 15°C (59°F) is about 1 week. There is lower morbidity/mortality at low (<10°C [50°F]) temperatures. Mortalities may occur for up to 60 days. A high percentage (>75%) of recovered fish may become carriers (Busch and Lingg 1975). Subclinical carriers cyclically shed bacteria from the lower intestine (Busch and Lingg 1975). Approximately monthly shedding has occurred in experimentally affected populations, but the periodicity of the shedding cycle probably varies with environmental conditions (Stevenson et al. 1993). Cyclic shedding helps to explain fluctuation in pathogen prevalence in fish populations (Bruno and Munro 1989). The carrier state can be maintained indefinitely (>100 days) with an average 10% infection (Busch and Lingg 1975). High (15–18°C [59–64°F]) temperature can cause carriers to begin shedding, leading to clinical disease (Rucker 1966). Clinical signs can develop within several days of the stress. Grading (Rucker 1966) and copper exposure (Knittel 1981) may also initiate outbreaks. There may be up to 70% mortality initially. The mechanisms responsible for virulence are unknown.

Clinical Signs/Pathogenesis
GROSS LESIONS
Affected fish are dark, anorexic, and lethargic. Acute ERM resembles aeromonad and vibrio infections, but reddening in the mouth (Fig. II-52, B) is especially diagnostic. Internal lesions are typical of other Gram-negative bacterial septicemias, including visceral petechiation, splenomegaly, and necrosis of the intestinal mucosa with a catarrhal exudate.

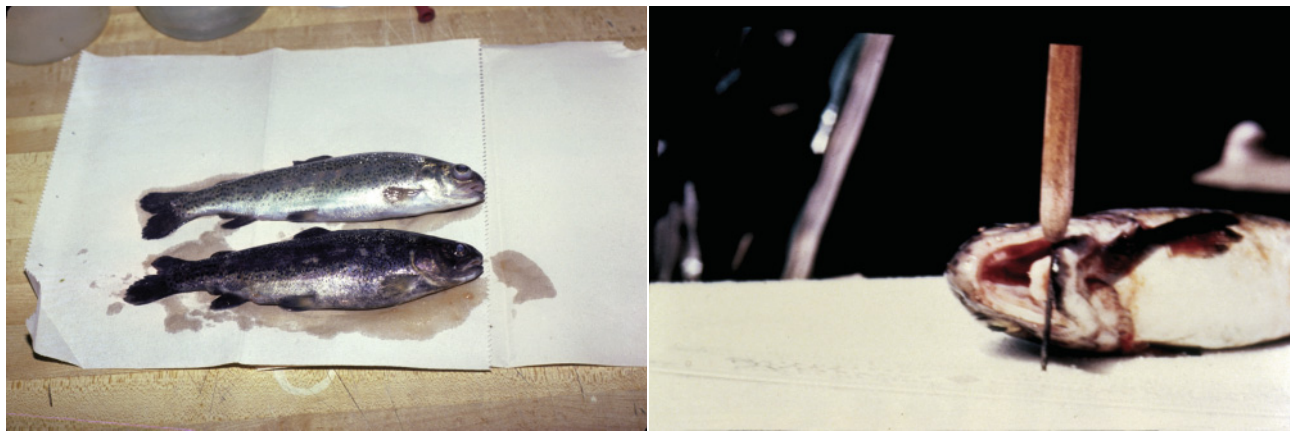


Fig. II-52. A. Rainbow trout with exophthalmos (*top fish*) and darkened color (*bottom fish*) exhibit common clinical findings with ERM. B. The lower jaw of a trout has been propped open, revealing hemorrhage in the roof of the mouth, a classical lesion of ERM. (*A* photograph by K. Townsend and E. Noga; *B* photograph courtesy of C.L. Davis Foundation for Veterinary Pathology.)

With chronic disease, there is also abdominal distension, unilateral or bilateral exophthalmos (Fig. II-52, A), and hyphema (blood spot). In this case, darkening is due to the ophthalmic lesions, which cause blindness, leading to lack of melanin pigment control (Fig. II-52, A). Fish also accumulate near the outlet screens of the raceway (see Fig. I-3, A).

CLINICAL PATHOLOGY/HISTOPATHOLOGY

Clinical pathological changes include leucocytosis, reticulocytosis, low hematocrit, and low total plasma protein. Histologically, there is bacterial colonization of well-vascularized tissues, causing hemorrhage and/or telangiectasis of gills, kidney, liver, spleen, and heart, as well as muscle. This leads to necrosis of the hematopoietic tissue, causing anemia. There is also necrosis and sloughing of the gastrointestinal tract.

Diagnosis

Gross lesions that differentiate ERM from other bacterial septicemias include reddened skin erosions found mainly on the head or mouth (Fig. II-52, B), especially the lower jaw, and blood spot (hyphema). The latter is characteristic of infections in Atlantic salmon. While these lesions are good presumptive evidence for ERM in salmonids, they are not always present (Frerichs et al. 1985).

Definitive diagnosis of ERM requires identification of the bacterium in target tissues, with attendant clinical signs. Kidney is the best organ for isolation during epidemics. Lower intestine appears to be better for isolating the bacterium from asymptomatic carriers (Busch and Lingg 1975). The bacterium is sometimes difficult to isolate, and enrichment by first incubating samples in trypticase soy broth for 2 days at 18°C (64°F) has been advocated (Stevenson et al. 1993). However, this may not be successful with intestinal samples because of the large numbers of other bacteria. Some other members of the Enterobacteriaceae (e.g., *Hafnia alvei* and *Serratia liquefaciens*) are phenotypically and even immunologically similar to *Y. ruckeri*. A selective medium based on positive Tween 80 hydrolysis and negative sucrose fermentation is useful for North American isolates (Waltman and Shotts 1984) but is not as successful in identifying isolates from other geographic areas (Austin and Austin 2007). A gene test has been developed for identifying *Y. ruckeri* and differentiating Type I from other serotypes and for detecting ERM carriers (Altinok et al. 2001).

Treatment

In the United States, oxytetracycline is the first choice for food fish, but many *Y. ruckeri* isolates are resistant. Ormetoprim-sulfadimethoxine is more expensive, but less resistance is present. Many isolates are also susceptible to oxolinic acid (Rogers and Austin 1982).

Carriers are the most important source of infection, especially when stressed (Hunter et al. 1980). Keeping the water supply free of carrier fish is the best method of control. Carriers can also be kept downstream of susceptible populations. Vertical transmission has not been demonstrated. Eggs from infected broodstock should be treated with antiseptic. Raising salinity to 9 ppt dramatically reduces mortality in rainbow trout (Altinok and Grizzle 2001), but is impractical. Maintain good sanitation and keep stress to a minimum to reduce recrudescence of carriers. Keep fish-eating birds and mammals away from culture facilities, since many can transport the bacterium in their intestines (Stevenson et al. 1993). Natural disease does not confer complete immunity, but commercial *Y. ruckeri* bacterins offer good protection and are important in managing populations at risk for ERM.

PROBLEM 53

Streptococcosis (*Streptococcus iniae* Infection, *Streptococcus* sp. Infection)

Prevalence Index

WF - 3, WM - 2, CF - 2, CM - 4

Method of Diagnosis

Culture of streptococci from typical skin and/or internal lesions

History

Acute to chronic morbidity/mortality

Physical Examination

Typical of hemorrhagic septicemia; especially exophthalmos, skin hemorrhages, bleeding near the vent

Treatment

Appropriate antibiotic

COMMENTS

Epidemiology

Streptococcosis (encompassing *Streptococcus* and related genera such as *Lactococcus*) is increasingly recognized as a serious disease of many marine and freshwater fish (Agnew and Barnes 2007). Fish-pathogenic cocci have been reported from cultured fish in Japan, the Middle East, Italy, South Africa, Australia, and the United States; streptococcosis might have also occurred sporadically in Great Britain and Norway (Austin and Austin 2007). Yellowtail, rainbow trout, tilapia and hybrid striped bass are commonly affected, especially in closed or intensive culture systems. Epidemics have also occurred in many other fish species (Table II-53). There is some evidence that streptococcosis might affect many tropical aquarium fish, including members of five families (characids, cichlids, cyprinids, monodactylids and pangasids) (Gratzek et al. 1992; Yanong 1995).

Epidemics in wild fish were first documented in the northwest Gulf of Mexico (southeast United States) and Chesapeake Bay (Plumb et al. 1974; Baya et al. 1990a). A massive kill of reef fish in the southeast Caribbean Sea was associated with *Streptococcus iniae* infection (Ferguson

Table II-53. Streptococcal infections of fish. All reports are from cultured fish unless noted otherwise.

Species	Hosts	Geographic range	References
Streptococcus iniae	Rainbow trout, tilapia, hybrid striped bass	Israel	Eldar et al. (1994)
"	Hybrid striped bass, rainbow trout	United States	Stoffregen et al. (1996)
"	Marine fish (wild)	Caribbean Sea	Ferguson et al. (2000)
"	White spotted rabbitfish	Bahrain	Yuasa et al. (1999)
"	Dusky spinefoot	Japan	Sugita (1996)
"	Barramundi	Australia	Bromage et al. (1999)
"	Marine fish (wild)	Red Sea and Mediterranean Sea (Israel)	Colorni et al. (2002)
"	Japanese flounder, yellowtail, rainbow trout, ayu, threadsail filefish, Pacific mackerel	Japan	Kanai et al. (2006)
Streptococcus difficilis	Rainbow trout, tilapia	Israel	Eldar et al. (1994)
"	Silver pomfret	Kuwait	Duremdez et al. (2004)
Streptococcus parauberis	Turbot	Spain	Doménech et al. (1996)
Streptoccus milleri	Koi	United Kingdom	Austin and Robertson (1993)
Streptococcus dysgalactiae	Amberjack, yellowtail	Japan	Nomoto et al. (2006)
Lactococcus garvieae	Striped mullet, sea trout, pinfish, spot, Atlantic croaker, Gulf menhaden, bluefish, silver sea trout, striped bass, hardhead sea catfish, stingray (wild)	Gulf of Mexico and Chesapeake Bay (United States)	Plumb et al. (1974) Baya et al. (1990a)
"	Yellowtail, Japanese eel	Japan	Kusuda et al. (1991)
"	Rainbow trout	Italy	Eldar and Ghittino (1999)
"	Yellowtail	Japan	Kumon et al. (2002)
"	Golden shiner	United States	Robinson and Meyer (1966)
"	Red Sea wrasse (wild)	Israel	Colorni et al. (2003)
Streptococcus sp.	Yellowtail, ayu, Japanese eel, pagrus sea bream, Japanese flounder, jacopever, dusky spinefoot, coho salmon, amago salmon	Japan	Shotts and Teska, 1989; Shiomitsu et al., 1980; Kitao, 1993b
Streptococcus sp.	Rainbow trout	South Africa Italy	Boomker et al. (1979)
Enterococcus faecalis subsp. *liquifaciens*	Brown bullhead	Croatia	Teskeredzic et al. (1993)
Lactococcus piscium	Salmonids	United States	Williams et al. (1990)

et al. 2000). There is evidence that *Streptococcus iniae* has been introduced into the Red Sea with infected exotic fish (red drum) for cage aquaculture. It has become established in native fish populations in the Red Sea and has also been suspected of being introduced via aquaculture into the Israeli Mediterranean coast (Colorni et al. 2002).

Some fish-pathogenic cocci are of uncertain taxonomic placement and thus the exact relationships among these many pathogens, isolated from a wide range of fish in different geographic and ecological locations, remains to be determined. Thus, some of the given species determinations might change in the future. One of the best characterized of the fish-pathogenic streptococci is *Streptococcus iniae* (= *S. shiloi*). Streptococcosis also includes other cocci, variously reported as members of the genera *Enterococcus* (e.g., *E. faecium*) or *Lactococcus* (e.g., *L. garvieae* [= *Enterococcus seriolicida;* Kusuda et al. 1991]) that can cause clinically similar disease (Austin and Austin 2007).

Streptococcosis is highly contagious and fish to fish transmission easily occurs. However, reservoirs of infection are not well defined. Streptococci can be isolated from seawater, a number of wild marine fish (sardines, anchovies, round herring, chub mackerel, black scraper), and for long periods in sediment in the vicinity of sea cages (Kusuda and Kawai 1982). However, whether these are fish pathogens or originate from another source is unclear (Austin and Austin 2007). Fresh and frozen fish carcasses can also harbor fish-pathogenic streptococci (Minami 1979). Co-infection with aquabirnavirus (PROBLEM 79) can increase the severity of infections (Packingking et al. 2003).

ZOONOTIC CONSIDERATIONS

Not all cocci infecting fish are zoonotic pathogens. Most human cases of streptococcosis linked to fish have been attributed to *S. iniae*. *Streptococcus iniae* was originally isolated from an Amazon freshwater dolphin (*Inia geoffrensis*). Infections in humans have been associated with skin abrasions or puncture wounds while handling freshwater or marine fish, including tilapia, barramundi, hybrid striped bass, coho salmon, trout and yellowtail (Greenless et al. 1998). Clinical signs include non-healing skin ulcers, cellulitis, arthritis, septicemia and meningitis.

Clinical Signs/Pathogenesis

Fish with streptococcosis may be dark and lethargic and have many gross signs of a typical bacteremia/septice-

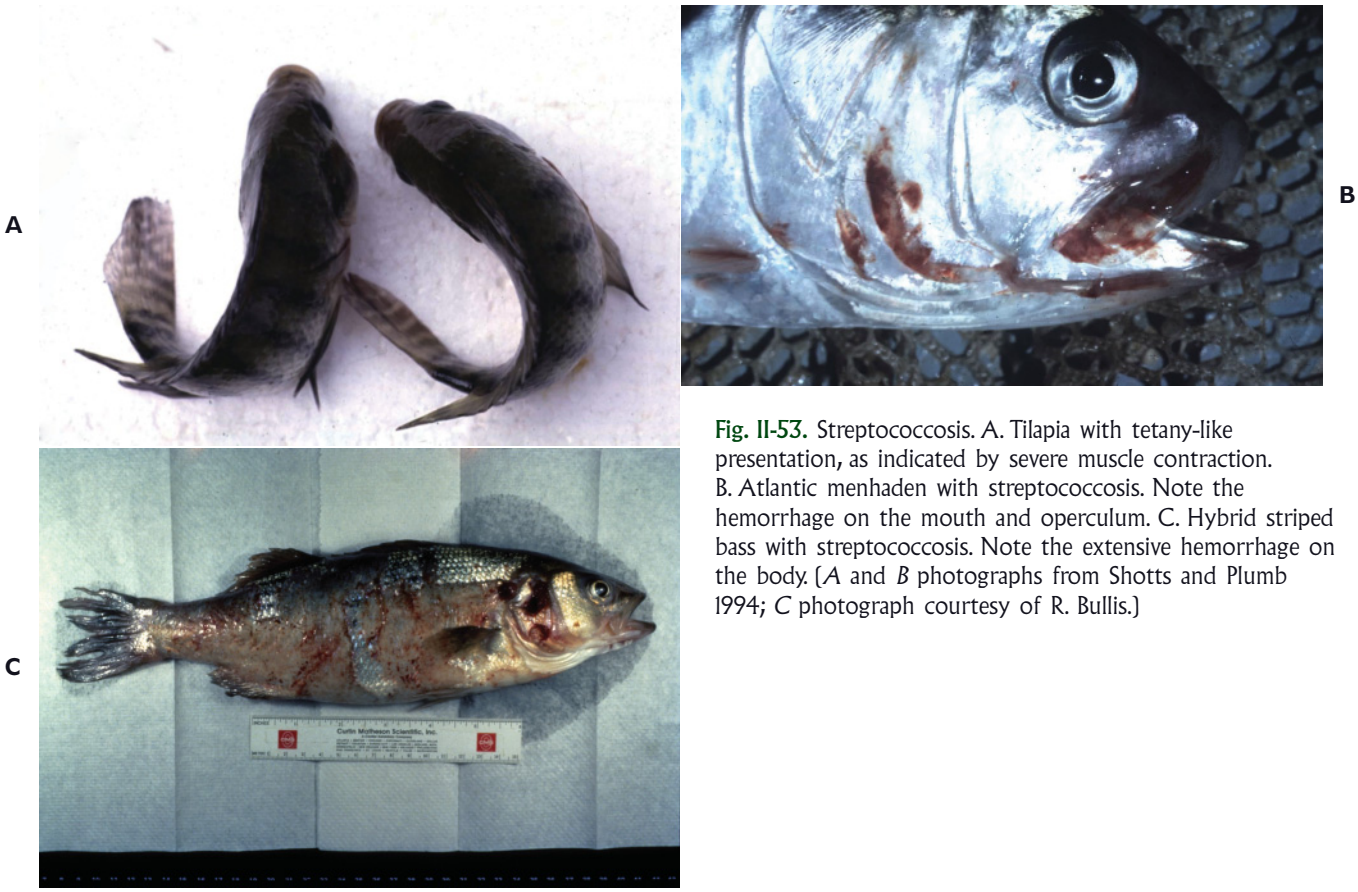


Fig. II-53. Streptococcosis. A. Tilapia with tetany-like presentation, as indicated by severe muscle contraction. B. Atlantic menhaden with streptococcosis. Note the hemorrhage on the mouth and operculum. C. Hybrid striped bass with streptococcosis. Note the extensive hemorrhage on the body. [*A* and *B* photographs from Shotts and Plumb 1994; *C* photograph courtesy of R. Bullis.]

mia. However, since the brain is commonly involved, fish may also swim erratically and show signs of dorsal rigidity (Eldar et al. 1994) or tetany-like (Fig. II-53, A). Streptococcosis is sometimes called "popeye" because exophthlalmos (with hyphema) is very common. There are often hemorrhages on the body (Fig. II-53, B, C) and serosanguinous fluid in the peritoneal cavity and intestine. The liver is pale and the spleen is dark red. However, the kidney may appear normal, as it is not always a major target organ (Shotts and Plumb 1994).

Diagnosis

Presumptive diagnosis of streptococcosis is based on presence of typical gross lesions having Gram-positive cocci, often in chains. Some may be ovoid rods. Histologically, when brain is involved, there is a granulomatous meningoencephalitis. Confirmatory diagnosis is usually based upon biochemical identification. The agent can be isolated from affected organs, especially brain, at room temperature or above (25–35°C [77–95°F]). Blood in the medium improves recovery. Dull grey to white, 1–2 mm colonies develop after 48 hours. Other cocci are involved in pseudokidney disease (see PROBLEM 57). A number of fish-pathogenic streptococci are Biosafety Level-2 organisms, and clinical material for culture should be handled accordingly.

Treatment

Amoxicillin, erythromycin, oxytetracycline and enrofloxacin, among other antibiotics, have been used to successfully treat streptococcosis. However, species and isolates vary in susceptibility. For example, *Lactococcus garvieae* isolates are commonly resistant to oxytetracycline, erythromycin and lincomycin (Kawanishi et al. 2005). Vaccines are under development (Agnew and Barnes 2007).

PROBLEM 54

Bacterial Kidney Disease (BKD; Dee Disease, *Renibacterium salmoninarum* Infection)

Prevalence Index

CF - 1, CM - 2

Method of Diagnosis

1. Culture of *Renibacterium salmoninarum* from typical skin and/or internal lesions
2. Identification of *R. salmoninarum* with antibody or gene probe

History

Acute to chronic morbidity/mortality

Physical Examination

Focal, white nodules in spleen, kidney, other viscera; pseudodiphtheritic membrane covering viscera; cavitations in muscle

Treatment

1. Appropriate antibiotic
2. Institute appropriate biosecurity

COMMENTS

Epidemiology

Renibacterium salmoninarum is an important pathogen of cultured salmonids, especially rainbow, brown, and brook trout and coho and chinook salmon. Any age salmonid is susceptible, but losses often do not occur until the fish are well grown (>6 months old), which makes it a potentially devastating disease. It occurs in virtually all areas where salmonids occur, except Australia, New Zealand, and Russia (Evelyn 1993; Austin and Austin 2007). It is a serious problem in the northeast Pacific (United States, Canada) and in Japan. Up to 80% losses in Pacific salmon and 40% losses in Atlantic salmon have been reported. It has also been observed in some wild salmonid populations that have not had any apparent contact with cultured fish (Souter et al. 1987; Jónsdóttir et al. 1998). Some nonsalmonids can be experimentally infected with *R. salmoninarum* (Traxler and Bell 1988), but risk of nonsalmonids as a significant reservoir of infection is not compelling. However, BKD has been documented in farmed ayu in Japan, possibly being transmitted from infected masu salmon (Nagai and Iida 2002).

The bacterium is an obligate pathogen and dies quickly in the environment (Evelyn 1993). Horizontal transmission can occur in both freshwater and seawater via cohabitation with infected fish, ingestion, skin wounds, or contact with contaminated water. Wire-tagged fish have a greater risk of infection, possibly because the wire-tagger becomes contaminated (Elliott and Pascho 2001). Feeding of raw viscera was responsible for epidemics in the 1960s. Vertical transmission is a major problem. The bacterium is commonly within the eggs of infected females (Evelyn et al. 1984). It resides in the yolk, protected from antiseptics (Evelyn et al. 1986). Infected peritoneal fluid is a major source of egg infection, but there is evidence that intraovum infections may also occur before ovulation (Evelyn 1993).

Clinical disease is most likely to develop during times of stress, especially during transfer of salmonids from freshwater to seawater, or during spawning (Fryer and Sanders 1981). While BKD is typically a chronic infection, stress may precipitate acute mortalities. Most epidemics occur during declining water temperatures (fall and winter). There can be a higher incidence of BKD in soft water, probably because of biological factors.

Clinical Signs/Pathogenesis

GROSS LESIONS

Fish with severe BKD may have no external signs. Affected fish may present with dark coloration, exophthalmos, pale gills, abdominal distension, or hemorrhages at the vent or base of the fins. Small vesicles on the flanks (Fig. II-54, A), filled with clear or turbid fluid, rupture to form small ulcers.

The major target organ is the kidney, which has white, nodular masses (Fig. II-54, B). Nodules may also occur in other viscera, especially spleen. There may be fluid in the abdomen. A pseudodiphtheritic membrane may be present over the abdominal viscera, most often at less than 10°C (50°F). A less common finding is large cavitations in skeletal muscle (Fig. II-54, C).

HISTOPATHOLOGY

Nodules are focal, often large, granulomas consisting of macrophages containing various numbers of phagocytized bacteria. In relatively resistant species (e.g., Atlantic salmon), granulomas are often encapsulated, indicating a successful host response. In more susceptible Pacific salmon, granulomas are rarely well encapsulated (Evelyn 1993; Fig. II-54, D). In advanced lesions, there is often caseous necrosis with numerous free bacteria (Bruno 1986).

Diagnosis

CLINICAL DISEASE

Tissue smears having 0.5 × 1–2 μm, coryneform-like, Gram-positive rods can be used for a rapid presumptive identification of BKD but are not reliable in light infections because of the difficulty in differentiating the bacterium from melanin granules.

Histopathology can also provide presumptive identification (Fig. II-54, D through F) but is best accompanied by antibody or gene probe confirmation.

Definitive diagnosis of clinical BKD requires identification of the bacterium in target tissues, with attendant clinical signs. The corpuscle of Stannius, a paired, white, endocrine organ in the anterior kidney, should not be mistaken for a BKD nodule. Kidney is the best organ for sampling during epidemics. The head kidney appears to be the best site for isolation (Chambers and Barker 2006).

Renibacterium salmoninarum is fastidious and extremely slow-growing, typically requiring 3–6 weeks to appear after primary isolation (Evelyn 1977). It grows best at 15–18°C (59–64°F) and does not grow at 25°C (77°F) (Evelyn 1993). It also requires a specialized medium for isolation, which is not commercially available. Several types of media have been tested (Shotts and Teska 1989). Highest recovery appears to be with Ten-M (Teska 1994; Chambers and Barker 2006), but it is complicated to prepare. Thus, clinical diagnosis of BKD is almost always based on immunological identification of *R. salmoninarum* antigen in tissues. The bacterium is

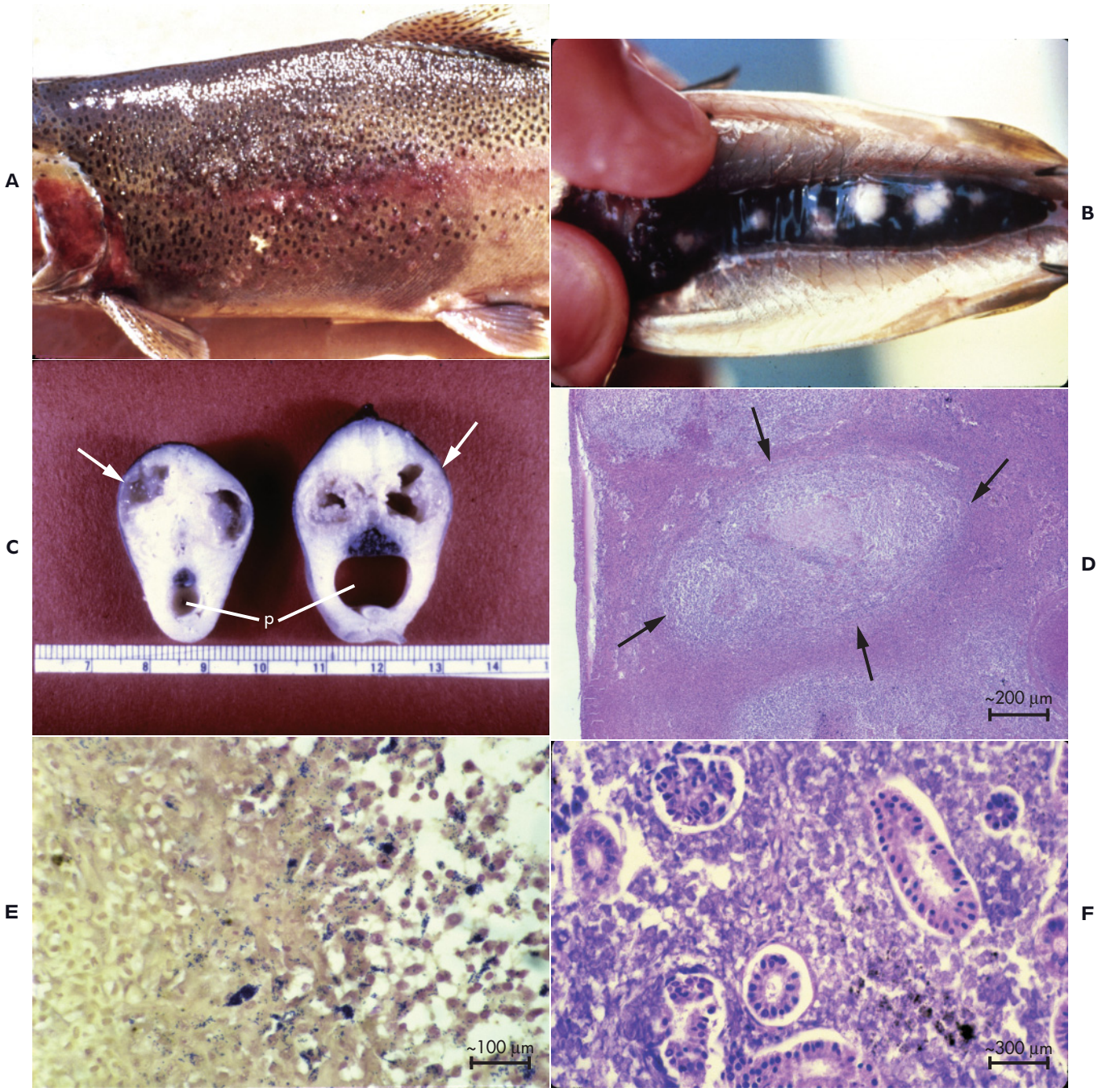


Fig. II-54. A. "Spawning rash" caused by dermal infection with *R. salmoninarum*. B. Salmon with abdominal cavity opened and viscera removed, revealing focal, white nodules in the kidney caused by BKD. C. Cross-sections through the body of a salmon, revealing large cavitations in muscle (*arrows*) caused by *R. salmoninarum* infection. *P* = peritoneal cavity. D. Histological section of anterior kidney, showing a large area of focal necrosis (*arrows*) caused by *R. salmoninarum* infection. Hematoxylin and eosin. E. Gram stain of section in Fig. II-52, *D,* with numerous, Gram-positive, short bacterial rods. Brown and Brenn. F. *R. salmoninarum* infection. Chronic interstitial nephritis. Kidney hematopoietic tissue has been replaced with a macrophage infiltrate. Compare with Fig. I-42, *E*. Hematoxylin and eosin. (*A* and *B* photographs courtesy of National Fish Health Research Laboratory, United States; *C* photograph courtesy of R. Wolke.)

an immunologically homogeneous taxon. Direct fluorescent antibody and ELISA are the the most widely used techniques (Pascho et al. 1987; Anonymous 1991, 2003). Antisera (Kirkegard and Perry Labs, Microtek), is commercially available for diagnosis. A gene test (PCR) can also be used to confirm the presence of *R. salmoninarum* in culture or tissue specimens. Tissue samples can also be sent to a specialized laboratory for confirmatory diagnosis. False positives are a serious problem, especially when small numbers of fish are positive (Austin and Austin 1987).

The only bacteria that may be mistaken for *R. salmoninarum* are a group of small, Gram-positive rods that cause pseudokidney disease (see PROBLEM 57). They are easily differentiated from *R. salmoninarum* based on their rapid growth at 30°C (86°F) on trypticase soy or brain-heart infusion agar (Hiu et al. 1984). *Renibacterium salmoninarum* is not acid-fast, which differentiates it from *Mycobacterium* (see PROBLEM 55).

CARRIERS

Detection of carriers is mainly focused on identifying infected broodstock. Ovarian fluid is the best material for identifying the bacterium from asymptomatic carriers during spawning because it is a known source of infection for eggs and bacterial load is proportional to the infection status of ovary tissue (another source of inoculum). However, fluorescent antibody is not always sensitive enough to detect all carriers (Evelyn 1993) and this is now used in conjunction with ELISA (Meyers et al. 1993) for screening (see "**Avoidance/Pathogen Reduction**" below).

Treatment

There are no proven therapies that can unequivocally cure fish of BKD (Elliott et al. 1989). The intimate association of the bacterium with host defenses, coupled with its chronic, insidious nature, make it difficult to control. Macrolide antibiotics (e.g., erythromycin) are the most effective agents in treating both clinical and asymptomatic infections (Austin 1985; Moffitt 1991). Only erythromycin thiocyanate or phosphate were effective prophylactically or therapeutically against BKD; other forms tested (stearate, ethylsuccinate, or estolate) were not (Austin 1985).

CLINICAL DISEASE

Oral erythromycin thiocyanate appears to reduce severity of outbreaks but has not been proven to cure fish of the infection (Austin 1985). Oral oxytetracycline has also been used to try to control infections, since it is less expensive (Kent 1992). However, oxytetracycline was ineffective in either prophylaxis or treatment of experimental BKD (Austin 1985).

ASYMPTOMATIC INFECTIONS

Injection of erythromycin base (as Erythro® 100 or Erythro® 200) into female broodstock before spawning significantly reduces the incidence of infected eggs (Moffitt 199l). This occurs because it kills bacteria in the fish and because the procedure "loads" antibiotic into the eggs. Erythromycin is detectable into the alevin stage (Evelyn 1993). There is preliminary evidence that this procedure may entirely eliminate the infection from broods (Lee and Evelyn 1991). Female broodstock should be injected with erythromycin between 9 and 56 days before spawning (Armstrong et al. 1989). In one preliminary study, there was no evidence of bacteria when fish were injected about 28 days before spawning (Lee and Evelyn 1991). As an added precaution, eggs should also be treated with potentiated iodine antiseptic after spawning (Evelyn 1993). Treating eggs only with antiseptic is ineffective because antiseptics do not penetrate the egg (Evelyn et al. 1986). Male broodstock are not treated, since they do not seem to be a significant source of vertical transmission, even when milt is heavily infected with the bacterium (Evelyn 1993).

Exposure of eggs to erythromycin phosphate before water hardening is not effective in reducing infection incidence, since therapeutic antibiotic levels are not maintained long enough and the antibiotic does not penetrate the yolk where some bacteria occur (Elliot et al. 1989).

AVOIDANCE/PATHOGEN REDUCTION

Use of specific-pathogen-free stock is the best means of control, but this may be difficult to achieve with anadromous stocks that are frequently exposed to feral fish harboring the bacterium. Clean stocks should be kept away from waters having feral salmonids. If possible, only one age group should be kept on a farm at one time.

To reduce vertical transmission, broodstock segregation and culling is now standard practice to select lots of eggs for producing juveniles in hatcheries (Anonymous 2003). Mating pairs that have little or no detectable *R. salmoninarum* infection are chosen for spawning. While it can reduce the incidence of disease, this procedure is unlikely to eliminate the bacterium from a population. Successful screening relies heavily on sensitive and specific testing for asymptomatic carriers. The membrane filtration-fluorescent antibody technique (MF-FAT), in combination with ELISA (Elliott and Barila 1987), can be used to segregate potential parents having either very high or very low *R. salmoninarum* burdens (Pascho et al. 1991). New tests are also being developed to improve screening methods (Anonymous 2003). A live vaccine (Renogen) is commercially available, but it is low efficiacy (Salonius et al. 2005).

PROBLEM 55

Mycobacteriosis (*Mycobacterium* Infection, "Fish Tuberculosis")

Prevalence Index

WF - 1, WM - 3, CF - 4, CM - 3

Method of Diagnosis

1. Culture of *Mycobacterium*
2. Histology of lesions (spleen, liver, kidney, skin)

History

Chronic morbidity/mortality

Physical Examination

Nonhealing, shallow to deep skin ulcers; corneal ulcers; pale coloration; emaciation; white nodules on viscera

Treatment

Disinfect and quarantine

COMMENTS

Epidemiology

HOST RANGE

Mycobacteriosis is probably the most common chronic disease that affects aquarium fish. Virtually all freshwater and marine aquarium fish are probably susceptible, especially members of the freshwater families Anabantidae, Characidae, and Cyprinidae (Nigrelli and Vogel 1963; Smith 1997; Decostere et al. 2004; Zanoni et al. 2008). Mycobacteriosis has also recently become a serious problem in laboratory research facilities that maintain medaka (Teska et al. 1997) and especially zebrafish (Astrovsky et al. 2000, Harriff et al. 2007). Aquarium fish mycobacteriosis has previously been associated only with *Mycobacterium marinum* and *M. fortuitum*, but now includes a number of other species (Table II-55).

Mycobacteriosis has also recently become a serious problem in several species of cultured food fish (Table II-55), such as European sea bass (Colorni et al. 1993, 1998), tilapia, and striped bass (Hedrick et al. 1987a), especially in intensive culture systems.

Mycobacteriosis was historically a serious problem in salmonids, when they were fed raw fish offal (Ross et al. 1959). Although mycobacteriosis is now relatively uncommon in salmonids, asymptomatic *Mycobacterium* infections have been common in some populations; over 25% of some hatchery salmonids were infected with *M. chelonae* subsp. *piscarium* along the northeastern Pacific coast (Arakawa and Fryer 1984). And recently, *Mycobacterium neoaurum* has been isolated from a mixed culture from chinook salmon with ocular lesions (Backman et al. 1990) and *M. chelonae* from cage-cultured Atlantic salmon (Brocklebank et al. 2003).

Mycobacteriosis has also caused epidemics in wild striped bass in Chesapeake Bay, USA (Rhodes et al. 2004). There is some evidence that *Mycobacterium marinum* has been introduced into the Red Sea from the introduction of infected exotic fish for cage aquaculture, although it is also possible that there is instead simply an increased prevalence of an endemic strain of the bacterium that has become more prevalent due to the introduction of a highly susceptible exotic fish species (i.e., Mediterranean seabass) (A. Colorni, personal communication). In any case, it has recently become much more common in native fish populations (Diamant 2001). Some fish-pathogenic mycobacteria (e.g., *M. marinum*) can also produce granulomatous disease in amphibians (Ramakrishnan et al. 1997). Vertical transmission can also occur in mycobacteriosis.

TRANSMISSION

Shedding of bacteria from infected skin ulcers, as well as the intestine, is probably a major source of contagion. Ingestion is probably the major source of infection (Harriff et al. 2007), including fish that have recently eaten dead tankmates. The bacteria can survive for 2 years in the environment (Reichenbache-Klinke 1972). Transovarian transmission has been demonstrated in some fish, such as platyfish (Conroy 1966; Chinabut 1999) but does not occur in salmonids (Ross and Johnson 1962).

ZOONOTIC CONSIDERATIONS

Fish-pathogenic mycobacteria are mainly known as atypical mycobacteria, nontubercular mycobacteria or environmental mycobacteria. Some species can infect humans, usually causing localized, nonhealing ulcers (fish tank

Table II-55. Examples of *Mycobacterium* infections of fish. All reports are from cultured fish unless noted otherwise.

Host	Species	References
Freshwater aquarium fish	*M. marinum*, *M. fortuitum*, *M. chelonae*, *M. gordonae*, *M. peregrinum*,	Pate et al. (2005), Sakai et al. (2005), Austin and Austin (2007)
Moray eel	*M. montefiorense*	Levi et al. (2003)
Zebrafish	*M. marinum*, *M. peregrinum*	Astrofsky et al. (2000), Harriff et al. (2007)
Medaka	*M. abscessus*	Teska et al. (1997)
European sea bass	*M. marinum*	Colorni et al. (1998)
Chinook salmon	*M. neoaurum*	Backman et al. (1990)
Atlantic salmon	*M. chelonae*	Brocklebank et al. (2003)
Salmonids (asymptomatic)	*M. chelonae* subsp. *piscarum*	Arakawa and Fryer (1984)
African catfish	*M. marinum*	Antychowicz et al. (2003)
Milkfish	*M. abscessus*	Chang et al. (2006)
Striped bass (wild)	*M. shottsii*, *M. pseudoshottsi*, *M. interjectum*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*, *M. triplex*	Rhodes et al. (2004, 2005)
Atlantic menhaden (wild caught)	*M. marinum*, *M. fortuitum*, *M. gordonae*	Stine et al. (2005)

granuloma, swimming pool granuloma [Kern et al. 1989]) that may be difficult to treat because of the resistance of some isolates to most antituberculosis drugs (Noga et al. 1990b). Owners should be cautioned about contacting potentially infected fish or fomites. The ubiquity of fish mycobacteriosis coupled with the apparently low numbers of human cases suggest that it fortunately appears to be a low risk for healthy humans. However, a small but significant number of persistent infections (e.g., osteomyelitis, arthritis, periocular infection) by atypical mycobacteria have been reported in humans due to trauma followed by exposure to infected surfaces or in immunosuppressed individuals. These infections often require lengthy systemic antibiotic treatment and surgical debridement (Astrofsky et al. 2000). Also, a small number of *M. marinum* infections have been reported from HIV-infected persons (Glaser et al. 1994) whom all acquired the infection from contact with pet fish, usually when cleaning the aquarium. Rarely, infections can become systemic (Streit et al. 2006). Gloves should be worn by persons at risk when cleaning an aquarium or when handling fish (Angulo et al. 1994).

Clinical Signs/Pathogenesis

GROSS LESIONS

Emaciation, poor growth, retarded sexual maturation, or decreased reproductive performance may be the only clinical signs of mycobacteriosis. Other lesions include skeletal deformities; chronic, nonhealing, shallow to deep ulcers or fin erosion (Figs. II-55, A and B). Internally, 1–4 mm white nodules may be present on the viscera, especially hypertrophic kidney or spleen (Fig. II-55, C). A more acute form of the disease, associated with abdominal distention and dermal edema (Astrofsky et al. 2000), is less common.

HISTOPATHOLOGY

There is a chronic inflammatory response with epithelioid macrophages surrounding the bacteria. Lesions often have necrotic centers and may have melanomacrophages or melanocytes. Bacteria are typically located in the center of the inflammatory focus.

Diagnosis

Mycobacteriosis is strongly suggested by the typical clinical signs in combination with the presence of large numbers of granulomas in wet mounts (Fig. II-55, D), especially spleen and kidney. Granulomas can be caused by many other pathogens, but if large numbers are present, histological material should be stained for acid-fast bacteria (Fig. II-55, E, F, and G). Fite-Faraco is often better than Ziehl-Nielsen for demonstrating piscine mycobacteria (Wolke and Stroud 1978). Note that tissue decalcification with acid (e.g., hydrochloric acid or picric acid [Bouin's]) can block the acid-fast reaction (Kent et al. 2006), so should not be used. Tissue smears can also be stained, but this is less advisable, since fresh, infective lesion material must then be handled, risking infection of the clinician. Note that an occasional granuloma is a common incidental finding on necropsy. Granulomas also look similar to melanomacrophage centers (see Fig. I-38, A). When in doubt about the significance of wet mount lesions, samples should be processed for histology. Mycobacteria are ~0.4 × 1.0–4.0 µm long, acid-fast, and often stain unevenly. In zebrafish, the presence of acid-fast positive bacteria in histological lesions is very highly correlated with positive cultures.

Mycobacteria are also Gram-positive but often do not stain well (Frerichs 1993). Other acid-fast rods (i.e., *Nocardia* [see PROBLEM 57]) are longer and branching (mycobacteria are never branching). Nocardiosis is also much less common than mycobacteriosis. Isolation on Löwenstein-Jensen (Fig. II-55, H) or Middlebrook 7H10 agar allows definitive diagnosis by biochemical identification, as well as determination of the species involved.

Isolation may take up to 30 days and positive confirmation up to 90 days; sometimes organisms cannot be cultured even when large numbers are seen in lesions (Frerichs 1993). Some isolates may grow on blood agar or trypticase soy agar, if the inoculum is heavy (Shotts and Teska 1989). Atypical mycobacteria are Biosafety Level-2 organisms and clinical material for culture should be handled accordingly. Culture is usually not necessary unless treatment is anticipated. Gene tests are often much more sensitive than culture in detecting infections (see "**Treatment**").

Mixed infections involving up to several *Mycobacterium* species have been observed in some cases (Rhodes et al. 2004; Pate et al. 2005; Stine et al. 2005), and the relative importance of each species to the clinical signs and disease is usually not entirely clear.

Treatment

Many drugs have been advocated for treating this disease, but there are few rigorous clinical trials yet published. In one study, erythromycin, rifampicin, or streptomycin was effective against experimental infections (Kawakami and Kusuda 1990). There is some clinical evidence that kanamycin may be effective in reducing clinical disease in some cases (Conroy and Solaro 1965; Conroy and Conroy 1999), but eradication of the infection remains unproven. Also, some strains are resistant in vitro (Noga et al. 1990b), and we know nothing of the bioavailability of any antimycobacteriosis drugs for fish. This disease can be insidious and difficult to eradicate. Freezing does not kill bacteria in carcasses (Ross et al. 1959).

As with so many fish diseases, mycobacteriosis usually gains a foothold under suboptimal environmental conditions. Once established, it can be difficult to control. The apparently high prevalence of subclinical disease in feral (and sometimes cultured) fish also makes it difficult to exclude (Beran et al. 2006). However, gene tests for several piscine mycobacteria appear to be highly sensitive

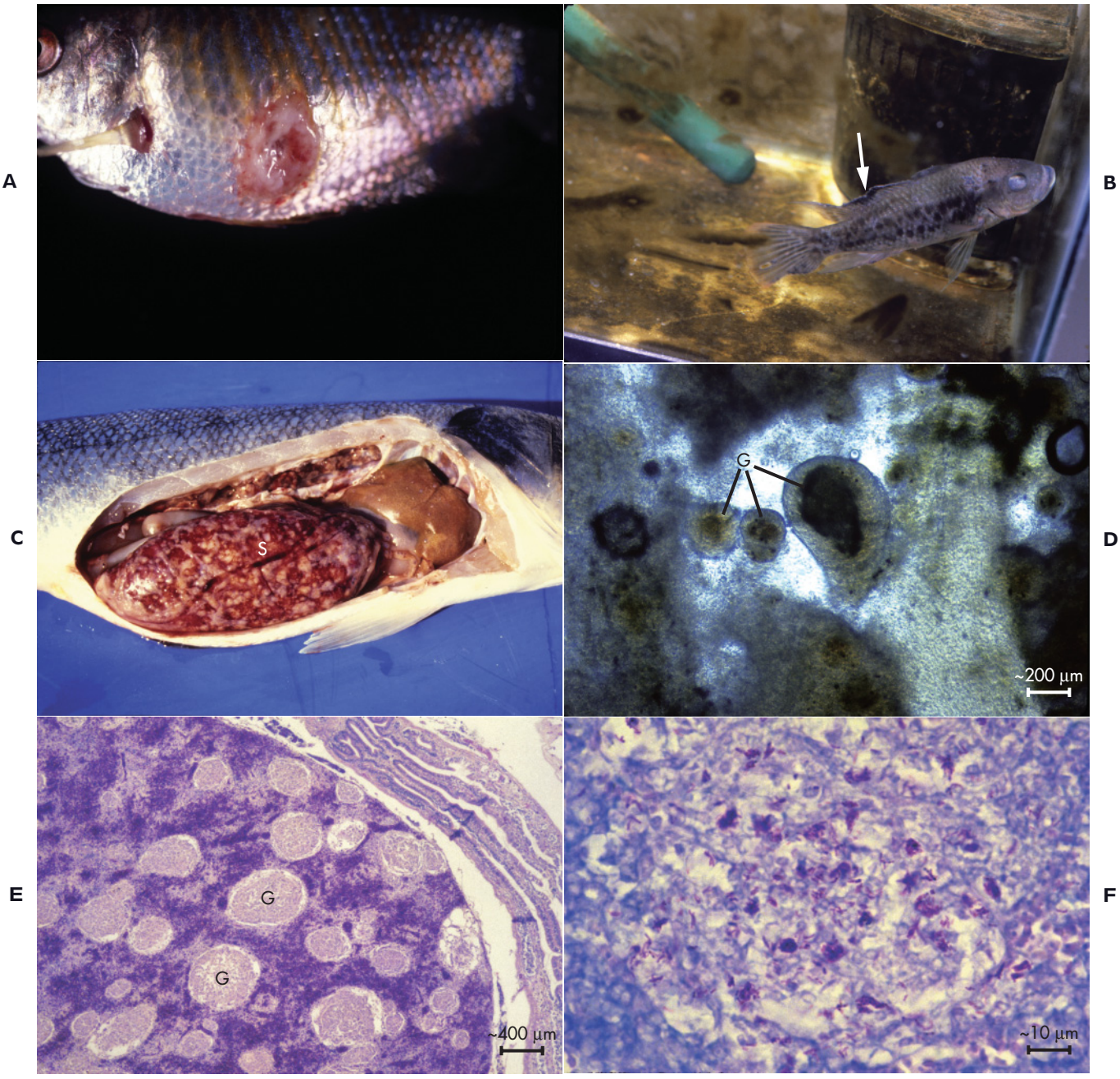
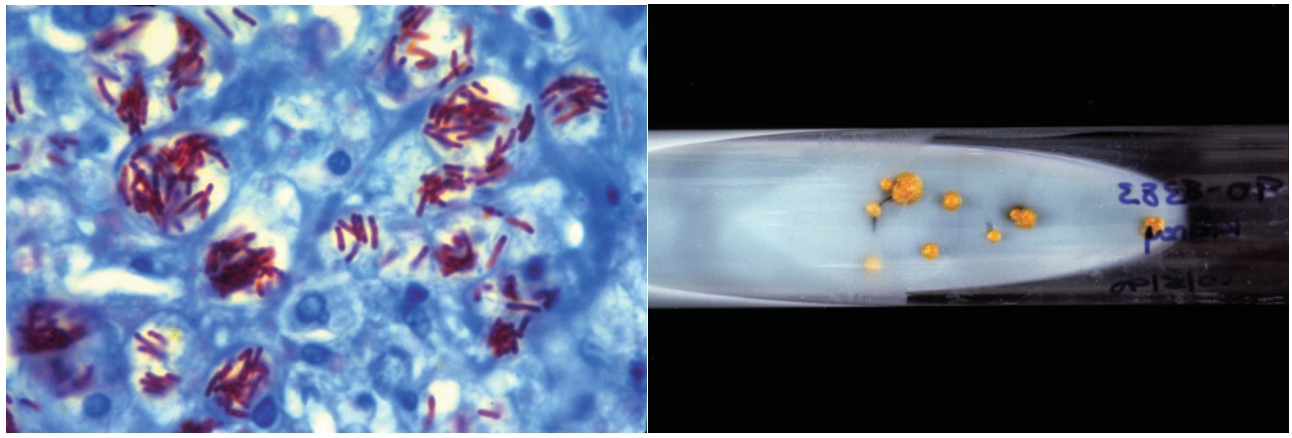


Fig. II-55. A. Dwarf gourami with a large chronic skin ulcer caused by mycobacteriosis. B. Mozambique tilapia with corneal ulceration and chronic, hyperpigmented erosions and ulcerations on the flank and fins (*arrow*) caused by *Mycobacterium marinum*. C. Massively hypertrophied spleen (*S*) of a European sea bass with multifocal granulomas caused by *Mycobacterium marinum*. D. Wet mount of the spleen of a Mozambique tilapia with mycobacterial granulomas (*G*). Note the dark, necrotic center and lighter periphery of inflammatory cells, which is diagnostic for granulomatous response. E. Granulomas (*G*) in the spleen of the fish in Fig. II-55, *A*. Hematoxylin and eosin. F. Numerous acid-fast mycobacteria in a granuloma of the fish in Fig. II-55, *A*. Fite-Faraco.



G H

Fig. II-55.—cont'd. G. High magnification of a granuloma with numerous acid-fast mycobacteria. Fite-Faraco. H. Culture of *Mycobacterium marinum* on Löwenstein-Jensen agar. Note bright yellow (photochromogenic) colonies. (*C* and *G* photographs courtesy of A. Colorni; *H* photograph by L. Khoo and E. Noga.)

for detecting asymptomatic infections (Colorni et al. 1993; Pate et al. 2005; Kaattari et al. 2006), and some appear to show promise in monitoring culture facilities for carriers (Astrofsky et al. 2000).

Disinfection is the best method for control of an epidemic. High level disinfection is required (see "**Pharmacopoeia**") and contact times vary greatly with the disinfectant, as well as the particular isolate/species of *Mycobacterium*. To kill all *M. marinum*, at least 60 minutes of contact time was needed when using 200 mg/L sodium hypochlorite (Chlorox®), while only one minute contact time was 100% lethal when using 1% benzyl-4-chlorophenol-2-phenylphenol (Lysol®), 50% ethanol, or sodium chlorite (Clidox-S; 1:5:1 ratio of base:water:activator). In addition, some *Mycobacterium* species have developed resistance to chlorine (Vaerewijck et al. 2005). Intermediate or low level disinfectants (e.g., quaternary ammonium compounds or Virkon-S) are ineffective (Mainous and Smith 2005).

When using heat sterilization in research laboratories, it is best to treat materials for 90 minutes at 121°C (250°F) and 16–18 psi pressure, which is about a two hour autoclave run including startup and cool down times (U.S. National Animal Disease Laboratory, Ames, Iowa, personal communication).

The common presence of environmental mycobacteria in soil and water, as well as their residence in asymptomatic fish for long periods, suggest that total elimination of exposure risk might be impossible and that keeping chronic stress low is essential for management. *Mycobacterium fortuitum* and *M. chelonae* can be readily isolated from biofilms and aquaria in the absence of severe disease (Schulze-Röbbecke et al. 1992). There is some evidence that the disease can regress under certain circumstances, but recovered fish still probably carry the infection (Colorni et al. 1998).

Methods for managing mycobacteriosis in zebrafish facilities should be dictated by the type of isolate encountered. For highly virulent strains, screen incoming fish and quarantine. If this type of strain appears, depopulate the stock and disinfect the equipment. For the ubiquitous opportunists, maintain a rigorous screening program, where moribund fish are removed and necropsied, and the infection identified. If a fish is positive, screen additional fish from the same population and keep fish, water and equipment from that population under quarantine. Subclinical infections in tankmates can persist for months (Astrofsky et al. 2000).

Eradication of some species (e.g., *M. chelonae*) is probably impractical.

PROBLEM 56

Piscirickettsiosis (Salmonid Rickettsial Septicemia [SRS], Coho Salmon Septicemia, Huito Disease, *Piscirickettsia salmonis*, Rickettsia-Like Organism [RLO])

Prevalence Index

WM - 4, CF - 4, CM - 2

Method of Diagnosis

1. Specific identification of *Piscirickettsia salmonis* from culture or tissue
2. Histology of lesions (liver, kidney, blood) with characteristic bacterium

History

Chronic morbidity/mortality, lethargy; anorexia

Physical Examination

Pale coloration (anemia); skin ulcers; swollen abdomen; nodules and/or depressions on liver

Treatment

1. Appropriate antibiotic
2. Institute appropriate biosecurity

COMMENTS: *Piscirickettsia salmonis*

Epidemiology

First discovered in Chile in the late 1980s, and later Norway, Ireland, Scotland, and Greece, as well as the east and west coasts of Canada, *Piscirickettsia salmonis* causes a chronic disease of mainly marine-cultured salmonids. It is the most important disease in the Chilean salmon farming industry, with annual losses sometimes exceeding $100 million. It is of minor importance in other countries. While coho are most susceptible, it also affects Atlantic, Chinook, pink and masou salmon, as well as rainbow trout (Turnbull 1993b; Anonymous 2003). Some freshwater salmonid farms have also experienced epidemics (Gaggero et al. 1995). All ages of fish (smolts to market size) are susceptible. Clinical signs typically appear about one month after placing fish in marine cages. Monthly mortalities can average 1% to 20%, with up to 90% cumulative losses. Horizontal transmission occurs in both seawater and freshwater, but the mechanism of transmission is unclear. Vectors have not been ruled out. It is not known if vertical transmission occurs. *Piscirickettsia salmonis* might be a marine bacterium, with salmonids an aberrant host. The bacterium can be prevalent in coastal waters, including near netpens (Mauel and Fryer 2001). Meriterranean sea bass in Europe (McCarthy et al. 2005) and white sea bass in California (Arkush et al. 2005) also can be affected.

Clinical Signs/Pathogenesis

Severely affected fish are dark, anorexic, and lethargic, swimming on the surface at the edge of the cage. Fish might swim erratically. External signs include anemia, skin ulcers (small white foci that progress to shallow ulcers) and abdominal distension due to ascites. Fish with milder infections may have no gross external lesions. Internally, there may be petechial hemorrhages on the visceral fat, stomach, swim bladder and muscle. Spleen and kidney are often swollen. The intestine may be filled with yellowish, mucoid material. The liver may have large white or yellow, multifocal, coalescing nodules (Fig. II-56, A); these lesions often rupture, resulting in circular, crater-shaped depressions. In more acute infections, the necrotic liver foci result in a more mottled appearance rather than discrete nodules.

Histologically, liver, kidney, spleen and intestine are most severely involved. Lesions in brain, heart, ovary or gill may also be present. Lesions are caused by a slowly developing septicemia with vascular damage (Olsen et al. 1997); there is a systemic vasculitis, often necrotizing, with granulomatous inflammation. In liver, there is multifocal hepatocyte necrosis with chronic mononuclear cell infiltrate, vascular and perivascular necrosis, and thrombi in major vessels (Fig. II-56, B). Similar changes also occur in spleen, kidney, intestine, and sometimes other organs.

Diagnosis

The most consistent gross external sign is anemia (as low as 2% PCV). The whitish-to-reddish skin ulcers and ascites are also characteristic, but all are nonspecific signs. The typical gross liver lesions of chronic infections (multifocal nodules and depressions) are highly diagnostic for piscirickettsiosis, but not always present.

For presumptive diagnosis, tissue smears or sections (liver, kidney, blood are best [Lannan and Fryer 1991]) should be examined at high power to observe aggregates of the bacterium in the cytoplasm of degenerated hepatocytes and in macrophages. Macrophages are usually hypertrophied and have abundant cell debris. In sections stained with hematoxylin and eosin, they appear as small (0.4–1.5 μm) basophilic or amphophilic spheres, like those of *Francisella* (PROBLEM 57). They also stain with Giemsa and are Gram-negative.

Definitive diagnosis requires observation of typical organisms in either culture or affected tissues, followed by confirmation via antibody (FAT, ELISA) or gene (PCR) test. Fluorescent antibody test (FAT) is the most commonly used method. A commercial ELISA is available but its use has not been published. *Piscirickettsia salmonis* is highly fastidious and can only be isolated using cell culture. If samples are to be submitted for culture, they must not be treated with antibiotics since this inhibits growth. *Francisella* (PROBLEM 57) is highly similar in clinical presentation.

Treatment

Isolates are susceptible to many antibiotics in vitro, including aminoglycosides, tetracyclines, erythromycin and quinolones, but not to penicillins. However, oral antibiotics have been mostly ineffective, probably because *P. salmonis* is an intracellular pathogen. Due to the possibility of vertical transmission, Gram-staining or FAT of broodstock tissues has been used to screen for the organism (Turnbull 1993b) and antisepsis of eggs is advisable. Injection of broodstock with antibiotics before leaving seawater in order to control the typical outbreak occuring in summer is common. Eggs of individual broodstock should be segregated and screened for infection. Commercial vaccines are available but their efficacy is not yet established.

COMMENTS: RICKETTSIA-LIKE ORGANISMS (RLOs)

Several fish develop infections (RLO) that are highly similar to piscirickettsiosis, including melanostigma grouper (Chen et al. 2000) in Taiwan, tilapia in Hawaii, USA (Mauel et al. 2003), Mediterranean sea bass in Europe (Comps et al. 1996) and Atlantic salmon in Canada and Tasmania (Corbeil et al. 2005). An aquarium catfish (blue-eyed plecostomus), imported

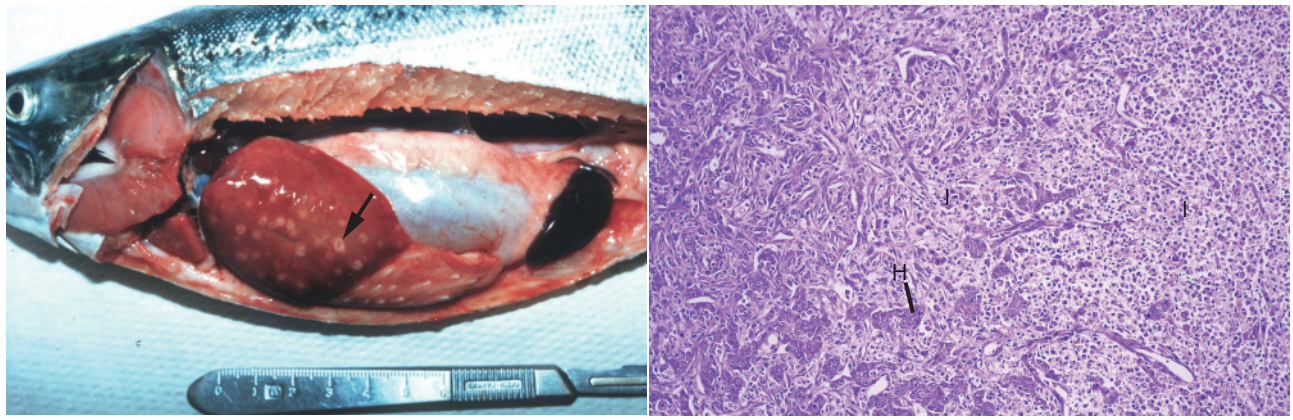


Fig. II-56. Piscirickettsiosis. A. Gross photograph of liver lesions in a salmonid. Note the multiple, white foci (*arrow*). B. Histological section of liver in an Atlantic salmon with piscirickettsiosis showing replacement of most of the normal, basophilic hepatocytes (*H*) with inflammatory infiltrate (*I*). Hematoxylin and eosin. (*A* photograph from House and Fryer 2002; *B* photograph courtesy of H. Ferguson.)

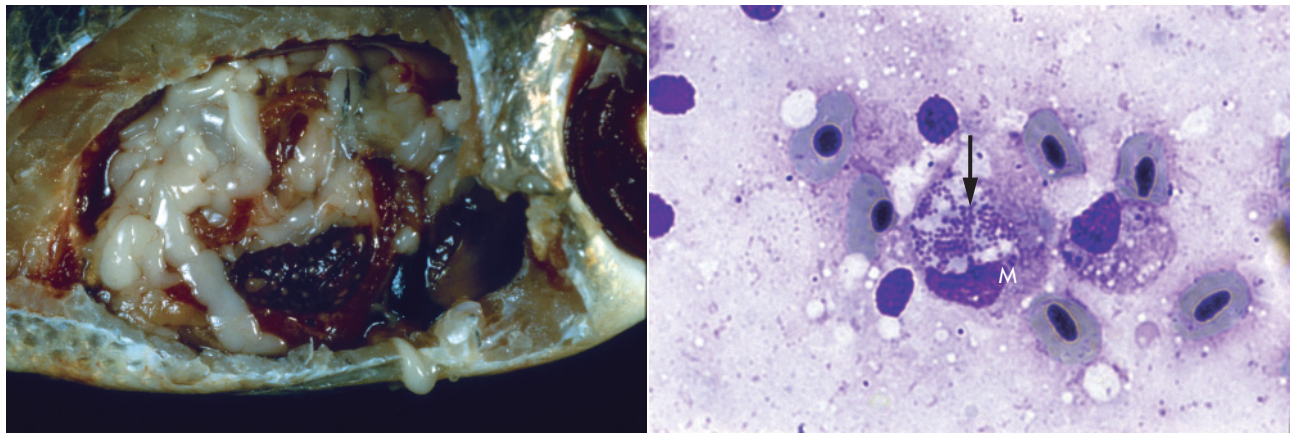


Fig. II-57. A. Spleen of tilapia with multiple granulomas due to *Francisella* infection. B. Blood smear of *Francisella* bacteria (*arrow*) in a tilapia macrophage (*M*). Giemsa. (*A* and *B* photographs courtesy of C. Tu.)

from South America, has also displayed similar pathology (Khoo et al. 1995). The relationship of these RLO to *P. salmonis* is uncertain. Note that an organism previously infecting Nile tilapia that was previously considered to be an RLO (Chen et al. 1994) is now considered to be a *Francisella* species (Hsieh et al. 2006; PROBLEM 57, Fig. II-57), suggesting that not all RLO are rickettsias.

PROBLEM 57
Miscellaneous Systemic Bacterial Infections

The most clinically important diseases in this group include botulism, nocardiosis, pseudokidney disease, and pseudomonad infections. Most other agents have been isolated only sporadically or in some instances from only a single epidemic (Table II-57).

Table II-57. Miscellaneous bacterial infections of fish.

Disease / pathogen	Hosts	Geographic / ecological range	Key diagnostic features	Treatment	References
ANEROBES					
Eubacteriaceae					
Eubacterium meningitis (*Eubacterium tarantellae* = *Catenabacterium* sp.)	Grey mullet, snook, redfish, flounder	Southeast United States marine	Chronic onset of neurological signs (spiral swimming; Filamentous, asporogenous, gram + rods in brain smears or histological sections	None proven	Udey et al. (1977) Henley and Lewis (1976)
Clostridiaceae					
Botulism (*Clostridium botulinum* Type E)	Rainbow trout, coho salmon, rockfish	Northwest United States England, Denmark freshwater	Chronic mortality with intermittent depression alternatively float and sink until death Common in sediment and fish intestines; thus, diagnosis requires presence of clinical signs, not just isolation of pathogen (bacteria are common in sediment, gastrointestinal tract)	Oxytetracycline oral; destroy infected stock; remove detritus; lime pond	Eklund et al. (1982) Cann and Taylor (1984) Schiewe et al. (1988)
GRAM-POSITIVE BACTERIA—LACTIC ACID BACTERIA					
Pseudokidney disease (*Carnobacterium piscicola* = *Lactobacillus piscicola*; also *Lactococcus piscium* and *Vagococcus salmoninarum*)	Salmonids, common carp, striped bass, catfish	United States Canada Europe Australia freshwater	Post-spawning fish; large amount of fluid in peritoneal cavity; liver, spleen, kidney damage; clinically similar to BKD (PROBLEM 54); Adults affected in North America; trout fry / fingerlings and common carp in Europe; chronic, stress-related disease	None proven	Hiu et al. (1984) Michel et al. (1986) Evelyn (1993) Baya et al. (1991b)
GRAM-POSITIVE AEROBIC RODS AND COCCI					
Nocardiaceae					
Nocardiosis (*Nocardia asteroides*)	Brook trout, steelhead trout, Pacific salmon, rainbow trout, paradise fish (E), 3-spot gourami (E), neon tetra, green sunfish (E), bluegill (E), blue minnow (E), jack mackerel (E), Formosa snakehead (E), largemouth bass (E),	Worldwide freshwater marine	Clinically resembles mycobacteriosis Short, coccobaccilary to long, slender, branching rods in chronic inflammatory lesions (*Mycobacterium* not branching); usually acid-fast in histological sections; abundant bacterial filaments in routine stains; growth on Löwenstein-Jensen agar after 21 days; important disease	None proven Destroy stock	Conroy (1964) Van Duijn (1981) Wood and Ordal (1958) Chen (1992)
Nocardiosis; gill tuberculosis (*Nocardia seriolae* = *N. kampachi*)	Yellowtail	Japan marine	See *N. asteroides* above	None proven Destroy stock	Kusuda et al. (1974) Kudo et al. (1988)
Nocardia salmonicida (= *Streptomyces salmonis* = *Streptoverticillium salmonis*)	Salmonids	United States freshwater	Gram-positive mycelia; only sporadic cases	None proven	Rucker (1949) Isik et al. (1999)
Rhodococcus erythropolis	Atlantic salmon	Norway Scotland freshwater and marine	Systemic infection (especially peritoneal cavity) associated with IP-injected, oil-adjuvated vaccines	None proven	Olsen et al. (2006b)
Rhodococcus sp.	Atlantic and chinook salmon	Canada freshwater	Corneal damage; exophthalmos; kidney granulomas with Atlantic salmon (may confuse with BKD, PROBLEM 54); only sporadic cases	None proven	Backman et al. (1990) Claveau (1991)

Continued.

Table II-57. Miscellaneous bacterial infections of fish, cont'd.

Disease / pathogen	Hosts	Geographic / ecological range	Key diagnostic features	Treatment	References
Bacillaceae					
Bacillus cereus	Common carp, striped bass	United States Poland freshwater	Gill necrosis	None proven	Pychynski et al. (1981) Baya et al. (1992a)
Bacillus mycoides	Channel catfish	United States freshwater	Skin ulcers and muscle necrosis	None proven	Goodwin et al. (1994)
Bacillus subtilis	Common carp	Poland freshwater	Gill necrosis	None proven	Pychynski et al. (1981)
Bacillus sp.	Various species	Nigeria Vietnam	Necrosis and/or granulomatous foci in liver, spleen, kidney	None proven	Oladosu et al. (1994) Ferguson et al. (2001)
Corynebacteriaceae					
Corynebacterium aquaticum	Striped bass	Maryland (United States) freshwater	Nervous signs due to infection of the CNS; disease only reported once	None proven	Baya et al. (1992b)
Staphylococcaceae					
Staphylococcus epidermis	Red seabream, yellowtail	Japan marine	Exophthalmos; skin ulcers; disease only reported once	None proven	Kusuda and Sugiyama (1981)
Staphylococcus aureus	Silver carp	India freshwater	Corneal damage progressing to phthisis bulbi; disease only reported once	None proven	Shah and Tyagi (1986)
Staphylococcus warneri	Rainbow trout	Spain freshwater	Skin ulcers, exophthalmos, swollen abdomen	None proven	Gil et al. (2000)
Planococcaceae					
Planococcus sp.	Atlantic salmon, rainbow trout	England freshwater	Associated with RTFS and skin lesions in trout, kidney damage in salmon; sporadic cases	None proven	Austin and Stobie (1992a) Austin and Austin (1993)
Micrococcaceae					
Micrococcus luteus	Rainbow trout	England freshwater	Isolated from fish with RTFS; disease only reported once	None proven	Austin and Stobie (1992a)
GRAM-NEGATIVE AEROBIC RODS					
Pseudomonadaceae					
Fin rot (*Pseudomonas fluorescens*, *P. putida*, *P. pseudoalcaligenes*, *P. chlororhaphis*, *P. plecoglossicida*, *Pseudomonas* sp.)	Goldfish; silver, bighead, grass, and black carp; tench hybrid striped bass; tilapia white catfish; rainbow trout; probably many other species	Worldwide freshwater marine	Typical bacterial septicemia Often fin erosion, ulceration Often pathogenic at low temperature Often resistant to antibiotics (need to test sensitivity of isolate)	Appropriate antibiotic	Bauer et al. (1973) Csaba et al. (1981) Noga (Unpublished data) Shotts and Teska (1989) Lio-Po and Sanvictores (1987) Roberts and Horne (1978) Meyer and Collar (1964) Austin and Stobie (1992b)

Sekiten-byo (*Pseudomonas anguilliseptica*)	Japanese and European eels, bluegill (E), common carp (E), goldfish (E), loach (E), ayu (E), crucian carp (E)	Japan Scotland marine	Petechiae around mouth, operculum, and ventrum Internal gross signs may not be present; 1mm pale grey, round, raised, shiny colonies after about 7 days; acute, often high mortalities	Raise temperature to 27°C for 2 weeks, then drop to <20°C Nalidixic acid Oxolinic acid Piromidic acid	Muroga et al. (1977) Ellis et al. (1983a)
Alteromonadaceae					
Shewanella (= *Pseudomonas*) *putrefaciens*	Rivulatus rabbitfish	Red Sea, Egypt marine	High mortalities in caged fish; disease only reported once	Killed vaccine may protect	Saeed et al. (1987)
Campylobacteraceae					
Arcobacter cryearophilus	Rainbow trout	Turkey freshwater	Skin ulcers, abnormal color, pale gills, hemorrhage in muscle and intestine	None proven	Aydin et al. (2002)
Francisellaceae					
Francisella sp.	Atlantic cod, hybrid striped bass, three-lined grunt, tilapia (Fig. II-57A,B)	Japan, Taiwan, Norway, United States Latin America marine and freshwater	Systemic granulomatous disease Ddx: Pisciririckettsia/RLO (PROBLEM 56)	None proven	Olsen et al. (2006a) Kamaishi et al. (2005) Ostland et al. (2006) Hsieh et al. (2006) Mauel et al. (2007)
Enterobacteriaceae					
Serratia plymuthica	Rainbow trout	Spain, Scotland freshwater	Often no external signs; may only be skin lesions; sporadic cases	None proven	Nieto et al. (1990) Austin and Stobie (1992b)
Serratia liquefaciens	Atlantic salmon, lake trout, brook trout	Scotland (marine) Ontario, (Canada) marine and freshwater	Few external signs; nodules on kidney, spleen; mottled liver; only sporadic cases	Oxolinic acid Possibly oxytetracycline	McIntosh and Austin (1990) Stevenson et al. (1993)
Serratia marcescens	White perch	Black River, Chesapeake Bay (United States) freshwater	Isolated only from clinically normal fish during a disease survey	None proven	Baya et al. (1992c)
Hafnia alvei	Rainbow trout	Bulgaria freshwater	Typical of hemorrhagic septicemia; *Brucella*-like organism; also see PROBLEM 52; disease only reported once	None proven	Gelev et al. (1990)
Citrobacter freundii	Marine sunfish, Atlantic salmon, rainbow trout, carp	Japan Spain Scotland India marine and freshwater	Erratic swimming; eroded and hemorrhagic skin; focal nodules (granulomas) in kidney; other lesions typical of hemorrhagic septicemia	None proven	Sato et al. (1982) Austin et al. (1992b) Austin and Austin (1993) Baya et al. (1991a)
Pantoea (= *Enterobacter*) *agglomerans*	Dolphin	Florida Bermuda marine	Hemorrhages in eyes and muscles; disease only reported once	None proven	Hansen et al. (1990)
Providencia (= Proteus) rettgeri	Silver carp	Israel freshwater	Red ulcers on body (head, fin bases, and abdomen); bacterium associated with poultry feces; disease only reported once	None proven Handle fish carefully	Bejerano et al. (1979)

Continued.

Table II-57. Miscellaneous bacterial infections of fish, cont'd.

Disease / pathogen	Hosts	Geographic / ecological range	Key diagnostic features	Treatment	References
Salmonella enterica subsp. *arizonae (= S. choleraeuis* subsp. *arizonae = S. arizonae)*	Piracuru	Japan (aquarium) freshwater	Corneal opacity; mild gross signs of hemorrhagic septicemia; disease only reported once	None proven	Kodama et al. (1987)
Plesiomonas (= Proteus) shigelloides	Rainbow trout	Portugal freshwater	Emaciation; red anus with yellow exudate; petechiation of muscle lining peritoneum; ascites in peritoneal cavity; only reported once, but may be fairly common	Sulfadiazine-Trimethoprim PO	Cruz et al. (1986)
Escherichia vulneris	Various fish	Turkey freshwater	Pale gills, hemorrhage in skin, intestine, gonads	None proven	Aydin et al. (1997)
Klebsiella pneumoniae	Rainbow trout	Scotland freshwater	Fin erosion	None proven	Daskalov et al. (1998)
Yersinia intermedia	Atlantic salmon	Australia	Lethargy, dark body, fin erosion	None proven	Carson and Schmidtke (1993)
Moraxellaceae					
Acinetobacter sp.	Atlantic salmon	Norway marine	Hemorrhage, hyperemia, ulceration, and edema of skin; hemorrhage in peritoneum and swim bladder; isolated on blood agar with 0.5% NaCl; disease only reported once	Oxytetracycline IM	Roald and Hastein (1980)
Moraxella sp.	Striped bass, rainbow trout (E)	Potomac River, Maryland (United States) freshwater	Large skin hemorrhages, missing scales; hemorrhage in swim bladder; pale liver, possibly with adhesions; disease only reported once	None proven	Baya et al. (1990b)
Halomonadaceae					
Halomonas (= Deleya) cupida (= *Alkaligenes cupidus*)	Schlegeli black seabream	Japan marine	Heavy mortalities in fry; isolated from mixed bacterial culture of fry homogenate; disease only reported once	None proven	Kusuda et al. (1986)
Oxalobacteriaceae					
Janthinobacteriun lividum	Rainbow trout	Scotland freshwater	Fry (RFTS): exophthalmos; hyperpigmentation; pale gills; swollen abdomen; swollen spleen and kidney Larger fish: skin ulcers; disease only reported once; produces purple colonies *Flavobacterium psychrophilum* (PROBLEM 37) is the major cause of RTFS	None proven	Austin et al. (1992a)
Mycoplasmataceae					
Mycoplasma mobile	Tench	United States freshwater	"Red disease" (gill infection)	None proven	Kirchhoff et al. (1987)
Pasteurellaceae					
Pasteurella skyensis	Atlantic salmon	Scotland marine	Inappetance	None proven	Jones and Cox (1999)
Unknown					
"*Flavobacterium piscicida*"	Marine fish	Florida (United States) marine	Mass mortality associated with phytoplankton bloom ("red tide"); disease only reported once	None proven	Meyers et al. (1959)

None proven = No clinical trials have been published that determine if a particular treatment will control the disease. Most of the these bacteria have been tested for susceptibility to various antibiotics in vitro, but in vivo trials that substantiate the usefulness of those specific antibiotics have not been published.
RTFS = rainbow trout fry syndrome (see Austin and Austin 2007).
E = experimental infection.
All isolated using routine procedures (blood agar or simple nutrient agar at room temperature) unless noted otherwise.

CHAPTER 11

PROBLEMS 58 through 76

Diagnoses made by necropsy of the viscera and examination of wet mounts or histopathology of internal organs

58. Digenean trematode infection: general features
59. Digenean gill infection
60. Nematode infection
61. Cestode infection
62. Acanthocephalan infection
63. Myxozoan infection: general features
64. Proliferative gill disease
65. *Ceratomyxa shasta* infection
66. *Hoferellus carassii* infection
67. Proliferative kidney disease
68. Whirling disease
69. Miscellaneous important myxozoan infections
70. Microsporidian infection
71. Ichthyophonosis
72. True fungal infections
73. Diplomonad flagellate infection
74. Tissue coccidiosis
75. Miscellaneous endoparasitic infections
76. Idiopathic epidermal proliferation/neoplasia

PROBLEM 58

Digenean Trematode Infection (Digenean Fluke Infection, Metacercarial Infection, Black Spot, White Grub, Yellow Grub): General Features

Prevalence Index

Larvae: WF - 2, WM - 4, CF - 4, CM - 3
Adults: WF - 4, WM - 3, CF - 4, CM - 4

Method of Diagnosis

1. Wet mount of gut contents or affected tissue that has adults or larvae
2. Histological section of gut contents or affected tissue having adults or larvae

History

Wild-caught or pond-raised fish

Physical Examination

Larvae: White, yellow, or black, flat to raised, about 1–4 mm nodules in skin, muscle, or viscera
Adults: Worms, usually 1–5 mm, in gut lumen

Treatment: Larvae

1. Keep infected birds or mammals away from ponds
2. Disinfect and quarantine
3. Copper (as molluskicide)
4. Slaked lime (as molluskicide)
5. Bayluscide® (as molluskicide)
6. Praziquantel oral
7. Praziquantel injection
8. Praziquantel bath

COMMENTS

Epidemiology

Digeneans are common, usually asymptomatic infections in wild fish. About 1,700 species of adult digeneans infect fish. Metacercariae are even more common than adults. Digeneans are uncommon in cultured fish, except when the other hosts needed for the life cycle are present. Freshwater aquarium fish are commonly infected because they are often collected in the wild; such infections do not progress in aquaria but there is the potential for fish to become infected from parasites released by the snail intermediate host. Some aquarium snails used to control algae in aquaria can transmit cercariae that cause grub diseases in fish.

Life Cycle

Adult digeneans produce large, usually operculated eggs that pass out of the gut of the final host (fish, bird, or mammal); each egg hatches into a miracidium and infects a mollusk (usually a snail). In the snail, a cercaria develops, is released by the mollusk host, and penetrates a fish. After reaching the host's target tissue, the cercaria differentiates into a metacercaria, which usually produces a cyst. When the fish is eaten by the final host, the metacercaria differentiates into an adult. Variations to this life cycle are shown in Fig. II-58, A.

Pathogenesis

ADULTS

Adult digeneans mostly inhabit the gastrointestinal tract, rarely infecting the swim bladder, ovary, peritoneal cavity, urinary bladder, or circulatory system. All but the hemoparasites (see PROBLEM 44) are usually an incidental finding.

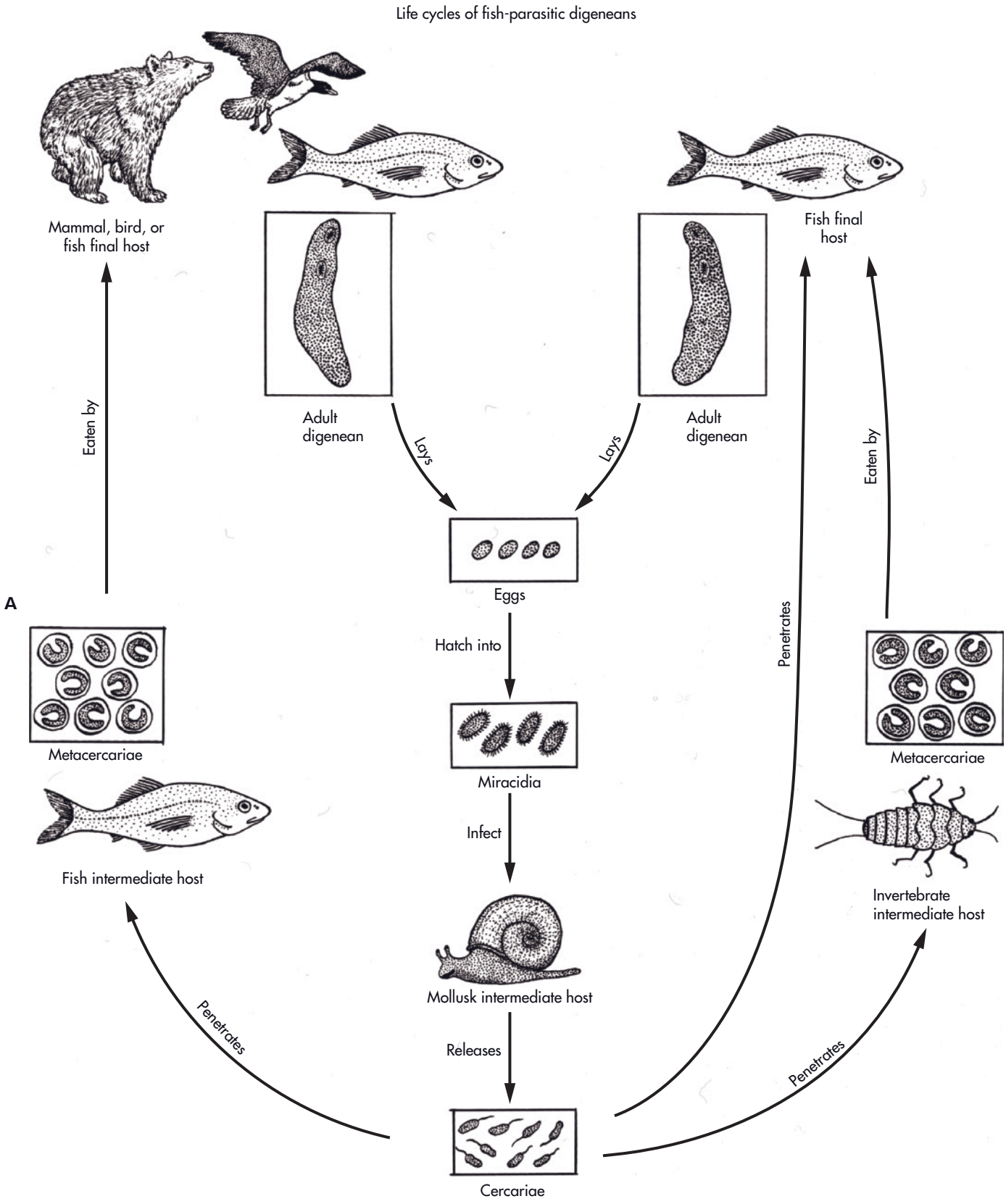


Fig. II-58. A. Life cycles of digeneans infecting fish.

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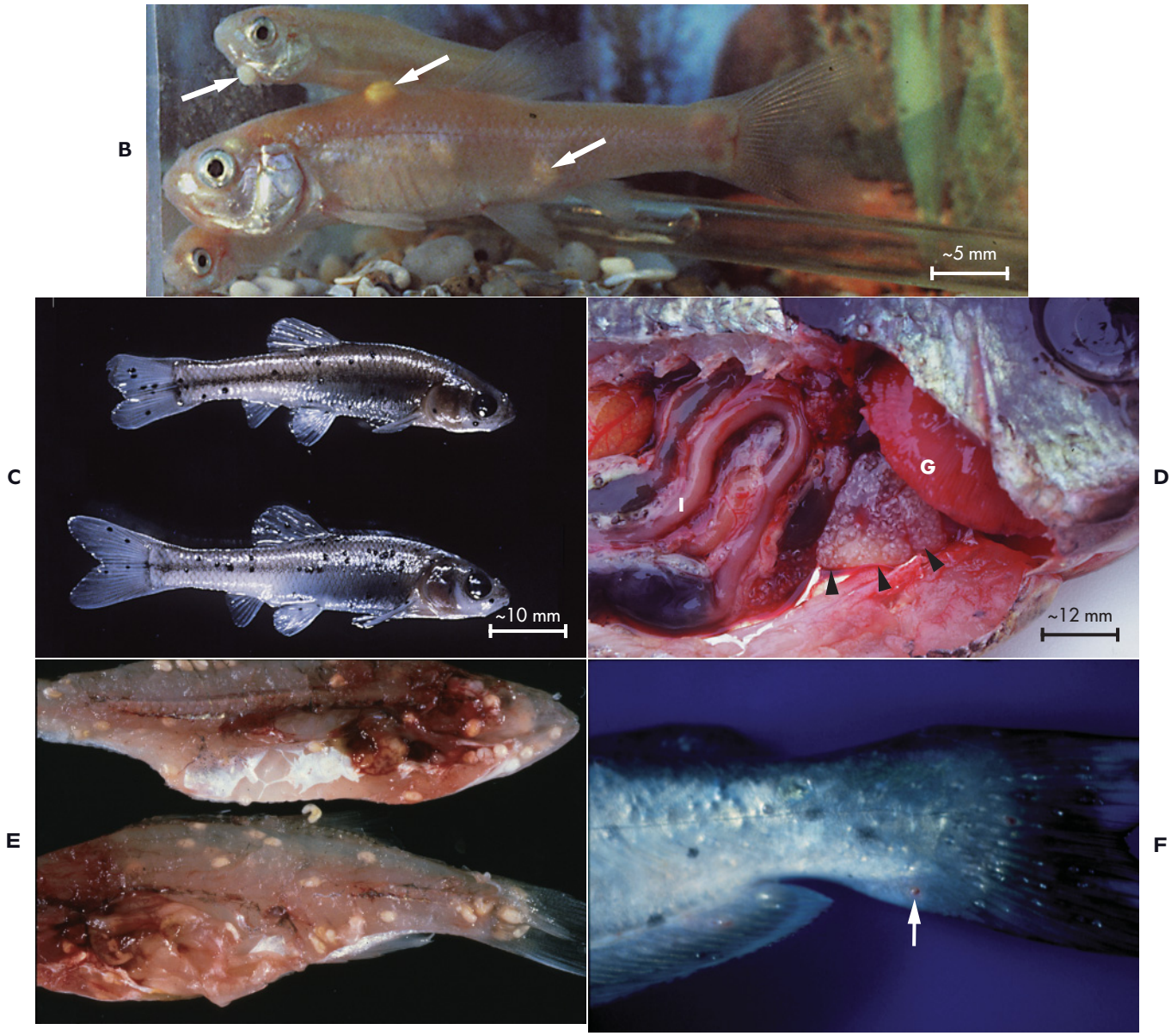

Fig. II-58.—cont'd. B. Minnows with yellow grub metacercariae (*arrows*) encysted in muscle and just below the skin. Note that some cysts protrude above the skin surface. C. Minnows with numerous black spots caused by the host's reaction to invading metacercariae (*Neascus*). D. Feral bluegill with body wall dissected away. Massive metacercarial infection of heart (*arrowheads*). Each white focus is a single metacercaria. The ventral portion of the heart is white because of the massive number of white worms. This fish was clinically normal when collected. *I* = intestine; *G* = gill. E. Heavy yellow grub infection in muscle (fillets) of hybrid striped bass. The appearance of the fish makes them unmarketable for human consumption. F. Pathology due to grub (*Bulbophorus*) infection in a channel catfish. Note the multiple, raised nodules; one nodule is surrounded by hemorrhage (*arrow*).

Continued.

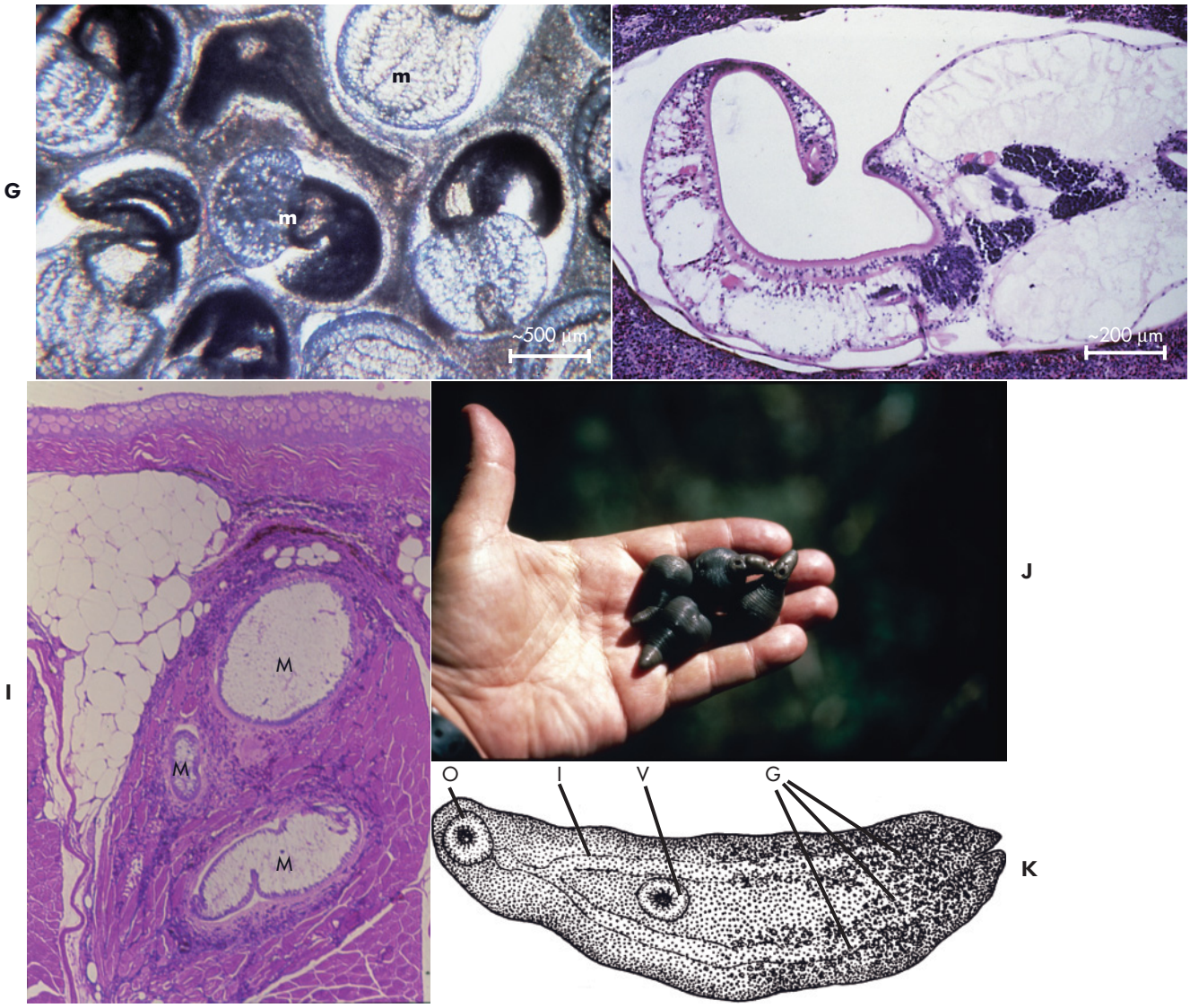

Fig. II-58.—cont'd. G. Wet mount of metacercaria (*m*) of *Posthodiplostomum minimum* in a tissue squash. H. Histological section of a metacercaria (*Posthodiplostomum minimum*). Hematoxylin and eosin. I. Histological section though the caudal peduncle of fish in *F.* Note the hemorrhage and inflammation surrounding the encysted, developing metacercariae (*M*). Hematoxylin and eosin. J. Large adult digeneans (*Hirudinella ventricosa*) from the gastrointestinal tract of a pelagic marine fish. K. Diagram of a typical digenean trematode. Diagnostic features: oral sucker (*O*), ventral sucker (*V*), blind gut (*I*), gonads (*G*). Metacercariae usually do not have mature gonads. (*B* photograph courtesy of T. Wenzel; *C* photograph by L. Khoo and E. Noga; *E* photograph by M. Levy; *F* and *I* photographs by L. Khoo; *G* photograph courtesy of G. Hoffman; *J* photograph by R Goldstein.)

CERCARIA

Host damage from most larval digeneans is most likely to occur during cercarial migration, causing hemorrhage, necrosis, and inflammation along the migration path (Sommerville 1981). Heavy, acute infections can be fatal, especially to small fish (Hoffman 1967, 1999; Sindermann 1990; Overstreet and Curran 2004).

METACERCARIA

Metacercariae can be found in virtually any tissue, depending on the infecting digenean species.

Metacercariae are usually innocuous and fish can carry amazingly high worm burdens without any apparent ill effects (Fig. II-58, B through D), probably because of the stable host-parasite relationship. Cyst formation is probably responsible for the characteristic lack of host response to metacercariae. While they are usually harmless, metacercariae are often disfiguring (Fig. II-58, B and C) and may render fish unpalatable or aesthetically unpleasing (Fig. II-58, E). Lesions may be white or yellow (white grub, yellow grub) because of the color of the worms, or they may be black (black spot disease) because of a hyperpigmentation host reaction (Fig. II-58, C). In aquarium fish this pigmentation may be mistaken by the owner as the host's normal color pattern.

Some metacercariae are dangerous; *Diplostomum* (eye fluke) metacercariae infect the lens and other ocular tissues of salmonids and other fish, causing blindness and a subsequent inability to find food. Heterophyid metacercariae cause severe gill damage, decreased respiratory tolerance, and mortality in pond-raised fish in the subtropics and tropics (see PROBLEM 59). In addition, some visceral metcercarial infections have caused significant morbidity and/or mortality in cultured fish, such as Japanese eels in Taiwan (Ooi et al. 1999), cichlids in Israel (Paperna 1996), and channel catfish in the United States (Terhune et al. 2002) (Fig. II-58, F). Sublethal effects such as decreased growth rate have also been documented in some cases (Lo et al. 1981). Even relatively mild infection with *Bolbophorus* results in greatly decreased yields in pond-cultured channel catfish due to decreased feed consumption (Hanson and Wise 2005).

ZOONOTIC POTENTIAL

Many heterophyids (e.g., *Heterophyes*, *Haplorchis*, *Metagonimus*) and opisthorchids (e.g., *Chlonorchis*, *Opisthorchis*) can infect humans that eat metacercaria-infected fish if the fish are not cooked well or are not heavily salted.

DIAGNOSIS

Worms are easily identified as digeneans by using wet mounts or tissue sections (Fig. II-58, G through I). Almost all metacercariae are encysted in tissues, while adults are usually free in the gut lumen (Fig. II-58, J). Digeneans typically have anterior (oral) and ventral suckers (Fig. II-58, K), although the suckers may be vestigial or completely absent in adults of some species (e.g., *Sanguinicola*). Worms are typically 1–5 mm, although adult parasites of some large, oceanic fish may be several centimeters or more (Fig. II-58, J). For more details on identifying a parasite as a digenean in histological sections, see Gardiner and Poynton (1999).

Digeneans are distinguished from monogeneans (see PROBLEM 17) by the absence of chitinous hooks or polyopisthocotylean-type suckers (see Fig. II-17, A) and from cestodes (see PROBLEM 61) by the presence of a ventral sucker and a gut, as well as by the absence of body segmentation.

Metacercariae typically have most characteristics of adult digeneans but usually lack mature reproductive organs. Since the size and shape of the genital organs are used for species identification, it is usually impossible to key metacercariae to species, unless a specific molecular probe is available (e.g., Dzikowski et al. 2004). None are commercially available. However, all metacercariae are managed similarly.

Treatment

MEDICAL/SURGICAL

Adult digeneans are not usually a problem in cultured fish, although Di N butyl tin has been tested against infections in trout (Mitchum and Moore 1966). Praziquantel is very effective against at least some metacercariae, with elimination of 100% of *Diplostomum spathaceum* in grass and bighead carp via either bath or oral treatment (Székely and Molnár 1991). However, bath praziquantel was only partially successful in eliminating yellow grub metacercariae in channel catfish. Reduction in parasite burden was not evident until 5 months after treatment (Lorio 1989); effectiveness of a treatment probably depends upon susceptibility of a parasite species, as well as drug dose. Metacercariae close to the skin can be surgically excised by cutting down to the cyst with a sharp scalpel and then gently removing the worm with forceps. The worm should be completely removed to avoid excessive postoperative inflammation. Interestingly, when fish are heavily infected, parasites near the body surface may be expelled, resulting in a partial "cure" (Hoffman 1958).

ENVIRONMENTAL

To acquire metacercariae, fish must be exposed to the intermediate host infected with cercariae. This typically occurs in ponds or natural waters where there is exposure to the appropriate intermediate and final hosts (usually a specific snail- and fish-eating bird, respectively). Eradicating snails with molluskicides can be difficult because snails are often resistant to treatment.

Snails in ponds can be treated with copper sulfate or slaked lime. It is preferable to use this as a pondside treatment, but heavy infestations may require direct addition to the pond. These treatments do not provide complete snail eradication, especially the shoreline treatments. Thus, ponds must be treated multiple times, typically

two or three times during the growing season (Engle and Dorman 2006). Treatments are best done at night, when snails are more active (Francis-Floyd 1993).

Bayluscide® appears to be a more effective chemical, but is not approved for use in food fish in the United States. Also, fish must be removed before treating the pond (Francis-Floyd et al. 1997). Treatment at night does not seem to be as important when using Bayluscide® (Francis-Floyd et al. 1997). Snail-eating fish (e.g., black carp in freshwater; gilthead sea bream in seawater) have been used as a biological control (Paperna and Dzikokski 2006). Black carp are the most cost-effective long-term method for controlling snails in freshwater ponds (Engle and Dorman 2006). However, ponds might need to be chemically treated to initially get the infestation under control. Sterile (triploid) black carp must be used in areas where this fish is not native; in some countries (e.g., United States), a permit must be obtained before using this fish. Aquarium fish that eat snails include clown loaches and freshwater puffers (but the latter are very aggressive).

Snails usually proliferate in oligotrophic or mesotrophic ponds containing solid substrates, such as earth or gravel, and having low fish density (i.e., ponds used for broodstock, spawning, or nursery). They also proliferate in extensive systems, such as impounded lakes. Snails are not a problem in intensive culture systems that have a muddy bottom and high organic loads (Paperna 1991). Avoiding exposure of culture waters to the final host will prevent infections but may be equally difficult. Small snails are often introduced into aquaria or other bodies of water while attached to plants.

PROBLEM 59
Digenean Gill Infection (*Centrocestus* Infection)

Prevalence Index

Larvae: WF - 2

Method of Diagnosis

1. Wet mount of gill tissue that has larvae
2. Histological section of gill tissue having larvae

History

Wild-caught or pond-raised fish; dyspnea

Physical Examination

Dyspnea; flared opercula; deformed gill lamella

Treatment: Larvae

1. Keep infected birds away from ponds
2. Disinfect and quarantine
3. Bayluscide® (as molluskicide)
4. Copper (as molluskicide)
5. Slaked lime (as molluskicide)

COMMENTS

Epidemiology

Unlike the great majority of digeneans, the gill digenean (a heterophyid tentatively identified as *Centrocestus formosanus*) has caused significant morbidity and mortality in many wild and cultured fish (Scholtz and Salgado 2000; Mitchell et al. 2005). In the United States, the gill digenean was first observed in the late 1950s in exotic fish in Hawaii. In the 1980s, heavy losses were observed in other exotic fish (cichlids, tetras and tropical cyprinids) on tropical fish farms in Florida. By the 1990s, the parasite was seen in several rivers (San Antonio, San Marcus, and Comal) in Texas, causing serious gill disease in both exotic and native fish (including some endangered species). In 2003, it was discovered in warm water springs in Utah. In Mexico, metacercariae infect the gills of many fish species and occasionally the intestinal wall and muscle of frogs (*Rana* spp.) (Salgado-Maldonado et al. 1995).

The intermediate host for the gill digenean is the aquatic snail *Melanoides tuberculatus* (known as the red-rim melania, Malaysian burrowing snail, Malaysian trumpet snail or cornucopia snail) (Mitchell et al. 2005). Originating in Asia, where it causes a similar disease in cultured eels and grass carp (Yanohara and Kagei 1983; Zeng and Liao 2000), it has been spread to not only the United States but many other subtropical and tropical regions, including Israel, where the gill digenean has caused mortalities in cultured fish (Paperna 1991; Dzikowski et al. 2004). A *Centrocestus* species has also caused mortality in common carp fry in India (Mohan et al. 1999).

In the United States, fish in the centrarchid, cichlid, cyprinid, cyprinodontid, gobid, ictalurid, mugilid, percid, percichthyid and poeciliid families can be hosts, including several endangered species (Mitchell et al. 2005). The adult worms reside in the gastrointestinal tract of piscivorous birds and mammals; in the United States, the green heron (*Butorides virescens*) and the great egret (*Ardea alba*) have been confirmed as final hosts; both are federally protected species.

Pathogenesis

The migration of the gill digenean into the gill tissue and formation of a metacercaria induce a reactive chondroplasia (increase in cartilage cells) in the gill, resulting in the formation of a thick, cartilagenous capsule around each parasite (Fig. II-59, A through D). Inflammation may also be present (Blazer and Gratzek 1983). This can severely damage the structure and function of the gill, resulting in highly impaired respiratory capacity (Fig. II-59, A through D). Grossly, the opercula can be flared out and the gill damage may be grossly visible. The severity of the host response to the gill digenean is probably due to all these fish being aberrant hosts. This is also suggested by presence of dead metacercariae in the cysts of some hosts (Mitchell et al. 2005). The natural fish host(s) of this parasite has not been identified.

Zoonotic Potential

The red-rim melania is a vector for the human liver fluke (*Opisthorchis sinensis*) and the oriental lung fluke

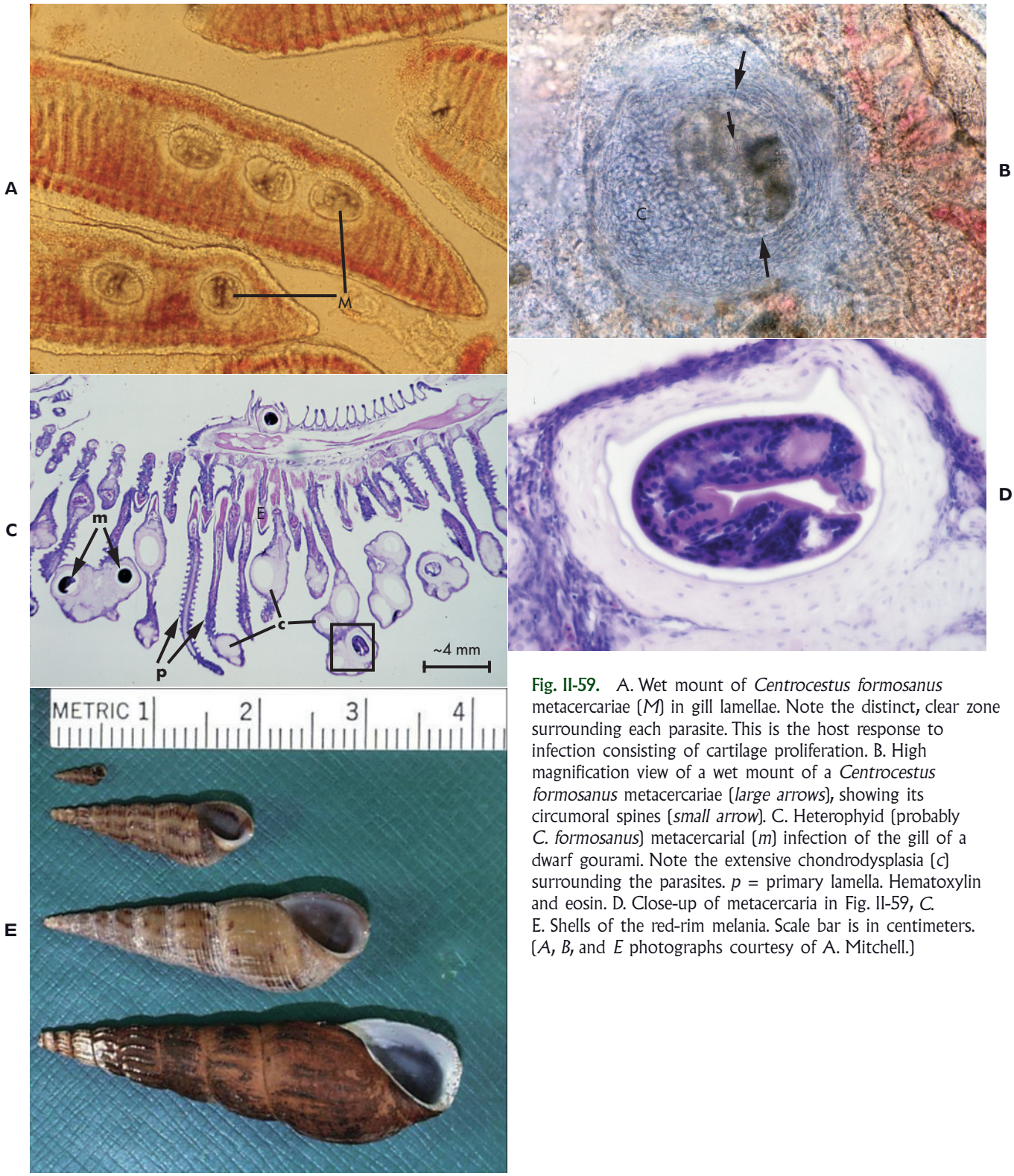

Fig. II-59. A. Wet mount of *Centrocestus formosanus* metacercariae (*M*) in gill lamellae. Note the distinct, clear zone surrounding each parasite. This is the host response to infection consisting of cartilage proliferation. B. High magnification view of a wet mount of a *Centrocestus formosanus* metacercariae (*large arrows*), showing its circumoral spines (*small arrow*). C. Heterophyid (probably *C. formosanus*) metacercarial (*m*) infection of the gill of a dwarf gourami. Note the extensive chondrodysplasia (*c*) surrounding the parasites. *p* = primary lamella. Hematoxylin and eosin. D. Close-up of metacercaria in Fig. II-59, *C*. E. Shells of the red-rim melania. Scale bar is in centimeters. (*A*, *B*, and *E* photographs courtesy of A. Mitchell.)

(*Paragonimus westermani*), but snails in the United States have not yet been shown to have these parasites.

Diagnosis

Metacercariae are easily identified by using wet mounts or tissue sections (Fig. II-59, A through D). The presence within the gill cartilage is pathognomonic for the gill digenean. However, there is molecular genetic evidence that two different species of *Centrocestus* cause this pathology in the United States and Israel (Dzikowski et al. 2004). Also, similar but not identical metcercariae have been observed in cage-cultured steelhead trout in the Willamette River, Oregon (Olson and Pierce 1997). Which taxon infects eels or Mexican fish is unknown. Nucleic acid probes have been developed to identify some *Centrocestus* species, but are not yet commercially available.

Treatment

As with other digeneans, eradication is focused on the snail intermediate host (Fig. II-59, E) (also see PROBLEM 58). However, this has proven extremely difficult, especially given the need to avoid damage to protected and endangered species involved in the gill digenean life cycle. The red-rim melania has an operculum, allowing it to seal tightly shut in its shell, protecting it against noxious agents. This makes it highly resistant to dessication, molluskicides, and disinfectants. While it is a freshwater snail, it can survive in 30 ppt salinity. As with other nuisance snails, Bayluscide® is currently the most effective chemical for pond treatment.

The major effort at this point is attempting to prevent further geographic spread of the parasite. The snail is restricted to waters that remain warm (probably >17°C [>63 °F]) year-round but at least 15 states in the United States have established populations of the snail and not all might currently harbor the parasite. The snail is commonly sold in aquarium stores and aquarium owners should be advised not to release it into natural waters. Persons frequenting waters infested with red-rim melania should be advised to reduce spread of the snail by not spreading aquatic plant material and by immersing all fomites (nets, buckets, boots, etc.) in hot (50°C [122°F]) water for at least 5 minutes to kill the snail. This is the temperature of most residential and commercial hot water systems (Mitchell and Brandt 2005). A low dose of quaternary ammonium (10 ppm of Roccal D Plus® for 24 hours) might also be lethal (Mitchell et al. 2005).

PROBLEM 60

Nematode Infection (Roundworm Infection)

Prevalence Index

Larvae: WF - 2, WM - 2, CF - 4, CM - 4
Adults: WF - 3, WM - 4, CF - 4, CM - 4

Method of Diagnosis

1. Wet mount of gut contents or viscera with adults, larvae, or eggs
2. Histology of gut contents or viscera with adults, larvae, or eggs
3. Fecal sample with eggs

History

Gradual weight loss; lethargy; pond-raised or wild fish

Physical Examination

Emaciation; worms protruding from anus

Treatment: Larvae

No proven treatment for encapsulated forms

Treatment: Adults

1. Fenbendazole oral
2. Levamisole oral
3. Piperazine oral

COMMENTS

Epidemiology

Fish are either intermediate or final hosts for nematodes. About 650 species of nematodes parasitize fish as adults and many others use fish as intermediate hosts (Williams and Jones 1994). While nematodes are common in wild fish, neither adult nor larval nematodes are usually a problem in most cultured fish because of the absence of other hosts in the life cycle (Fig. II-60, A). However, pond-raised fish or those fed live and wild-caught arthropods can become infected. Also, some nematodes infecting aquarium fish might have a direct life cycle (see Fig. II-60, A) (Moravec 1994; Molnár et al. 2006).

Freshwater fish are often infected by members of the Camallanoidea and Ascaroidea. Marine fish are usually infected by members of the Ascaridoidoiea (*Contracecum*, *Pseudoterranova*, *Anisakis*), Camallanoidea (*Camallanus*, *Culcullanus*), Dracunculoidea (*Philonema*, *Philometra*), and Spiruroidea (*Metabronema*, *Ascarophis*). Most of the camallanoids, dracunculoids, and spiruroids have two host life cycles where fish are the final host. *Spirocamallanus* can be pathogenic to tropical marine fish (Rychlinski and Deardorff 1982).

Life Cycle

Sexes are separate in nematodes. Most fish-parasitic nematodes are oviparous; eggs usually hatch in the water, releasing a free-swimming larva. Some (*Camallanus*, *Philometra*) are viviparous, with females releasing live young. In either case, the larva is ingested by an intermediate host, often a crustacean (sometimes an annelid, coelenterate, mollusk, or fish), and then by a fish, where it either matures to an adult or encysts. Larvae encysted in fish are ingested by a bird, mammal, or another fish as final host (Rohde 1984; Hoffman 1999). Paratenic hosts are very common in nematode life cycles.

Some nematodes appear to have a direct life cycle, although this has not yet been experimentally substantiated (Molnár et al. 2006): *Capillaria pterophylli* infects

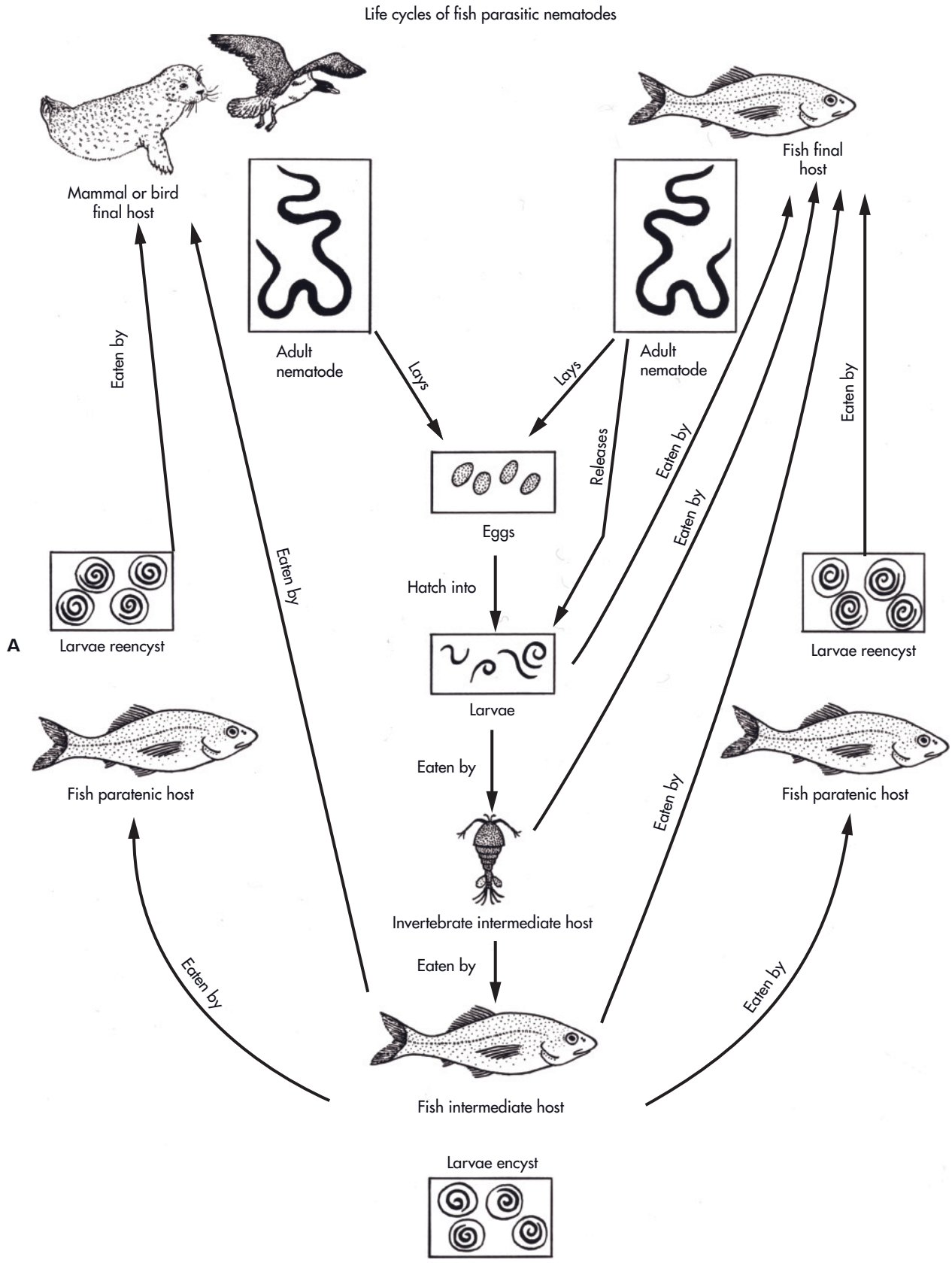

Fig. II-60. A. Life cycles of nematodes infecting fish.

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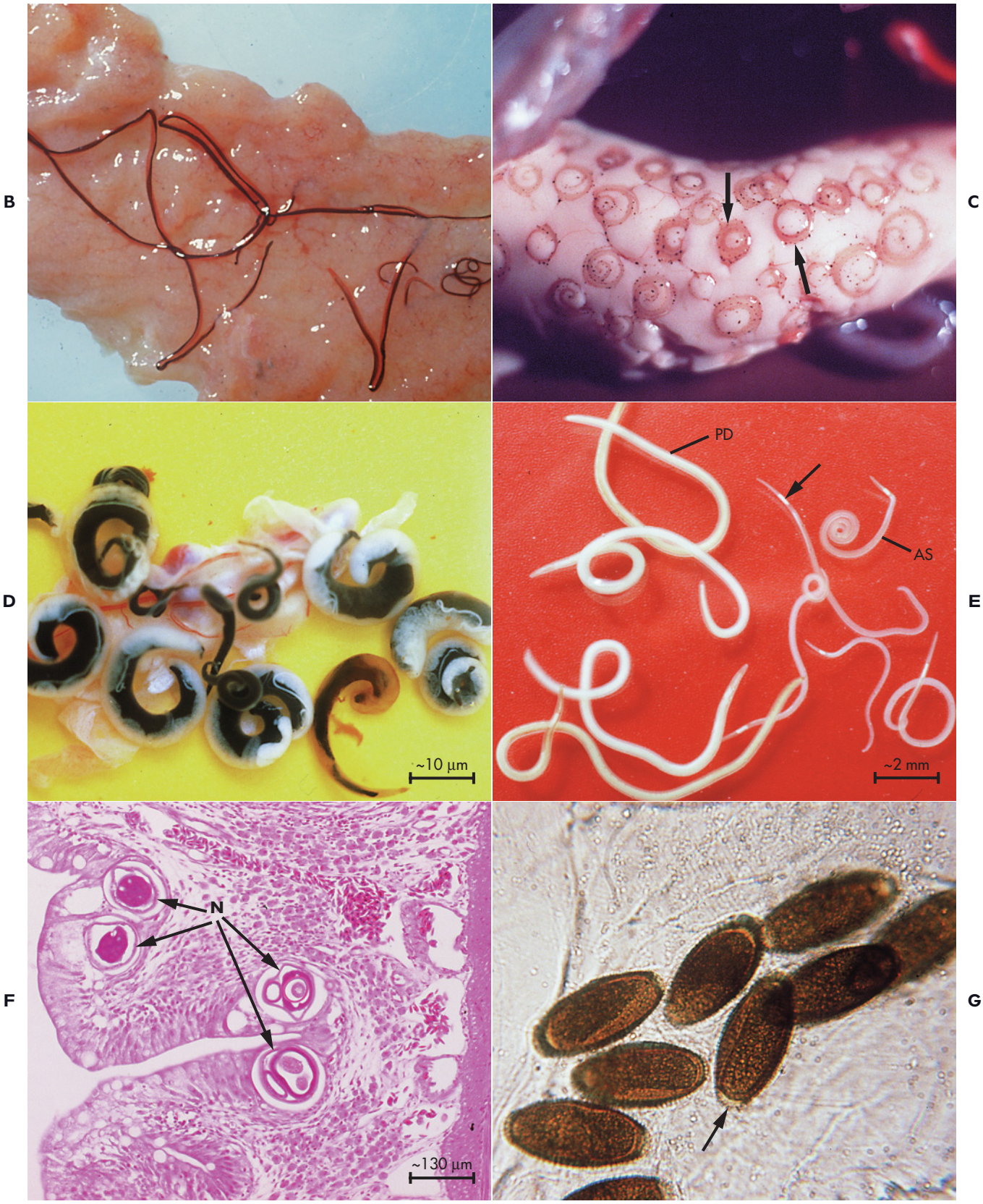

Fig. II-60.—cont'd. B. Adult red worm (*Philometra* sp.) in the ovary of a croaker. C. Liver of Atlantic cod with encysted, anisakid, nematode larvae. Each larva (*arrows*) is curled and in a capsule. D. Swim bladder worms (*Anguillicola*). Dark color is due to feeding on blood. E. Nematodes responsible for anisakiasis: *Pseudoterranova decipiens* (*PD*) and *Anisakis simplex* (*AS*). The milky white ventricle (*arrow*) is characteristic of *A. simplex*. F. Freshwater angelfish intestine with nematodes (*N*) invading the mucosa. Diagnostic features: cylindrical shape; pseudocoelom, giving appearance of a "tube-within-a-tube." Hematoxylin and eosin. G. Wet mount of intestinal squash from a fish with *Capillaria* sp. eggs. Note the plug on each end (*arrow*). (*B*, *C*, *D*, and *E* photographs courtesy of H. Möller.)

freshwater angelfish and other cichlids (Moravec 1983); at 20–23°C (68–73°F), eggs embryonate in 3 weeks, and the prepatent period is 3 months. *Capillostrongyloides ancistri* infects ancistrid (bushymouth) catfish (Moravec et al. 1987) and probably also has a direct life cycle. *Pseudocapillaria tomentosa* has a broad host range; it causes disease in tropical cyprinids (including zebrafish in research facilities) and also can infect anguillid eels, cod, salmonids and silurid catfish (Kent et al. 2002). Transmission might be both direct and via ingestion of an oligochaete worm (*Tubifex tubifex*), which acts as a paratenic host (Lomakin and Trofimenko 1982). *Tubifex* worms can also harbor *Eustrongyloides* (Yanong 2001). Other capillarids infect gouramies (Moravec et al. 1987). *Camallanus*, a bright-red livebearer, affects poeciliids. They typically present as red worms protruding from the anus. Fish lice (PROBLEM 15) can transmit some nematodes to fish (Molnár et al. 2006).

Pathogenesis

Adults are almost always found in the digestive tract, where some (e.g., *Capillaria*) can cause chronic wasting if present in high numbers. Some can be highly invasive, penetrating the gut wall and inducing significant inflammation (Fig. II-60, F) (Kent et al. [2002]). *Pseudocapillaria tomentosa* has also been associated with increased prevalence of neoplasia in zebrafish (Kent et al. 2002). Some adult nematodes inhabit the peritoneal cavity, gonads (Fig. II-60, B), or swim bladder, but none are highly serious problems in cultured fish except the swim bladder nematode *Anguillicola* (Fig. II-60, D).

Anguillicola crassus (from Japan) has caused serious problems in freshwater cultured and wild European eels, apparently after introduction with exotic eels (Paperna 1991; Molnár et al. 1993). First discovered in the U.S. in an American eel aquaculture operation in Texas in 1995, it was first observed in the wild in South Carolina, and has subsequently been identified in New York, New Jersey and Maryland (Morrison 2001). In Chesapeake Bay, prevalence and infection severity can sometimes be very high (up to 82% of sampled eel populations with as many as 52 worms per eel) (Barse 1999).

In its native Japan, *A, crassus* infections occur during grow-out in earthen ponds but rarely occur in intensive systems, since the copepod intermediate host cannot survive (Hirose et al. 1976). *Anguillicola crassus* is much more pathogenic to European and American eel than to Japanese eel. Adults inhabit the lumen of the swim bladder, while third and fourth stage juveniles are found in the swim bladder wall. Lesions are most evident in postjuvenile eels. The swim bladder has a foamy fluid that later becomes brown-red. The swim bladder wall is thickened and opaque. There is up to 20% mortality from secondary bacterial infections after swim bladder rupture. Adult worms are grossly visible (~20–70 mm); juveniles are ~600–800 µm and can be seen with a magnifying glass in the swim bladder wall, often near capillaries.

Wild or pond-raised fish are common hosts for larval nematodes, which rarely cause any problem, even in high numbers. However, migrating larvae of *Anisakis*, *Contracaecum*, *Eustrongyloides*, and *Philonema* may cause tissue damage. Larval worms may be present in virtually any organ, most commonly the skin, muscle, viscera, or peritoneal cavity (Fig. II-60, C).

Zoonotic Potential

Some larval nematodes are serious public health problems and can cause larva migrans when ingested by humans (e.g, *Anisakis*, *Pseudoterranova*) (Fig. II-60, E). Most zoonotic problems are caused by infections of feral, cold water marine fish.

Diagnosis

Fecal exam can be used to identify eggs in the digestive tract (Fig. II-60, G). Worms are easily identified as a nematode (both adults and larvae) by using wet mounts or tissue sections. The main criteria used to identify species are size, fine structure of the head and tail, position of the excretory pore, and structure of the transitional area between the esophagus and intestine. Most of these criteria are also valid for older larval stages. For more details on identifying a parasite as a nematode in histological sections, see Gardiner and Poynton (1999). Species confirmation is best done by sending samples to a reference laboratory.

Free-living nematodes may occasionally colonize chronic skin lesions or recently dead fish. Parasites are distinguished from free-living nematodes by the lack of long sensory setae on the head (Moller and Anders 1986).

Treatment

Anthelminthics can control adult nematodes. Fenbendazole, levamisole, and piperazine have been used with some success. A commercial water-borne preparation containing trichlorphon and mebendazole (Fluke-Tabs™, Aquarium Products) appear to cure fish of *Pseudocapillaria tomentosa* infection (Pack et al. 1995). Ivermectin has also been used for treatment (Heckmann 1985) but has a low therapeutic index in fish and is thus dangerous to use.

Encysted nematodes are difficult to treat. For example, levamisole kills adults of the eel swim bladder worm, *Anguillicola*, but not the L3 larvae, which are in the swim bladder wall and are not hematophagous. The glass eel stage cannot eat the L3 because their digestive tract is still closed. Thus, the best prevention for anguillcolosis is to catch the glass eels before they begin to eat (Blanc et al. 1992).

To prevent infections having an intermediate host, avoid feeding organisms that may harbor larvae. Live copepods or fish are the most common sources. Live oligochaete worms might be a source in some aquarium

fish. Fish can even become infected when fed frozen fish (Gaines and Rogers 1971). Proper sanitation should help to mitigate infections with a direct life cycle.

PROBLEM 61
Cestode Infection (Tapeworm Infection)

Prevalence Index
Larvae: WF - 4, WM - 4, CF - 4, CM - 4
Adults: WF - 3, WM - 4, CF - 4, CM - 4
Method of Diagnosis
Wet mount of affected tissue having cestode larvae or adults
History
Wild-caught or pond-raised fish; worms in tissue or body cavity; feeding live copepods or other intermediate hosts
Physical Examination
Adult worms in intestine or larvae in peritoneal cavity, liver, or muscle; emaciation with heavy worm burdens; usually asymptomatic
Treatment: Larvae
No proven treatment
Treatment: Adults
1. Disinfect pond and exclude intermediate host from water supply
2. Praziquantel oral
3. Praziquantel bath

COMMENTS
Epidemiology/Pathogenesis
With a complex life cycle that requires one or two intermediate hosts, cestodes are relatively uncommon in cultured fish. Fish can be an intermediate host, definitive host, or both (Fig. II-61, F). While a few cestodes that infect elasmobranchs and sturgeons are in the Cestodaria, the great majority of fish-infecting cestodes are in the Eucestoda, which are characterized by having an attachment organ (scolex), as well as internal and external segmentation (proglottids) (Fig. II-61, G). Proglottids increase in size and maturity toward the end of the parasite's body. The scolex may have hooks, suckers, grooves, and/or spines (Fig. II-61, G). The less common Pseudophyllidea usually infect elasmobranchs as adults, but the larvae of one species can damage salmonids (Kent 1992) and another is a serious exotic pathogen (Luo et al. 2003).
Pathogenesis
A few freshwater cestodes cause serious disease in wild fish. Larval cestodes (plerocercoids), also known as metacestodes (Freeman 1973), can be some of the most damaging parasites to freshwater fish and decrease carcass value if present in muscle (e.g., muscle infection of clupeaformis whitefish with *Triaenophorus crassus* [Dick et al. 2006]). Migrating plerocercoids may cause adhesions and severely damage viscera or muscle because of pressure necrosis. *Ligula* (Fig. II-61, A, B) causes peritoneal adhesions and pressure atrophy of the liver, gonads, and body wall musculature of cyprinids worldwide. Many piscivorous birds or mammals can act as a final host for *Ligula*. *Gilquinia squali* metacestodes infect the eyes (vitreous humor) of net-pen cultured chinook salmon, causing blindness and idiopathic mortality (Kent et al. 1991). Impaired reproduction is also a common sequela. *Proteocephalus ambloplitis* infection of the ovary causes reduced fecundity in feral smallmouth bass (McCormick and Stokes 1982).

Adult cestodes infect the intestine or pyloric ceca and almost all species are asymptomatic. However, adult *Eubothrium* species have caused poor growth and chronic mortality in marine-cultured Atlantic salmon (Bristow and Berland 1991) and juvenile sockeye salmon (Boyce and Clark 1983).

One of the most serious adult cestodes that affect fish is the Asian tapeworm, *Bothriocephalus acheilognathi* (formerly known as *B. gowkongenesis*), having an unusually wide and currently expanding host range (including minnows, golden shiner, various carp species, channel catfish, and possibly aquarium fish, such as discus and other cichlids) (Dick et al. 2006; Luo et al. 2003). Originating in Siberia, it has been introduced into many parts of the world with grass carp and other cyprinids. In the United States, it has caused serious problems with bait minnow producers. It can cause up to 90% mortality in grass carp and juvenile common carp. Susceptible hosts also include fish in the centrarchid, atherinid, and goodeid families.

The Asian tapeworm is large, with two long, deep grooves (bothria) (Fig. II-61, D). Worms accumulate in the anterior intestine, which may become obstructed (Fig. II-61, E) or perforate, resulting in high mortalities (Hoffman 1980). The entire life cycle requires about 1 year in warm water environments and 2 or more years in cold waters. Development ceases at 12°C (54°F). Worms mature in about 21 days at 28°C (82°F) and in 2 months at 15°C (59°F) (Paperna 1991). The plerocercoids are transmitted by copepods. Several copepod genera can be intermediate hosts and the distribution of infections depends largely on the abundance of the intermediate host (Paperna 1991).
Diagnosis
Worms are easily identified as cestodes from wet mounts or histology. For more details on identifying a parasite as a cestode in histological sections, see Gardiner and Poynton (1999). Large worms can be identified grossly. Larval cestodes may not have segmentation, but a recognizable (Fig. II-61, C), although often rudimentary, scolex is usually present. Diagnosis of

intestinal cestode infections can presumably also be made from wet mounts of fecal contents having proglottids or eggs.

Identification of adult cestodes to species uses features of the scolex and organs of the mature proglottid; immature cestodes might only be classifiable to order. Schmidt (1986) provides identification of specific groups. Specimens are best sent to a reference laboratory if determining species is desired. Gene tests have been used to identify a number of species (Dick et al. 2006), but are not commercially available.

Treatment

Praziquantel is effective in treating adult cestode infections. There are no published studies of metacestode treatment. Aquarium fish should not be fed live foods that might transmit larval cestodes, especially if *Bothriocephalus acheilognathi* is prevalent. Ponds can be disinfected to eradicate the Asian tapeworm's intermediate host. In farms using surface water, filtering the water to excluse the intermediate host can prevent infections such as *Triaenophorus* larvae or *Proteocephalus* adults in the fish (Dick et al. 2006).

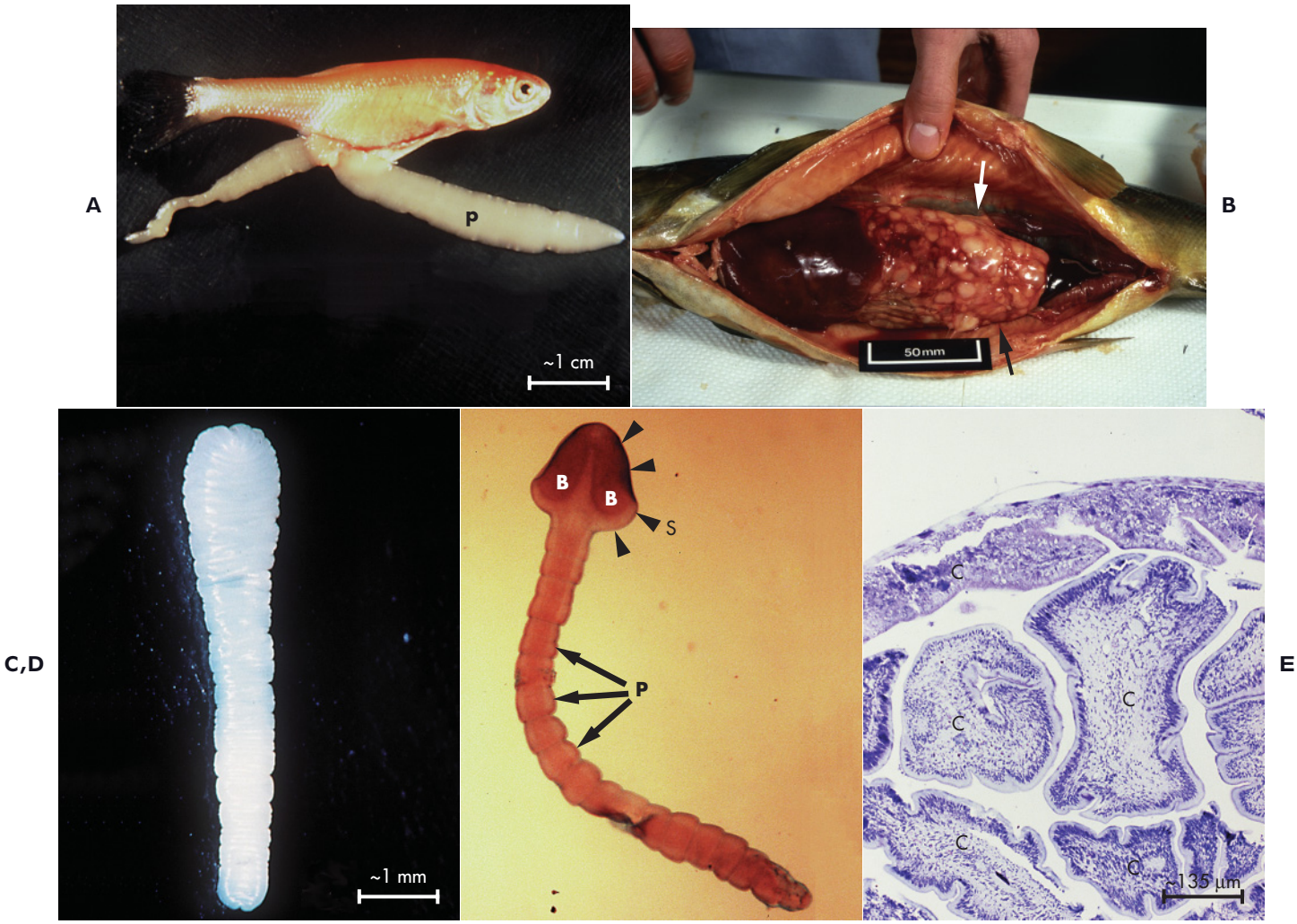

Fig. II-61. A. *Ligula intestinalis* in a cyprinid. The body wall has been cut, revealing the peritoneal cavity filled with a single plerocercoid (*P*). Part of the worm remains in the peritoneal cavity. B. Rainbow trout with a massive infection of *Diphyllobothrium dentriticum* plerocercoids (*arrows*). The larvae are encapsulated mainly around the stomach and pyloric caeca. C. Plerocercoid of *Diphyllobothrium latum*. D. *Bothriocephalus acheilognathi*. The pit viper–shaped scolex (s, arrowheads) with bothria (*B*) (grooves) is diagnostic. *P* = proglottids. E. Histological section through a cestode (*C*), *Bothriocephalus acheilognathi*, filling the lumen of the intestine (*I*) of a minnow. Giemsa.

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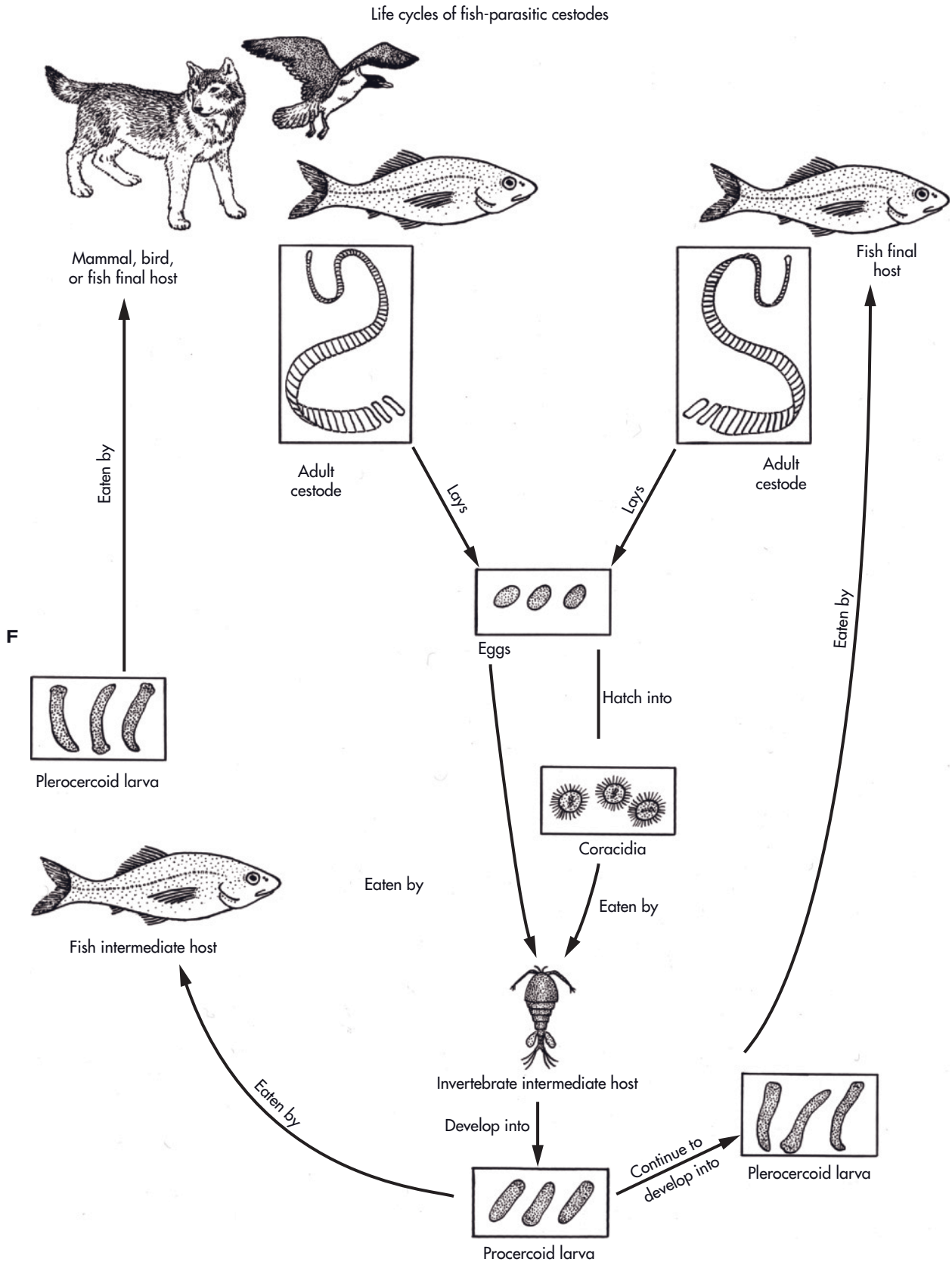

Fig. II-6l.—cont'd. F. Life cycles of cestodes infecting fish.

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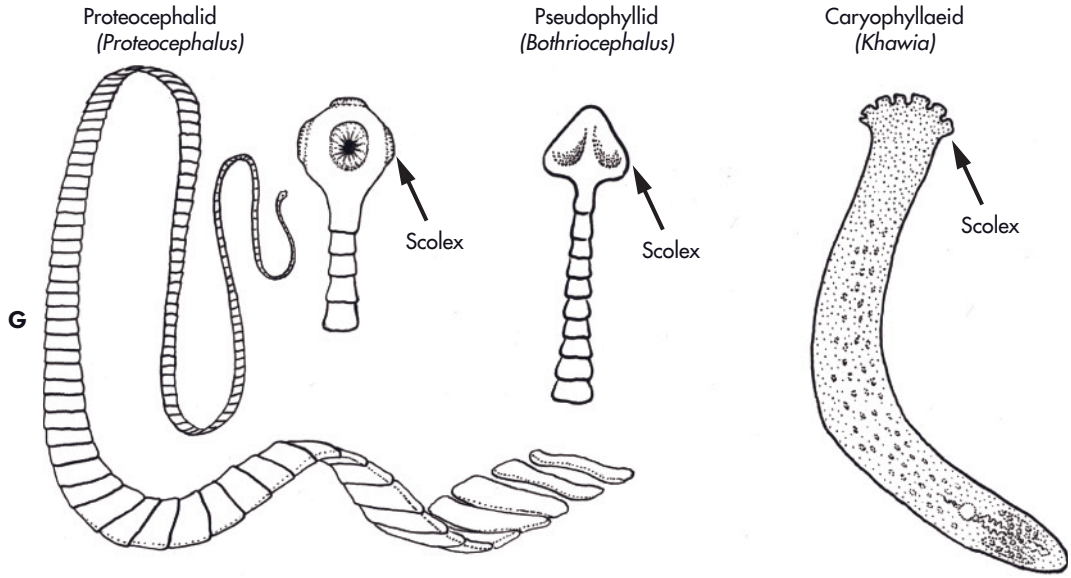

Fig. II-61.—cont'd. G. Diagram of typical cestodes (Eucestoda). (*A* photograph courtesy of A. Mitchell; *B* photograph courtesy of A. Pike; *C* photograph courtesy of H. Möller; *D* photograph courtesy of G. Hoffman.)

PROBLEM 62

Acanthocephalan Infection (Thorny-Headed Worm Infection)

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

Wet mount of affected tissue having acanthocephalans

History

Wild-caught fish

Physical Examination

Adult worms in intestine; larvae in mesentery or liver

Treatment

None reported

COMMENTS

Epidemiology/Pathogenesis

Acanthocephalan infections (~400 species affecting fish) are rare in cultured fish. With a complex life cycle that requires one or two intermediate hosts, fish may be intermediate or final hosts, depending on the acanthocephalan species. The egg that contains the larva (acanthor) is passed into the water, where it is ingested by an intermediate host (usually an amphipod or other crustacean). The acanthor enters the hemocoel of the intermediate host, forming a cystacanth. When the intermediate host is ingested by a fish, the cystacanth either matures into an adult or encysts in the fish's tissue. The fish may thus act as an intermediate or paratenic host, which is eventually ingested by the final host (fish, bird, or mammal).

Larval infections are usually located in the mesentery or liver, while adults always infect the intestine. Very little disease has been associated with acanthocephalan infections in fish, although heavy worm burdens would presumably have the potential to cause serious intestinal damage (Fig. II-62, B).

Diagnosis

Diagnosis of intestinal acanthocephalan infection can be made from wet mounts of intestinal tract. The most characterisitic feature is the spined (hooked) proboscis, which can also be seen in histological sections. Worms have a cuticle and have no digestive tract. For more details on identifying a parasite as an acanthocephalan in histological sections, see Gardiner and Poynton (1999). Species are identified mainly by the arrangement of hooks on the proboscis. Yamaguti (1963) and Petrochenko (1970) provide identification of specific groups. Specimens are best sent to a reference laboratory if determining the species is desired.

PROBLEM 63

Myxozoan Infection: General Features

Prevalence Index

WF - 2, WM - 3, CF - 2, CM - 2

Method of Diagnosis

1. Wet mount of affected tissue having spores
2. Stained smear of affected tissue having spores
3. Histology of affected tissue having spores

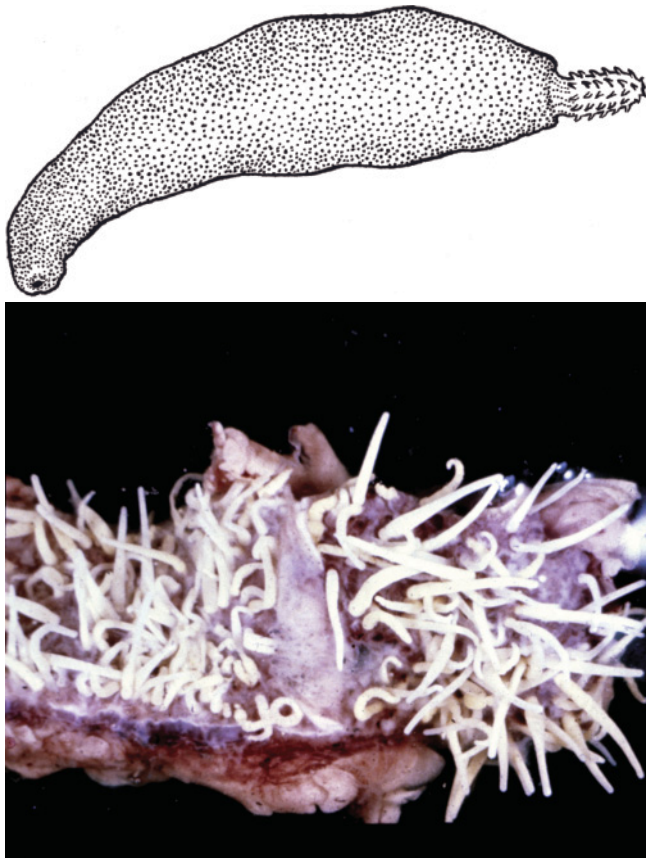

Fig. II-62. A. Diagram of typical adult acanthocephalan. B. Acanthocephalans embedded in the intestinal mucosa of a fish. [*B* photograph courtesy of Armed Forces Institute of Pathology.]

History

Usually wild-caught or pond-raised fish; exposure to natural waters; various-sized nodules that enlarge slowly, if at all

Physical Examination

Often white or yellowish, variously sized nodules (pseudocysts) that have firm-to-soft material; in species not forming pseudocysts, clinical signs depend on the organ system(s) affected

Treatment

1. Disinfect and quarantine
2. Fumagillin oral

COMMENTS

General Features

The Phylum Myxozoa is restricted to invertebrates (mostly annelid worms) and poikilothermic vertebrates; the vast majority infect fish. While the Myxozoa were initially considered to be protozoa, recent evidence shows them to be metazoans (Zravý and Hypša 2003). They are considered by most to be part of the Bilateria (Canning and Okamura 2004), but their exact taxonomic position is still unresolved. Virtually all Myxozoa that infect fish are members of the class Myxosporea, with two (one in carp and one causing PKD) being members of the class Malacosporea. Myxozoans are obligate parasites of tissues (histozoic forms that reside in intercellular spaces or blood vessels or reside intracellularly) or organ cavities (coelozoic forms that live primarily in the gall bladder, swim bladder, or urinary bladder). Most are intercellular parasites that are typically site specific, infecting only certain target organs, and taxonomically specific, usually infecting only one species or a closely related group. However, some have a broad host range.

Myxozoan Characteristics

Key characteristics of the Myxozoa include development of a multicellular spore, presence of polar capsules in their spores, and endogenous cell cleavage in both the trophozoite and sporogony stages. One of the most important characteristics of myxosporeans is that, except during autogamy (sexual reproduction), all of the stages are multinucleated forms that have enveloping (primary) cells that contain enveloped (secondary) cells.

Mode of Transmission and Life Cycle

The method of transmission and life cycle for the great majority of myxozoans has not yet been determined, but evidence to date suggests that many if not all of the freshwater fish-pathogenic myxozoans have an indirect life cycle (Fig. II-63, A) that involves asexual reproduction in the vertebrate (fish) host and sexual reproduction in an invertebrate host (usually an oligochaete worm, but sometimes a polychaete worm or a bryozoan) (Markiw and Wolf 1983; Wolf and Markiw 1984; Wolf et al. 1986; Feist and Longshaw 2006). Since sexual reproduction occurs in the invertebrate host, it is actually the final (definitive) host. In general, invertebrate hosts are most prevalent in environments having high dissolved oxygen, high water flow and good water quality (Feist and Longshaw 2006).

The life cycle of a number of freshwater myxozoans alternates between fish and invertebrate hosts (see Table II-69), and this also occurs for some marine myxozoans (Køie et al. 2008). However, evidence has shown that at least some marine myxozoans can instead be transmitted directly by ingestion of spores released in feces (Diamant 1997; Yasuda et al. 2002).

Developmental Stages

Spores released from the fish host have one binucleate or two uninucleate sporoplasms, one to six (usually two) polar capsules (refractile in live spores; each has a polar filament), and a shell with two to six valves. When the invertebrate host ingests the spore, this triggers the rapid release of the coiled polar filaments, which probably facilitates the adherence of the spore to its gut. The spore valves separate, releasing the infective sporoplasm. When the parasite hatches, fusion of the two uninucleate

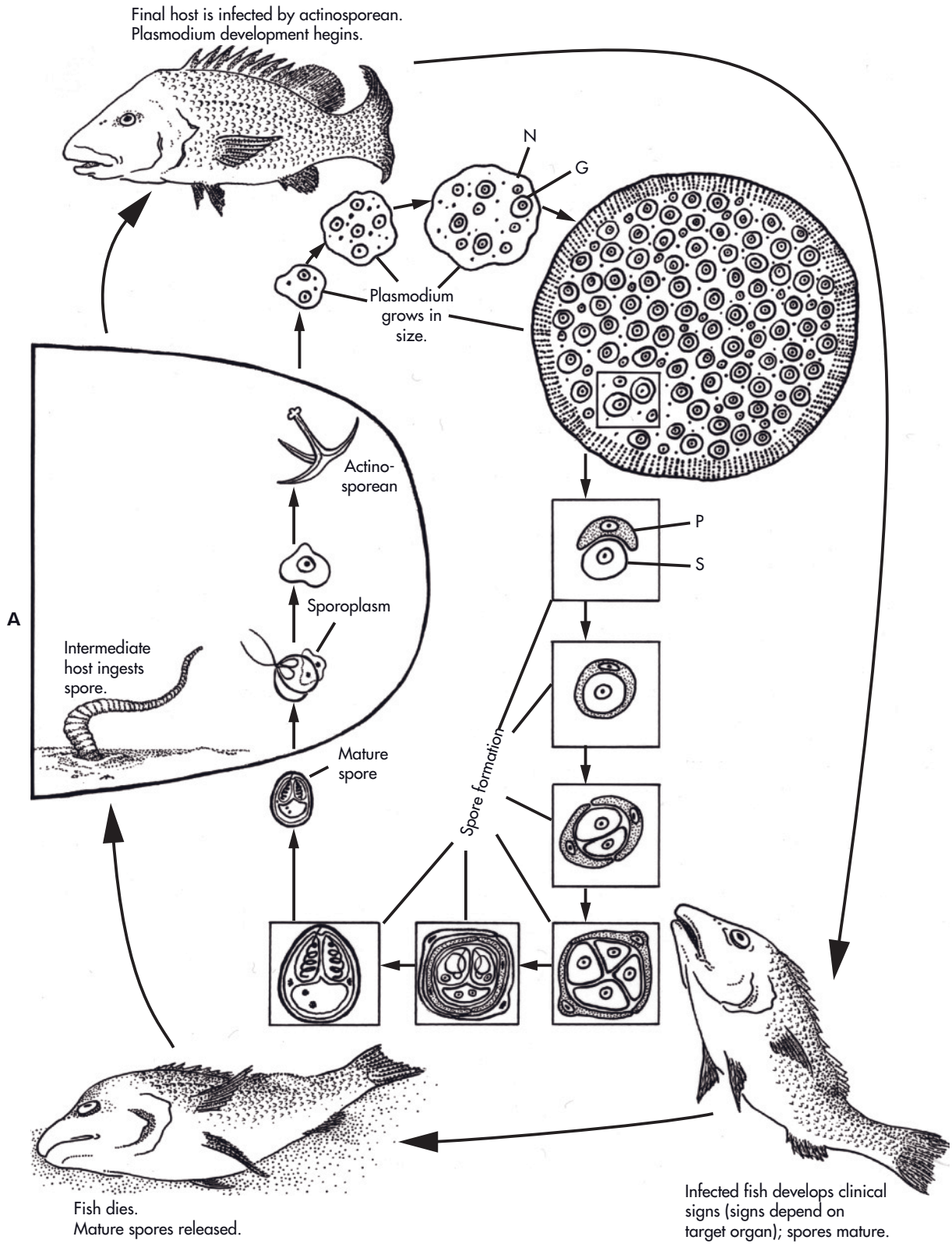

Fig. II-63. A. Generalized life cycle of myxozoan parasites. Fish host is infected with the actinosporean stage (infection is either by direct penetration of actinosporean into fish or by fish eating the intermediate host). Actinosporean transforms into a plasmodium, which grows in size in the fish. Plasmodium has generative cells (*G*), as well as many vegetative nuclei (*N*). Cells within the plasmodium then begin spore formation, first forming pansporoblasts, consisting of the union of two cells, a pericyte (*P*) and a sporogonic cell (*S*). These cells then divide, forming the various structures of the mature spore. As the plasmodium grows and matures, fish develop clinical signs of infection. Signs depend on the target organ infected. Eventually, the spores are released, usually when the fish dies. The spore is ingested by the intermediate host and the sporoplasm is released from the spore; it transforms into an actinosporean. Note that an intermediate host has not yet been identified for most myxozoan species.

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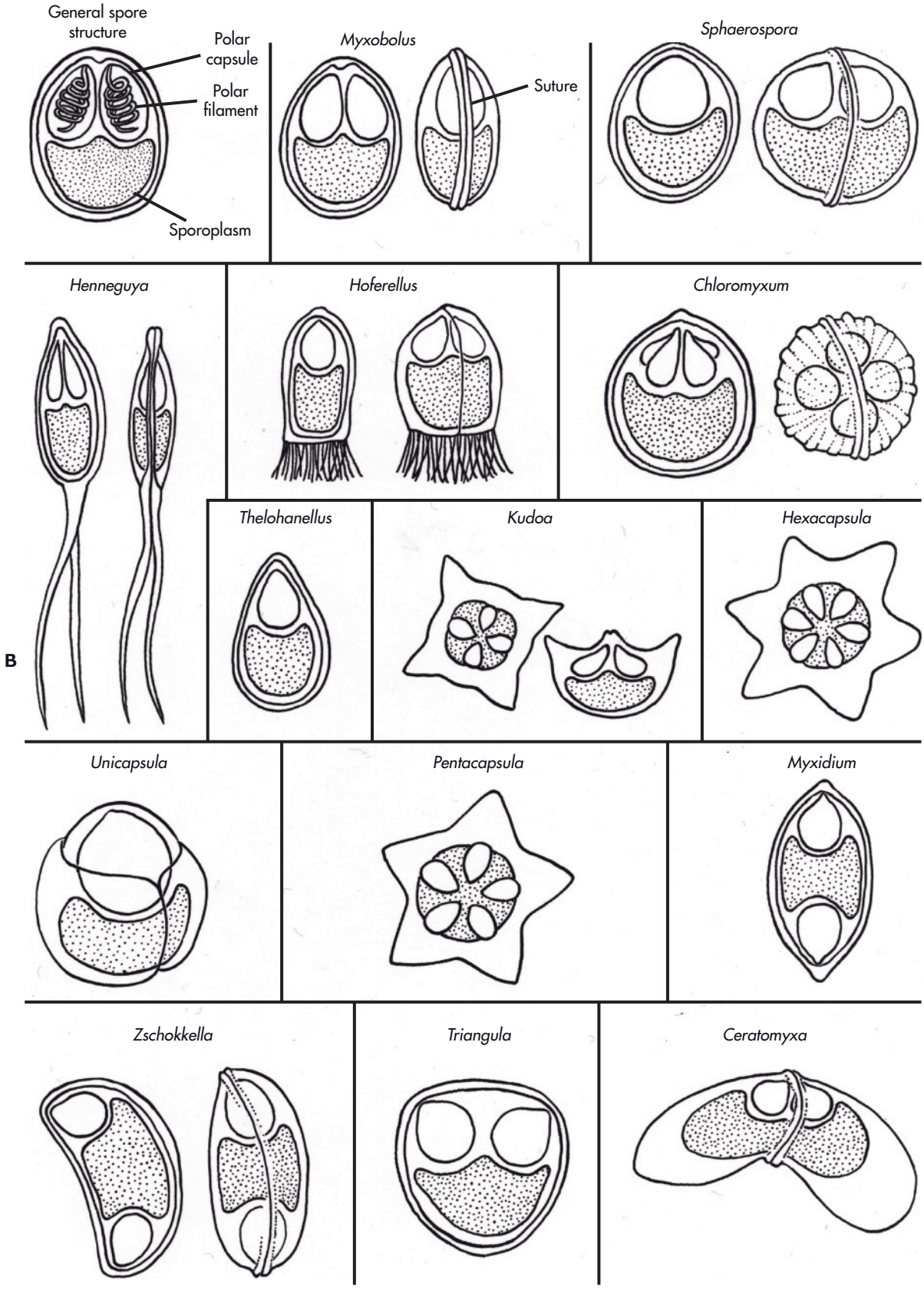

Fig. II-63.—cont'd. B. Spores of important myxozoan parasites. Key diagnostic features of myxozoan spores include: size (~10–100 µm), presence of polar capsules, and polar filaments. Polar filaments are not drawn for most spores. Polar filaments are not visible with routine light microscopy.

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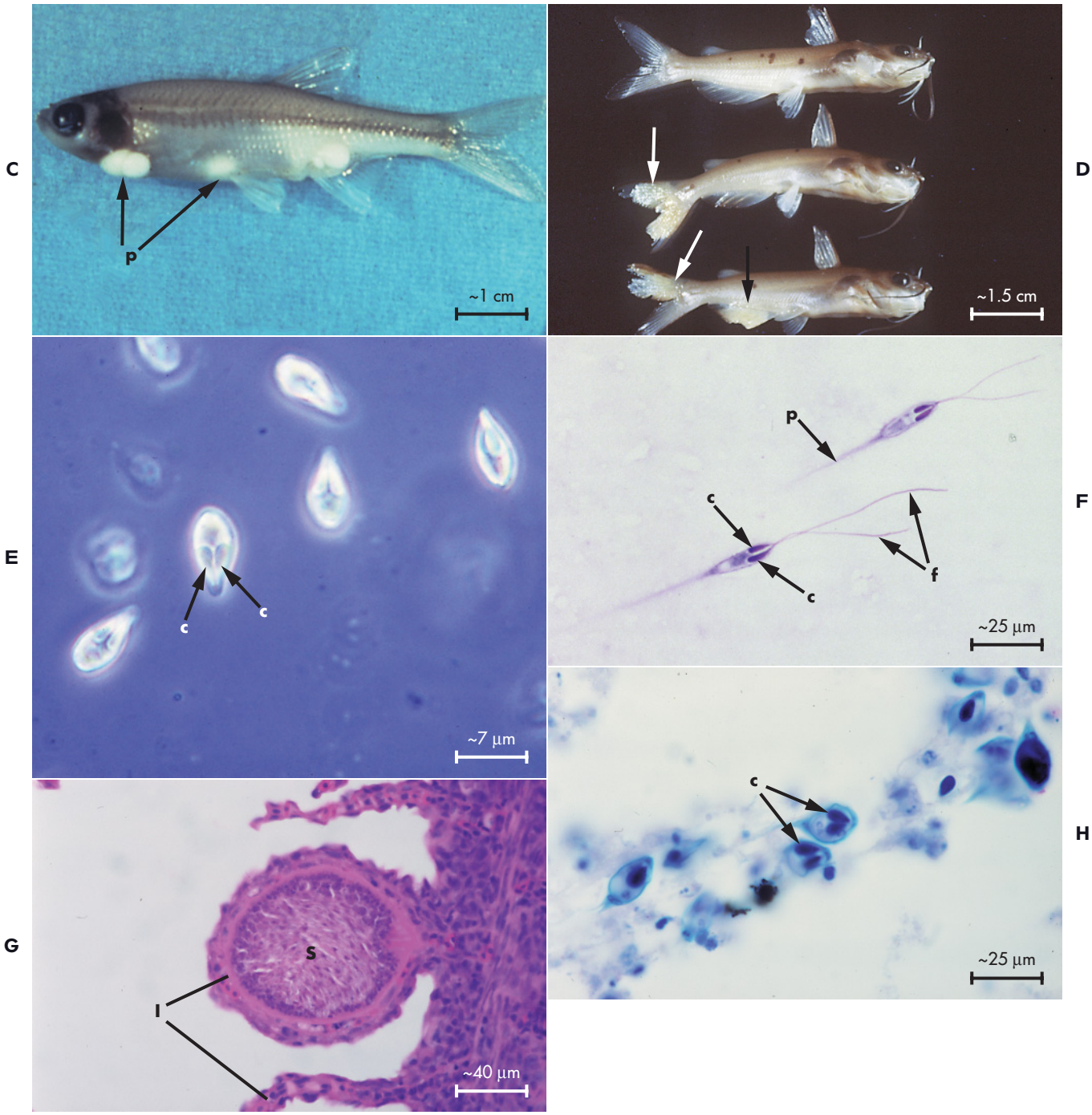

Fig. II-63.—cont'd. C. Pseudocysts (*p*) of *Myxobolus argenteus* in golden shiner. D. Pseudocysts (*arrows*) of *Henneguya* in channel catfish fin. E. Wet mount of a typical myxozoan spore (*Myxobolus* sp.). Note that polar capsules (*c*) may be difficult to see on some fresh spores. Polar filaments (within the polar capsule) are not visible without special microscopic techniques. F. Modified Wright's stain of *Henneguya* spores. Note the well-stained polar capsules (*c*). Polar filaments (*f*) have discharged during sample preparation. *P* = caudal process. G. Histological section of intralamellar *Henneguya* infection in channel catfish gill. Note enlarged secondary lamella (*l*) filled with spores (*S*). Compare with adjacent normal lamella. Hematoxylin and eosin. H. Histological section of *Henneguya* lesion in Atlantic menhaden muscle, showing spores with polar capsules (*c*). Giemsa.

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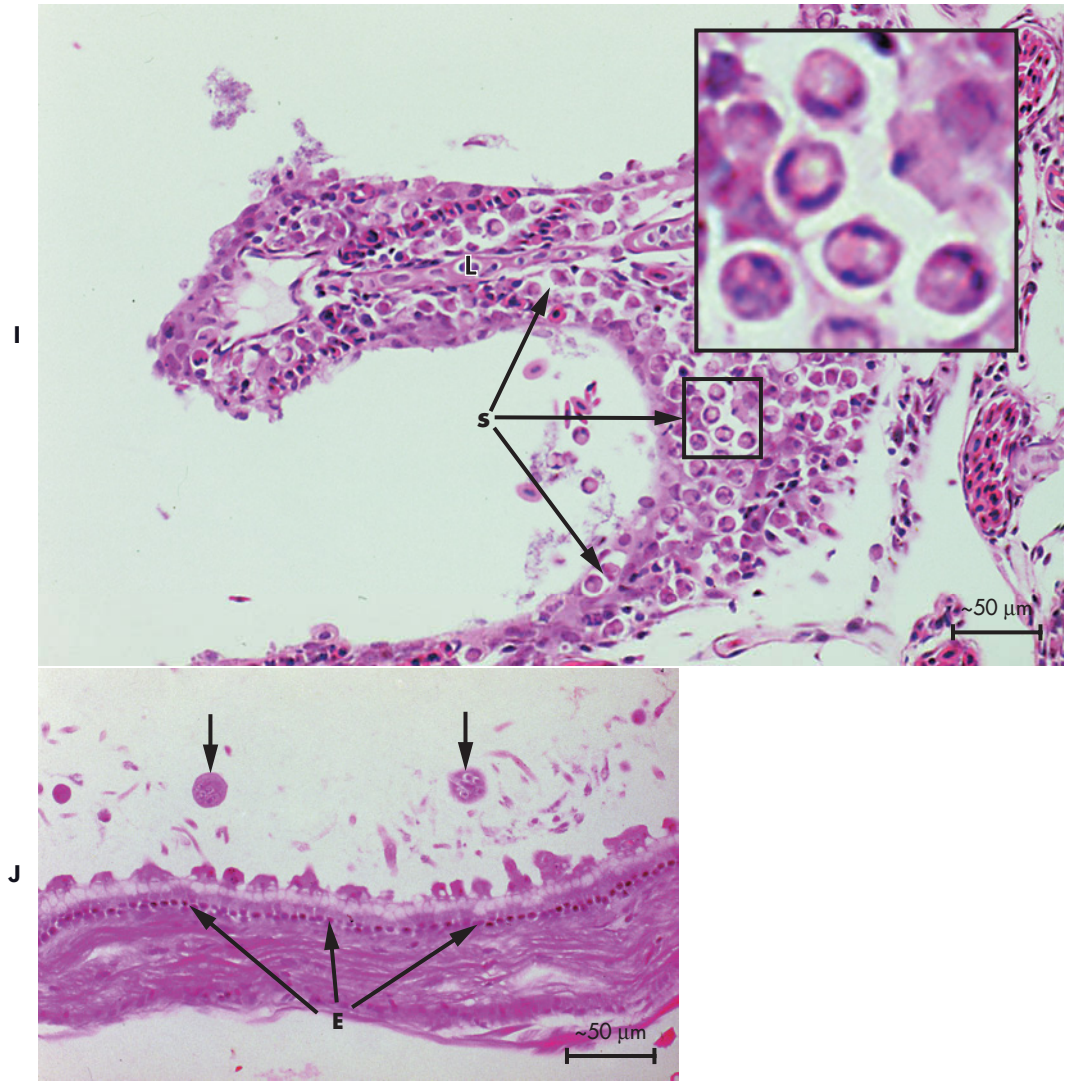

Fig. II-63.—cont'd. I. *Sphaerospora molnari* spores (*s*) filling the primary lamella (*L*) of a goldfish gill. Hematoxylin and eosin. J. Histological section through the gall bladder of a naso tang with a *Ceratomyxa* infection (coelozoic myxozoan). Developing spores are attached to the epithelium; maturing spores are in the lumen (*arrows*). *E* = gall bladder epithelium. Hematoxylin and eosin. (*A* modified from Lom and Dyková 1992; *C* and *E* photographs courtesy of G. Hoffman; *D*, *F*, and *I* photographs by L. Khoo and E. Noga.)

sporoplasms occurs, producing the only uninucleate stage in the parasite's life cycle (Fig. II-63, A).

The infective stage (actinosporean or malacospore) released from the invertebrate host (via defecation or death of the host) penetrates the epidermis or gill epithelium of the fish. In several genera (e.g., *Sphaerospora*, *Hoferellus*, *Myxidium*, *Kudoa*, *Myxobolus*), separate cycles of proliferation may also occur in epithelium and/or other organs besides the final target tissue. These extrasporogonic stages increase the number of parasites in the host without involving sporogenesis.

In the final target tissue, the trophozoite may reproduce in one of two ways. In some species the nucleus divides to produce a massive plasmodium containing generative cells, as well as many (vegetative) nuclei belonging to the plasmodium itself (Fig. II-63, A). In other species there are a large number of small plasmodia, each with only one vegetative nucleus that divides to produce many parasites before sporogony; each gives rise to one to two spores. In coelozoic species the plasmodia cover the walls of the lumen or attach to the epithelial surface, where they usually divide by cleaving into two or more parts or by producing multinucleate buds.

In coelozoic species (see PROBLEM 65), the plasmodia divide and produce myxospores continuously, result-

ing in infections that may last a long time. Conversely, myxospore production in histozoic plasmodia is synchronous, and thus eventually the plasmodium matures into a large packet of myxospores (Fig. II-63, A and G). Plasmodia situated near an external surface, such as the gills, skin, or intestine, may rupture, releasing the myxospores. Dissemination of myxospores from deeper tissue sites probably depends on the death of the host by predation or other means. Myxospores are typically very resistant to environmental conditions.

Pathogenesis

Most myxozoan infections of fish are relatively innocuous, eliciting only moderate host reactions. But heavy infections can be quite serious, resulting in mechanical damage from the pseudocysts or tissue necrosis and inflammation from trophozoite feeding. Young fish are usually most seriously affected by myxozoan infections. Histozoic forms usually cause more serious diseases.

The early stages of the life cycle usually incite little host reaction, but plasmodia with mature spores often induce considerable inflammation. Interestingly, in many cases, tissue damage is greatest after death of the host, when enzymes released by the parasites are believed to cause massive muscle liquefaction (e.g., tapioca disease of mackerel and tuna) (see PROBLEM 69). Muscle lysis can cause serious reduction in carcass value.

Taxonomic Identification

The classical taxonomy of the Myxosporea is based solely on spore structure, including spore size and shape, and the number and position of polar capsules. Spores can have from 2 to 12 shell valves and from 1 to 13 polar capsules. Spores usually range from about 8–25 μm (rarely as high as 100 μm), which is considerably larger than the typical microsporidian spore (see PROBLEM 70). The order Bivalvulida (e.g., *Myxobolus*) has spore walls with two spore valves. The order Multivalvulida has spore walls with three to six valves; most live intracellularly in myocytes (e.g., *Kudoa*). Spores may have projections of various sorts (Fig. II-63, B) that may facilitate their maintenance in the water column or passive attachment to food of potential hosts. Some recent genetic analyses indicate that the morphological criteria used to classify some myxozoans are not always correct in assigning taxa (Whipps et al. 2003).

Diagnosis

GROSS LESIONS

Myxozoan lesions (e.g., Fig. II-63, C and D) can look grossly similar to other diseases that cause focal masses, including microsporideans (see PROBLEM 70), ich (see PROBLEM 20), lymphocystis (see PROBLEM 40), and dermal metacercariae (see PROBLEM 58). Internal lesions may resemble focal granulomas (see Fig. II-55, C) and neoplasia (see PROBLEM 76); differentiation is easily done by examining wet mounts or histological material.

DEFINITIVE DIAGNOSIS

Diagnosis of myxozoan disease is based on identifying myxozoan spores (myxospores) in target tissues with appropriate clinical signs. Note that myxozoan pseudocysts are often an incidental finding. Counterstaining samples with India ink may help to identify spores in wet mounts but is usually not necessary. Spores with polar capsules (Fig. II-63, E, F, and H) are pathognomonic for myxozoan infection. Polar capsules can be seen in fresh wet mounts but are more easily seen in Giemsa or Wright's stained smears (Fig. II-63, F). If identification of species is desired, fresh (unfixed) spores are often needed. Fixation causes artifacts, including shrinkage, which affects size measurements. Note that spore size within a species may vary slightly from reported dimensions and malformed spores also occur (Feist and Longshaw 2006).

Histopathology may be better for detecting certain infections (Fig. II-63, G through J) (Dyková and Lom 2007), especially when inflammation against the parasite is extensive (e.g., when pseudocysts rupture), making individual spores difficult to find in wet mounts. Spores are refractile and difficult to see in hematoxylin and eosin sections, but polar capsules stain intensely with Giemsa or toluidine blue (Fig. II-63, H). Light microscopy can only definitively identify myxozoans to genus (not species).

In a few diseases, myxozoans are responsible or suspected to be involved, but mature spores are not formed or rarely visible. In such cases, diagnosis is based on the identification of trophozoites or other developmental stages in target tissues. Wet mounts of such suspected (or obviously infected) tissue may reveal small plasmodial stages or spores. Gene tests have been developed for some myxozoans but are not commercially available.

CLINICAL INTERPRETATION OF INFECTIONS

Many wild-caught fish harbor myxozoans. In aquarium fish, infection is fairly common, but epidemics have not been reported, possibly since the life cycle cannot be completed in aquaria because of the absence of an essential intermediate host (Wolf and Markiw 1984). In more natural environments, such as ponds (see PROBLEM 64), or where fish are exposed to natural waters (see PROBLEMS 65 and 68), myxozoans can be serious.

Treatment

There are no highly effective drugs for controlling myxozoan infections (Molnár 1993). Malachite green (Alderman and Clifton-Hadley 1988) is a clinically effective compound for some parasites (e.g., for PKD, PROBLEM 67), but not all (e.g., ceratomyxosis) and is highly illegal to use on food fish in virtually all countries. Fumagillin can either reduce the severity or slow the progression of certain myxozoan infections (Hedrick et al. 1988) (see "**Pharmacopoeia**" for typical doses), but many are resistant (Feist and Longshaw 2006). Also,

there is little evidence that it is completely curative and it is often toxic at the required dose.

Even disinfection and quarantine can be challenging, since myxospores are very environmentally resistant, requiring potent treatments (e.g., see PROBLEM 68). They are also long-lived; some can survive for well over 1 year (Hoffman et al. 1962). Actinospores should presumably be much more susceptible to disinfection than myxospores, but there is a dearth of studies examining their susceptibility.

Prevention of infection is the best approach. Myxozoans can be introduced not only via fish (live or dead) but also via their invertebrate host. Various species of oligochaetes (black worms, red worms, *Tubifex* and others) are often used as food, especially for aquarium fish; these commonly harbor actinosporeans (Lowers and Bartholomew 2003) (see Table II-69). Oligochaetes present in gravel, on aquatic plants, or on other fomites also might introduce actinosporeans into culture systems. The host and geographic ranges of many myxozoans have probably been expanded due to transfer of infected fish and/or infected invertebrate hosts (Feist and Longshaw 2006). Breaking the transmission cycle of myxozoans having a direct life cycle is also challenging.

PROBLEM 64

Proliferative Gill Disease (PGD; Hamburger Gill Disease, *Henneguya ictaluri* Infection)

Prevalence Index

WF - 2

Method of Diagnosis

1. Wet mount of affected tissue having life stages of *Henneguya ictaluri*
2. Histology of affected tissue having life stages of *Henneguya ictaluri*

History

Pond-raised channel catfish

Physical Examination

Pale, grossly thickened ("clubbed") and broken gill lamellae; dyspnea

Treatment

Supplemental oxygen for affected fish

COMMENTS

Epidemiology

Proliferative gill disease (PGD), due to infection by *Henneguya ictaluri*, causes acute branchitis and low-to-high mortality (1% to 95%) in all ages of (but primarily fingerling) channel catfish throughout the southeastern United States and California (Hedrick et al. 1990c; Pote et al. 2000). Blue catfish tend to be much more resistant (L. Khoo, personal communication). PGD occurs most commonly at 16–20°C (61–68°F), although epidemics have been seen between 14° and 26°C (57° and 79°F; MacMillan et al. 1989b). Thus, major outbreaks are most common in spring (April–May), with smaller outbreaks occurring in fall (September–October) in the southeastern United States (Pote et al. 2003). Development of PGD is typically associated with new ponds; however, recurrence in the same pond can occur after it has been drained and refilled (Styer et al. 1991) or when naive, young fish are added to an established pond (L. Khoo, personal communication).

The actinospore *Aurantiactinomyxon ictaluri*, develops in the invertebrate host, an aquatic oligochaete worm (*Dero digitata;* Groff et al. 1989; Styer et al. 1991; Hanson et al. 2001). Infected *D. digitata* then release the actinospore, which infects the fish either orally or through the skin. Development of the mature spore occurs in the gill; when the spores are released in the water, they then infect *D. digitata* to continue the life cycle (Pote et al. 2003).

Pathogenesis

Affected fish are depressed because of respiratory impairment. In early stages the lamellae are pale and swollen. In later stages, lamellae are thickened, blunted, and bleed easily (hamburger gill disease) (Fig. II-64, A). Histologically, there is severe epithelial hyperplasia and granulomatous branchitis, forming nodules around parasitic cysts (Fig. II-64, C). Lamellar fusion is common. Cartilage necrosis and liquefactive necrosis of cells within the nodules is characteristic. Cartilage necrosis results in breakage of the lamella. Cysts may also be present in liver, spleen, kidney, and brain, but there is typically little inflammation (Groff et al. 1989). Later, healing is characterized by chondroplasia and absence of cysts. A related species, *Henneguya exilis*, also causes severe gill pathology ("lamellar disease") (Lin et al. 1999; Table II-69).

Diagnosis

While swollen, clubbed, and broken lamellae in channel catfish provide a presumptive diagnosis of PGD, histopathology is required to identify the characteristic inflammatory response and trophozoite stage in the gill parenchyma (Fig. II-64, C). Several sections may need to be cut to locate the parasites. Parasites can rarely be seen in wet mounts, but only if samples are examined within minutes of excision. In early stages, focal areas of cleared cartilage are strongly suggestive of PGD (Fig. II-64, B). Note that in some cases, fish with apparently mild histological lesions may still experience high mortality (Pote et al. 2003). Several months after infection, spores will be produced, but gene tests are required to differentiate *H. ictaluri* spores from the other *Henneguya* species that produce similar spores (Fig. II-63, D, G) (Hanson et al. 2001).

Treatment

Fish with PGD are intolerant of stresses, such as handling, low dissolved oxygen, or high ammonia, so

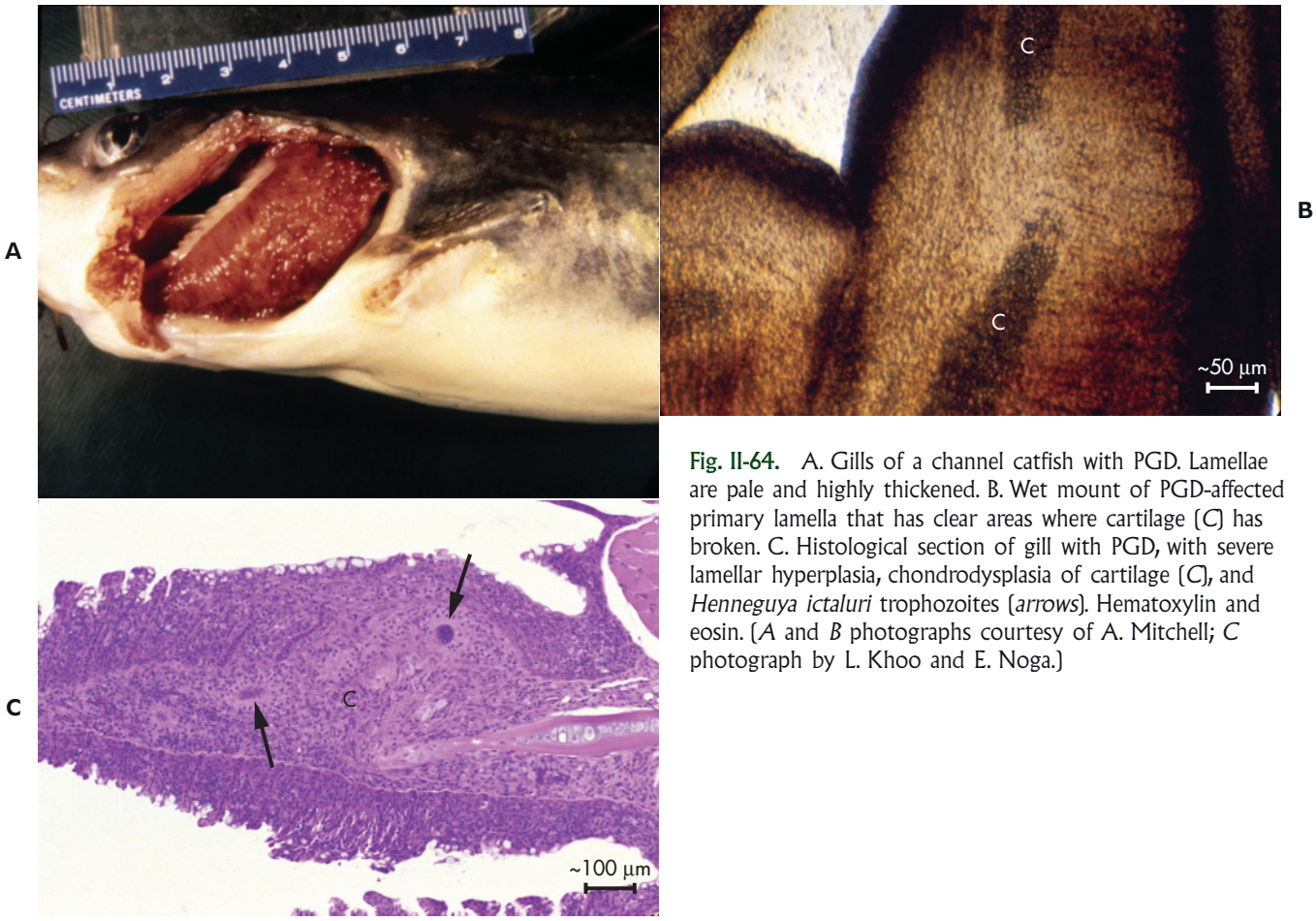

Fig. II-64. A. Gills of a channel catfish with PGD. Lamellae are pale and highly thickened. B. Wet mount of PGD-affected primary lamella that has clear areas where cartilage (*C*) has broken. C. Histological section of gill with PGD, with severe lamellar hyperplasia, chondrodysplasia of cartilage (*C*), and *Henneguya ictaluri* trophozoites (*arrows*). Hematoxylin and eosin. (*A* and *B* photographs courtesy of A. Mitchell; *C* photograph by L. Khoo and E. Noga.)

increased aeration and possibly water changes can be helpful. Treatment with chemical irritants, such as formalin, are contraindicated, since they can increase mortality. Many fish can recover spontaneously if undisturbed. Pond disinfection should be considered but may be contraindicated, since it may precipitate a new disease cycle.

PROBLEM 65
Ceratomyxa shasta Infection (Ceratomyxosis)

Prevalence Index

CF - 3

Method of Diagnosis

1. Wet mount of affected tissue having *Ceratomyxa shasta* myxospores
2. Histology of affected tissue having *Ceratomyxa shasta* myxospores

History

Salmonids exposed to parasite-endemic waters

Physical Examination

Swollen abdomen; necrotic muscle lesions

Treatment

1. Avoidance and quarantine
2. Disinfect incoming water

COMMENTS

Epidemiology

Ceratomyxa shasta affects salmonids, especially anadromous species, in the western United States and Canada (primarily the Columbia River basin, including Oregon, Idaho, California, Washington, and British Columbia). It can cause up to 100% mortality in young cultured and wild fish and is also an important cause of prespawning mortality in adult salmon. The most susceptible species are rainbow trout, cutthroat trout, chinook salmon, and chum salmon. Coho salmon, sockeye salmon, brown trout, and brook trout are less susceptible.

Endemic strains of salmonids from the Columbia River basin are relatively resistant, while exotic strains and the native-endemic crosses are more susceptible (Hoffmaster et al. 1985; Bartholomew et al. 1989; Ibarra et al. 1992). Thus, introducing susceptible strains into parasite-endemic areas could endanger the native stocks. Many

naïve, native stocks with no prior exposure are also vulnerable.

Fish can be infected at as low as 4–6°C (39–43°F) (Ching and Munday 1984a); however, at such low temperatures, the disease progresses slowly. Higher temperatures cause a faster onset of clinical signs, which can occur as quickly as 7 days at 18°C (64°F). At 10°C (50°F), infections may take over 3 months to kill fish. The temperature dependence accounts for the seasonal nature of the disease, peak prevalence being in warmer months (May–November).

Fish acquire the infection from actinospores released by the freshwater polychaete *Manayunkia speciosa* (Bartholomew et al. 1997; Bartholomew 2002). Fish are readily infected (within minutes) if exposed to parasite-endemic waters or mud (Johnson et al. 1979).

Pathogenesis

Clinical signs vary with fish species, but the main target tissue is usually the gastrointestinal tract, especially the intestine. Developing parasites induce a diffuse granulomatosis in many host tissues. Infections start in the intestine and pyloric caecae and then spread hematogenously to liver, kidney, spleen, gonads, and muscle. The abdomen is often distended because of granulomatous peritonitis (Fig. II-65, A), with many conical, widely arched spores in the exudate (Fig. II-65, B). The vent may be swollen, and necrotic abscesses ("boils") have been reported in muscle of some species (Wood et al. 1989).

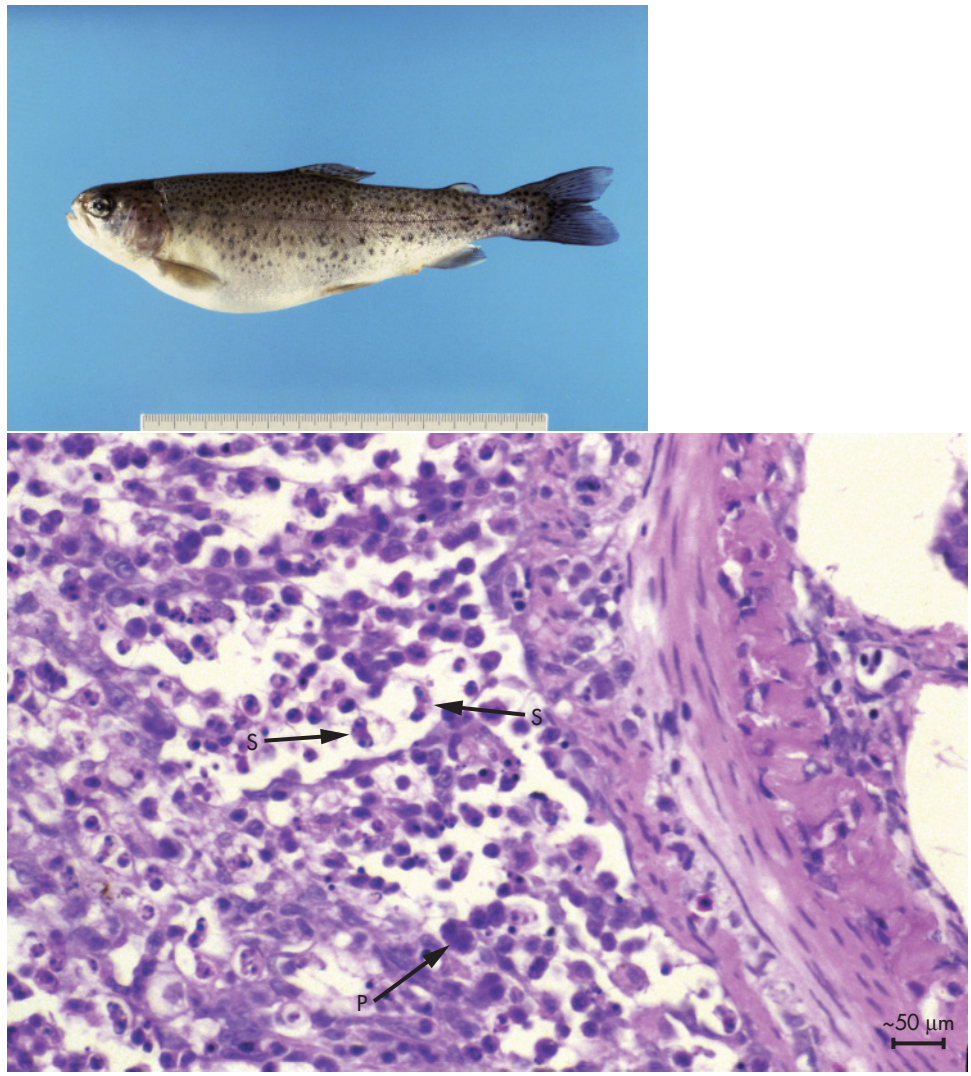

Fig. II-65. A. Rainbow trout with experimental *Ceratomyxa shasta* infection. Pronounced abdominal swelling caused by peritonitis. B. Histological section with severe, chronic peritonitis, with disporic, 13 × 19 µm plasmodia (*P*) and spores (*S*). Key diagnostic features of spores: size (14–23 µm long and 6–8 µm wide at the suture line), winged shape (ends of spores are rounded and reflect posteriorly), and polar capsules (2.2 µm). Hematoxylin and eosin. (*A* photograph by J. Landsberg and E. Noga; *B* photograph by L. Khoo and E. Noga.)

Diagnosis

Diagnosis is based upon identification of typical spores in lesions or in scrapings of the intestinal lumen or gall bladder. With Ziehl-Neelsen stain, polar capsules stain red and sporoplasm, blue. Note that mature spores may not develop until the terminal stages of the infection. In such cases, trophozoites can be identified by electron microscopy (*C. shasta* trophozoites cannot be differentiated from those of other myxozoans by light microscopy). Monoclonal antibodies have also been developed that can identify mild infections in fixed tissues by using antibodies that recognize the prespore stage (Bartholomew et al. 1989). A gene test can identify *C. shasta* (Bartholomew 2002) and allows nonlethal sampling of fish (Fox et al. 2000), but is not yet commercially available.

Treatment

There are no proven chemotherapies. Disease progression can sometimes be reduced by transfer to saltwater (Hoffmaster et al. 1985). This also prevents further infection. However, salmonids infected in freshwater and transferred to seawater may still exhibit high mortalities (Ching and Munday 1984b). Surviving fish are undersized and emaciated (Tipping 1988). Susceptible fish can be protected by filtration of infective water, followed by ultraviolet sterilization, chlorination, or ozonation (Sanders et al. 1972; Bower and Margolis 1985). Fish from *C. shasta*-endemic areas should not be moved to other areas unless certified free of the disease.

PROBLEM 66

Hoferellus carassii Infection (Kidney Enlargement Disease [KED], Kidney Bloater)

Prevalence Index

WF - 3

Method of Diagnosis

1. Wet mount of affected tissue having myxospores
2. Histology of affected tissue having myxospores or developmental stages

History

Pond-raised goldfish

Physical Examination

Moderate to severe abdominal swelling, often asymmetrical; usually normal otherwise

Treatment

None known

COMMENTS

Epidemiology

Hoferellus carassii, formerly known as *Mitraspora cyprini*, causes kidney bloater, a chronic renal infection that results in massive renal hypertrophy and concommitant abdominal distension in goldfish. The disease occurs in Europe, North America and Asia (Hoffman 1981; Trouillier et al. 1996), especially goldfish-producing areas (e.g., Japan, Israel). Fish typically become infected in ponds during summer but usually do not exhibit clinical signs until fall. Spores are produced early the following spring, when clinical signs are most severe and fish most often tend to die. The life cycle is believed to be about 1 year. Conflicting studies have implicated two different oligochaete worms as the final host: *Branchiura sowerbyi* (Yokoyama et al. 1993) and *Nais* cf. *elingius* (Trouillier et al. 1996).

Pathogenesis

While infections are invariably fatal, infected fish can live for months, especially if they are over 1 year old when clinical signs develop. Fish usually act and eat normally. The abdomen often protrudes asymmetrically (Fig. II-66, A) because of the swelling of the kidneys and ureters (Egusa 1978). The swim bladder may be displaced, causing balance problems, with the fish then floating on its side. There are no other internal lesions, despite the space-occupying, swollen kidney. The kidney appears cystic grossly (Fig. II-66, B) and is hypertrophic because of the extensive swelling and hyperplasia of renal tubules caused by infection of the tubular epithelium by the prespore stages. In the advanced stage a yellow fluid is found in the dilated tubules. Only some tubules are affected. After several developmental stages, trophozoites line the tubular epithelium and differentiate into myxospores in early spring, which are shed in the urine (Molnár et al. 1989).

Diagnosis

Diagnosis is based upon identification of typical myxospores in lesions. Myxospores are mitre-like, ~7.5 × 13 µm, with 4.5–6.0 µm long bristles (see Fig. II-63, B). However, there are typically few spores present, and if myxospores have not yet developed, identification of myxozoan trophozoites in typical lesions provides a strong presumptive diagnosis. Note that there are many causes of abdominal swelling in goldfish, including bacterial infection, viral infection, abdominal neoplasia, osmoregulatory failure and polycystic kidney.

Treatment

There are no proven treatments. Disinfecting ponds and restocking with known, uninfected goldfish may break the transmission cycle.

PROBLEM 67

Proliferative Kidney Disease (PKD; *Tetracapsuloides bryosalmonae* infection)

Prevalence Index

CF - 2

Method of Diagnosis

1. Histology of affected tissue having the myxozoan's life stages

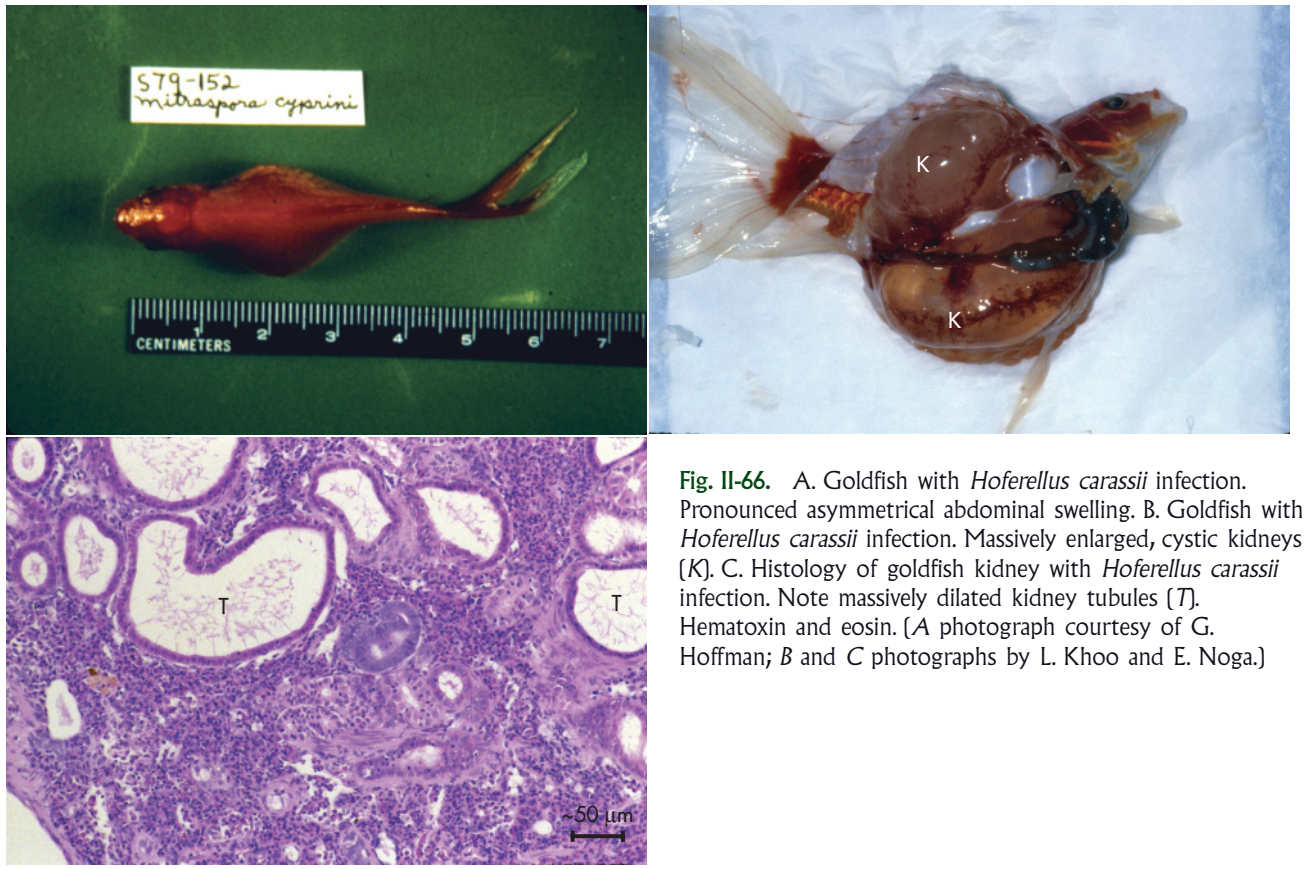

Fig. II-66. A. Goldfish with *Hoferellus carassii* infection. Pronounced asymmetrical abdominal swelling. B. Goldfish with *Hoferellus carassii* infection. Massively enlarged, cystic kidneys (*K*). C. Histology of goldfish kidney with *Hoferellus carassii* infection. Note massively dilated kidney tubules (*T*). Hematoxin and eosin. (*A* photograph courtesy of G. Hoffman; *B* and *C* photographs by L. Khoo and E. Noga.)

2. Impression smear of affected tissue having the myxozoan's life stages

History

Chronic morbidity/mortality in salmonids

Physical Examination

Hypertrophic kidney; anemia; swollen abdomen; splenomegaly

Treatment

1. Disinfection, avoidance, and quarantine
2. Malachite green bath
3. Salt bath

COMMENTS

Epidemiology

Proliferative kidney disease (PKD) is a serious disease of salmonids. It has been reported in the Pacific Northwest of the United States, including California, Idaho, and Washington, as well as British Columbia (Canada) and Europe (Hedrick et al. 1986, 1993; Beraldo et al. 2006). Most outbreaks occur in rainbow or steelhead trout, but disease in brown trout, Atlantic salmon, coho salmon, and chinook salmon are also common. Other species affected include cutthroat trout, marble trout, grayling and Arctic charr; it can experimentally infect Kokanee salmon and chum salmon. Brook trout can also be infected but do not show clinical signs. Juvenile fish are most susceptible but any age fish can be affected. Infections have been observed in both captive and feral salmonids. It also infects northern pike.

PKD is caused by *Tetracapsuloides bryosalmonae* (formerly known as PKX) (Canning et al. 1999). The pronounced inflammatory response, lack of typical mature myxozoan spores and inability to infect bryozoans with the spores from fish has also made some speculate that fish may be an aberrant or dead-end host (Tops et al. 2004).

PKD primarily occurs during summer. The infection is typically contracted between April and June, when fingerlings are stocked into infected waters (Foott and Hedrick 1987). Mortalities may range from 10% to 95%. Highest mortalities occur at 12–14°C (54–57°F). High parasite intensities are not always strongly correlated with high mortalities, suggesting that other factors (e.g., complicating infections) influence morbidity (Hedrick et al. 1985a). *Tetracapsuloides bryosalmonae* is one of only two known members of the class Malacosporea that infect fish. The final host of the malacosporean is the bryozoan *Fredericella sultana* (Feist et al. 2001).

Pathogenesis

Most pathology can be attributed to damage of the kidney, which is the primary target organ. Gross lesions

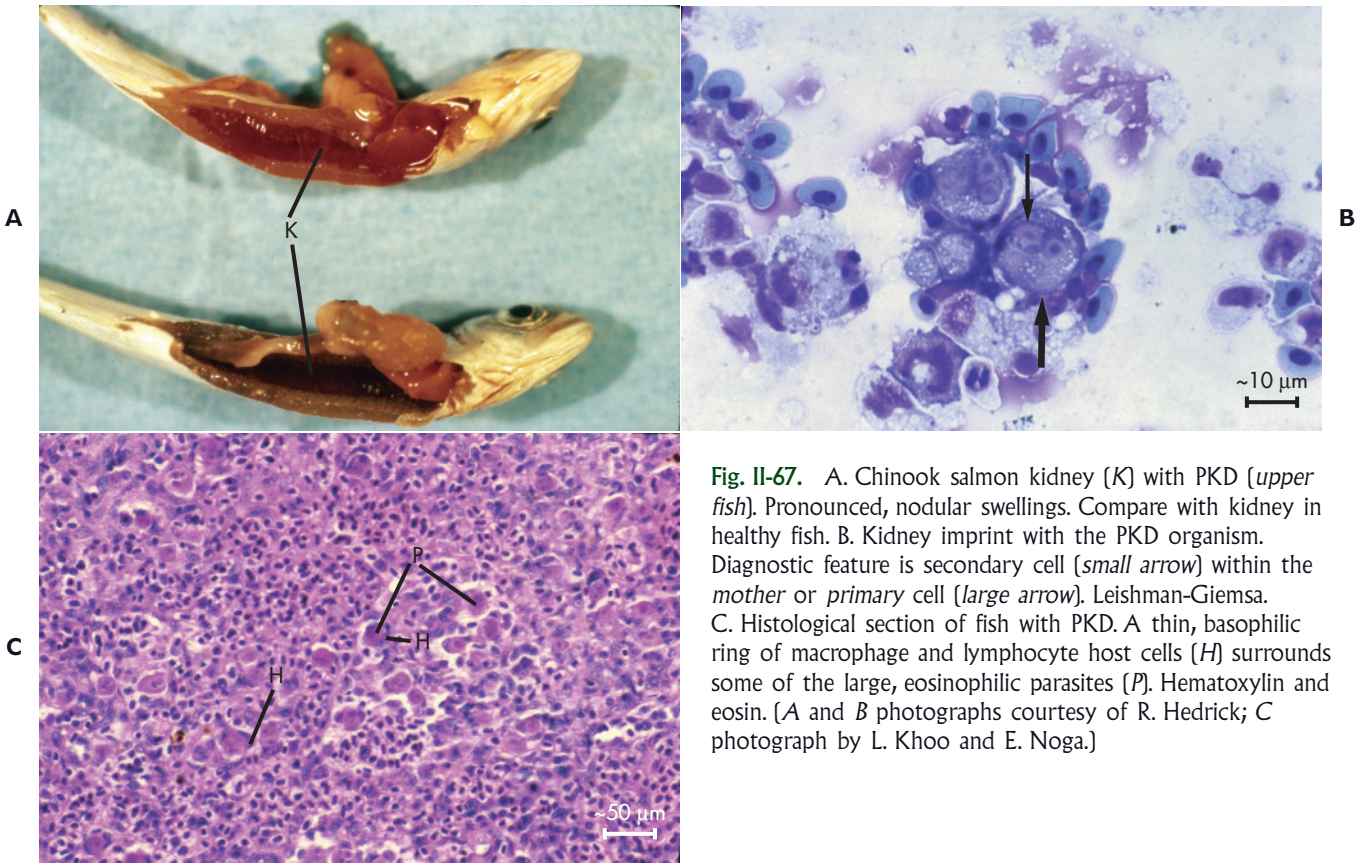

Fig. II-67. A. Chinook salmon kidney (*K*) with PKD (*upper fish*). Pronounced, nodular swellings. Compare with kidney in healthy fish. B. Kidney imprint with the PKD organism. Diagnostic feature is secondary cell (*small arrow*) within the *mother* or *primary* cell (*large arrow*). Leishman-Giemsa. C. Histological section of fish with PKD. A thin, basophilic ring of macrophage and lymphocyte host cells (*H*) surrounds some of the large, eosinophilic parasites (*P*). Hematoxylin and eosin. (*A* and *B* photographs courtesy of R. Hedrick; *C* photograph by L. Khoo and E. Noga.)

include darkened body color, exophthalmos, pale gills (anemia), abdominal swelling, ascites, splenomegaly, and renal hypertrophy (Fig. II-67, A) (Ferguson and Needham 1978). The kidney may be so enlarged that it forms a swelling just beneath the lateral line. Multifocal swellings give the kidney a nodular appearance.

PKD induces a diffuse, chronic interstitial nephritis, consisting primarily of macrophages and lymphocytes, often surrounding the amoeboid parasites (Fig. II-67, B and C). This results in necrotizing vasculitis and tubular atrophy. Other organs, especially spleen, but also intestine, gill, liver, pancreas and muscle, may be infected, presumably hematogenously.

Parasites penetrate the kidney tubule lumen and begin sporogenesis but do not produce mature spores. Parasites are often found singly or in aggregates in the renal portal vessels. Fish in North America that are recovering often have sporoblasts in the tubules (Kent and Hedrick 1985, 1986).

Diagnosis

Few mature myxospores are present, so diagnosis of PKD is based on the identification of typical amoeboid parasites in stained tissue smears (Fig. II-67, B) (Clifton-Hadley and Richards 1983) or histological sections (Fig. II-67, C). The primary cell is up to 15 µm, with one or more secondary (daughter) cells. They can be found within and between host cells. Monoclonal antibodies and lectin probes that can identify both the extrasporogonic (interstitial) and sporogonic (intraluminal) stages of the parasite have been developed (de Mateo et al. 1993). A nucleic acid test (PCR assay) has also been developed (Kent et al. 1998) but is not commercially available.

Treatment

Malachite green bath shows some efficacy (Alderman and Clifton-Hadley 1988) but is no longer legally approved for use on food fish. Fumagillin slows but does not stop disease progression (Hedrick et al. 1988). Increasing salinity to 8–12 ppt decreases morbidity and mortality. Reducing water temperature suppresses the effects of the disease. Fingerling salmonids should not be stocked into *T. bryosalmonae*–infected waters until at least July to avoid clinical disease; if fish are stocked late in the season but before temperatures decrease in fall, they also may display greater resistance in the following season (Feist and Longshaw 2006). Recovered fish are also resistant to reinfection (Foott and Hedrick 1987).

PROBLEM 68
Whirling Disease (Black Tail)

Prevalence Index

CF - 2, CM - 4

Method of Diagnosis

1. Wet mount of cartilage digest having typical spores
2. Histology of cartilage having typical spores

History

Whirling or tail-chasing behavior in young salmonids; fish raised on mud bottom

Physical Examination

Scoliosis, kyphosis, other axial skeletal deformities; postural deficits; regional pigment abnormalities

Treatment

1. Disinfect and quarantine
2. Raise stock in parasite-free water for first 6 months of life
3. Disinfect water source

COMMENTS

Epidemiology

Whirling disease is a chronic, debilitating disease caused by *Myxobolus* (syn. *Myxosoma*) *cerebralis*. First recorded as an infection of brown trout in Central Europe, it has subsequently been reported worldwide, including in North America, South Africa, Australia and New Zealand. Traditionally a problem only in cultured fish, it has recently caused epidemics in wild salmonid populations in the United States. This has caused considerable concern since there is evidence that some epidemics might be responsible for population declines (Hedrick et al. 1998; Allendorf et al. 2001). All salmonids in the genus *Oncorhynchus* (especially rainbow trout) are susceptible to varying degrees. Brown trout is more resistant and is considered a reservoir (Bartholomew and Reno 2002). Recently, presporogonic stages of *M. cerebralis* have been associated with neurological disease and mass mortality of marine-cultured Atlantic salmon smolts in Ireland. It was hypothesized that the infective stages originated from nearby rivers (Frasca et al. 1999).

The severity of the disease is inversely related to the age of the fish when exposed, varying from 100% mortality in newly hatched fry to little or no clinical signs in fish over 6 months old. After 1 year there is little cartilage available in the skeleton for infection, but even fish that are several years old can be infected via the gill cartilage and thus become carriers. In endemic areas, *M. cerebralis* typically causes a mild disease that is restricted to hatcheries and is not usually evident in feral populations.

Depending on temperature the entire life cycle may require over 1 year (Hoffman 1976), making it an insidious problem that may go undetected for a long time. Clinical signs usually develop 2–8 weeks after infection (longer at low temperatures). Spore formation in infected fish requires 4 months to complete at 7°C (45°F), 3 months at 12°C (54°F), and about 50 days at 17°C (63°F) (Halliday 1973).

Most myxospores remain trapped in the skeletal tissues until the fish dies (Hoffman and Putz 1969), but some can be released by live fish (Nehring et al. 2002). Myxospores can be spread in the feces of piscivorous birds. Myxopores must be ingested by an oligochaete final host, the sludge worm (*Tubifex tubifex*), which is common in organically polluted sediment. The myxopore releases the sporoplasm, which differentiates into an actinosporean. After completion of both asexual and sexual stages in tubifex (this requires about 3–4 months) the actinosporean directly penetrates a new host via the skin, gill, or buccal epithelium (see Fig. II-63, A). It then migrates from the epithelium to the peripheral nerves and then the central nervous system, finally reaching the cartilage.

Pathogenesis

The parasite feeds on cartilage of the axial skeleton and clinical signs are related to this damage. The first clinical sign is usually a black tail (Fig. II-68, A) caused by vertebral instability and the resultant damage to sympathetic nerves near the spinal cord. These nerves control melanin pigmentation. Black tail occurs only in 3- to 6-month-old fish.

Predilection for cartilage causes impaired balance and a frenzied, tail-chasing behavior (whirling) (Rose et al. 2000). Whirling is most obvious when the fish are fed or disturbed. Both black tail and whirling eventually disappear with time. However, survivors of these episodes often develop spinal curvature, pug-headedness, or an undershot jaw because of cartilage damage. Clinical signs seem to be more evident at ~17°C (63°F). Heavy infections may cause acute mortalities without clinical signs.

The parasites lyse cartilage and feed on chondrocytes; histologically, there is a reactive chondrosteal proliferation to infection. Damaged cartilage often has a chronic inflammatory response.

Diagnosis

Because spores are trapped in cartilage, it is difficult to make wet mounts of fresh tissues. Thus, diagnosis is usually made by histopathology of head, gill, or vertebral cartilage. To sample for *Myxobolus cerebralis*, a cross-section should be taken just behind the eye (approximately 5 mm posterior), so that the cartilage around the auditory capsule is included (this is a highly common site for *M. cerebralis* infection). Note that other *Myxobolus* species may be found in the connective tissues outside the cartilage, especially in brown trout and grayling (Bucke 1989). For asymptomatic infections, a more sensitive method is to enzymatically digest head cartilage

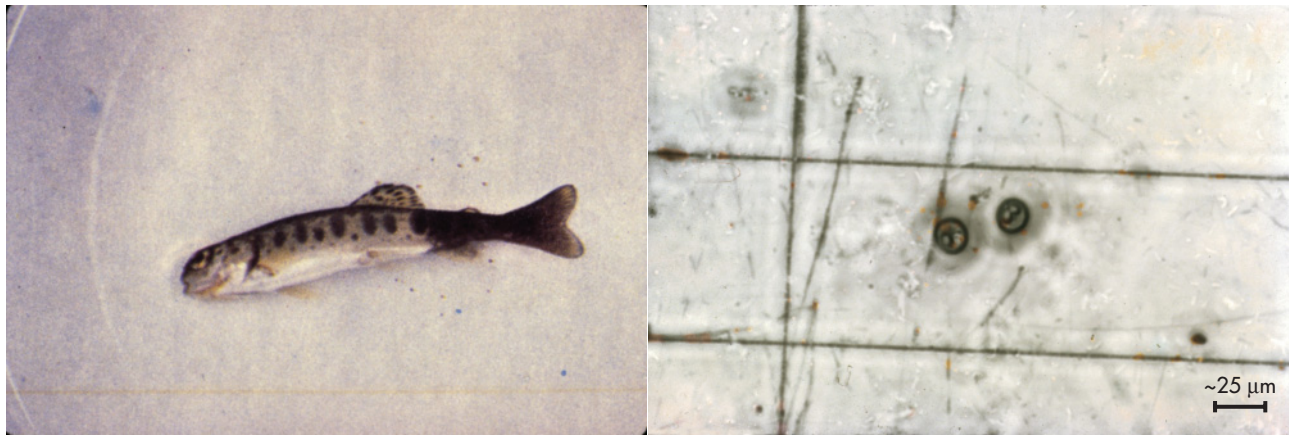

Fig. II-68. A. Rainbow trout with whirling disease. Black tail. B. Wet mount of cartilage digest from a fish with whirling disease, showing the characteristic spores that are almost round in front view, with two pyriform polar capsules. (*A* and *B* photographs courtesy of G. Hoffman.)

and concentrate spores by sedimentation (Markiw and Wolf 1980; MacConnell 2003) (Fig. II-68, B). Spores are highly variable, oval to circular in front view, 7.4–9.7 µm long × 7–10 µm wide × 6.2–7.4 µm thick, with a mucus envelope on the posterior half of the spore (Lom and Dyková 1992). The sporoplasm has an iodinophilous (glycogen) vacuole, which is best seen in fresh spores. This vacuole is characteristic of the genus *Myxobolus*. A nucleic acid test has been developed (Kelley et al. 2004).

Treatment

Whirling disease can be eliminated in culture facilities only by thorough disinfection and quarantine and repopulation with specific-pathogen-free stock. Raising fish in concrete raceways to avoid exposure to mud is also useful. When whirling disease is endemic in a watershed, eradication is impossible; thus, management of the disease requires stocking fish into affected waters only after 6 months of age or at least raising them in concrete vats during this time to reduce infective inoculum. Ultraviolet sterilization of incoming water can eliminate the infective (actinosporean) stage of at least some species (Hedrick et al. 2000) but is difficult to implement in many situations.

Whirling disease is a reportable disease in the United States and exotic salmonids must be certified free of the disease. The disease has been reported from 19 countries, including the United States, and it probably exists in all countries that have imported live or frozen salmonids or salmonid products (Whipps et al. 2004). Spores can survive in fresh and frozen fillets (over 3 months at –4°C [24°F]), posing a danger to nonendemic areas (El-Matbouli and Hoffman 1991a). Spores are killed after 10 minutes at 60°C (140°F), and thus are killed by hot smoking. They survive drying. Spores can survive in water for over 1 year. They also survive passage through the alimentary tract of northern pike and mallard ducks, which means that they could be spread via this route (El-Matbouli and Hoffman 1991b). All spores are killed in 2 days by treating with 25% unslaked lime in 3-cm-deep soil, by adding 380 g unslaked lime/m^2 (Hoffman and Hoffman 1972). They are also killed by 1,600 ppm chlorine for 24 hours or 5,000 ppm chlorine for 10 minutes (Wagner 2002).

PROBLEM 69
Miscellaneous Important Myxozoan Infections

Prevalence Index

WF - 2, WM - 3, CF - 2, CM - 2

Method of Diagnosis

1. Wet mount of affected tissue having typical spores
2. Histology of affected tissue having typical spores

History

Usually wild-caught or pond-raised fish; variously sized nodules that enlarge slowly, if at all

Physical Examination

Usually white or yellowish-colored, variously sized nodules having firm to soft material; other clinical signs depend on the organ system(s) affected (Table II-69, Fig. II-69)

Treatment

None proven

Table II-69. Miscellaneous important myxozoan infections of fish.

Pathogen / disease	Fish host(s)	Site(s)	Invertebrate host	Geographic range	Diagnostic features	References
Henneguya (several species)	Channel catfish	Skin, gills	See PROBLEM 64	United States	Macroscopic and / or microscopic pseudocysts; inter- and intralamellar pseudocysts in gills; interlamellar lesions most pathogenic (can cause severe branchitis and respiratory impairment); skin pseudocysts or diffuse cutaneous masses containing spores (Fig. II-69, A) are usually not important (no effect on carcass quality); also see PROBLEM 64	Minchew (1977) McCraren et al. (1975)
Chloromyxum truttae	Salmonids	Gall bladder, bile ducts	ND	Europe	Emaciation; icterus; hypertrophy of gall bladder; enteritis;	Bauer et al. (1969) Feist and Rintamäki (1994)
Henneguya zschokkei (= *H. salminicola*) (milky flesh)	Salmonids	Muscle	ND	Europe North America	Ulcers from breakdown of large pseudocysts; cysts with milky fluid; fillets unmarketable	Petrushevski and Shulman (1956) Boyce et al. (1985)
Kudoa, *Hexacapsula*, *Unicapsula*, *Pentacapsula* (various species) (tapioca disease; jellied flesh)	Various pelagic and benthic fish	Muscle	ND	Worldwide	Rapid muscle autolysis upon death of fish (within hours of capture; Fig. II-69, B) caused by myxozoans producing microscopic to small macroscopic white pseudocysts having characteristic spores (Fig. II-69, C); decreases carcass value; "soft,""milky," or "jellied" flesh; cysts turn dark (black) with age due to melanization; cooking may stop enzyme activity but for some, cooking softens flesh; *Kudoa* has most muscle-invading myxozoans	Egusa (1978) Lom and Dyková (1992) Moran et al. (1999)
Parvicapsula sp.	Salmonids	Kidney pseudobranch	ND	NW United States	Renal tubular necrosis; nephritis	Johnstone (1985) Yasutake and Elliott (2003)
Parvicapsula minibicornis	Pacific salmon	Kidney	ND	British Columbia, Canada	Slight renal swelling; associated with pre-spawning mortality	Jones et al. (2004)
Myxidium giardi (= *Myxidium matsui*)	American eel Japanese eel	Skin, gill, viscera	*Tubifex tubifex*	United States Japan	Pseudocysts usually not fatal but disfiguring, decrease carcass value	Ghittino et al. (1974) Paperna et al. (1987) Benajiba and Marques (1993)
Myxidium minteri	Salmonids	Kidney	ND	NW United States	Renal tubular degeneration	Yasutake and Wood (1957)
Chloromyxum majori	Rainbow trout chinook salmon	Kidney	ND	NW United States	Glomerulonecrosis	Yasutake and Wood (1957)
Myxobolus pavlovskii	Bighead carp silver carp	Gills	*Tubifex tubifex*	Asia Europe	Branchial necrosis	Molnár (1979) El-Matbouli and Hoffmann (1991b) Molnar (2002)
Myxobolus exiguus	Cyprinids mullets	Gills, skin, stomach, pyloric cecae	ND	Asia Europe Africa	Has caused mass mortalities in mullet	Pulsford and Matthews (1982)

Continued.

Table. II-69. Miscellaneous important myxozoan infections of fish, cont'd.

Pathogen / disease	Fish host(s)	Site(s)	Invertebrate host	Geographic range	Diagnostic features	References
Myxobolus notemegoni	Golden shiner	Skin	ND	United States	Pseudocysts lift scales, causing bristled appearance; increase susceptibility to infection; decreased market value as bait fish	Lewis and Summerfelt (1964) Moore et al. (1984)
Myxobolus argenteus	Golden shiner	Skin	ND	United States	Decreased market value as bait fish	Moore et al. (1984)
Sphaerospora renicola (swim bladder inflammation)	Common carp goldfish	Swim bladder, kidney, blood,	*Branchiura sowerbyi*, *Tubifex tubifex*	Eurasia Israel	0+ carp; locomotion dysfunction; swim bladder chronic inflammation, hemorrhage, thickening, hypertrophy (extrasporogonic stages); peritonitis; renal granulomas with hypertrophy, atrophy, necrosis; sporogonic stages in renal tubule lumen; don't confuse with viral swim bladder inflammation (PROBLEM 83)	Lom and Dyková (1992) Poimanska et al. (1998) Molnár et al. (1999)
Myxobolus koi	Goldfish, koi	Gills	ND	Japan Europe	Proliferative branchitis; can be fatal; infects connective tissue of gill filaments and subcutaneous tissue of head	Hoshina (1952) Crawshaw and Sweeting (1986) Yokoyama et al. (1997)
Hoferellus cyprini	Common carp	Kidney	*Nais* sp.	Europe Asia	Infects renal tubular epithelium in summer, produces trophozoites in fall, spores in winter; abdominal distension; exophthalmia	Bauer et al. (1981) Alvarez- Pellitero et al. (1982) Grossheider and Körting (1992)
Sphaerospora molnari (*S.* cf. *chinensis*)	Common carp goldfish	Gills, skin	ND	Europe Israel United States	Infects skin and gill epithelium, causing hyperplasia / necrosis; dyspnea; can be fatal	Svobodová and Groch (1986) Hedrick et al. (1990) Paperna (1991) Poimanska et al. (1998)
Myxobolus encephalicus	Common carp	Brain	ND	Europe	Encephalitis; locomotion dysfunction; emaciation	Lom and Dyková (1992)
Thelohanellus nikolskii (= *T. cyprini*)	Common carp	Fins	ND	Europe Asia	Pseudocysts on fin rays; rays may break off, causing secondary infections and impaired ambulation	Molnár (1982)
Thelohanellus hovorkai	Koi	Skin	*Branchiura sowerbyi*	Japan	Hemorrhage, ulceration, chronic mortalities	Yokoyama et al. (1998)
Thelohanellus kitauei	Common carp Israel carp	Intestine	ND	Japan Korea	Pseudocysts occlude intestine; emaciation; pressure atrophy of adjacent viscera	Rhee et al. (1993)
Myxobolus artus	Common carp	Skeletal muscle	ND	Japan	Pseudocysts with inflammation, muscle damage	Ogawa et al. (1992)
Chloromyxum cristatum	Common carp grass carp	Liver	ND	Eurasia	Liver necrosis	Lom and Dyková (1984)
Zschokkella nova	Goldfish other cyprinids	Liver	*Tubifex tubifex*	Eurasia	Bile ducts distended with plasmodia; liver atrophy	Lom and Dyková (1992) Uspenskya (1995)
Sphaerospora tincae	Tench	Kidney	ND	Europe (France; Germany)	Externally visible renal hypertrophy; no inflammation	Lom and Dyková (1992)
Triangula percae	Redfin perch	Brain	ND	Australia	Spinal curvature; brain damage	Langdon (1987b)

Continued.

Table. II-69. Miscellaneous important myxozoan infections of fish, cont'd.

Pathogen / disease	Fish host(s)	Site(s)	Invertebrate host	Geographic range	Diagnostic features	References
Myxobolus sandrae	Pike-perch redfin perch	Subcutaneous tissue of head; branchial cavity and gills; spinal cord	ND	Europe	Severe vertebral deformities; unmarketable	Lom et al. (1991b)
Myxobolus buri	Yellowtail	Brain	ND	Japan	Severe scoliosis	Egusa (1985)
Enteromyxum leei	Gilthead seabream	Intestine	Direct	Mediterranean Sea	Chronic, severe enteritis	Fleurance et al. (2008)
Polysporoplasma sparis	Gilthead seabream	Kidney		Mediterranean Sea	Glomerulonephritis	Palenzuela et al. (1999)
Sphaerospora testicularis	European sea bass	Testis	ND	Mediterranean Sea	Damaged seminiferous tubules; impairs male reproduction	Sitjà-Bobadilla and Alvarez-Pellitero (1990)
Kudoa ovivora	Tropical wrasses	Ovary	Possibly none	Caribbean Sea (Panama)	Infected eggs nonviable	Swearer and Robinson (1999)
Enteromyxum scophthalmi (enteromyxosis)	Turbot	Intestine	Direct	Europe	Severe enteritis	Redondo et al. (2003)

ND = not determined; Direct = direct life cycle.
For more details on myxozoan infections, see Feist and Longshaw (2006).

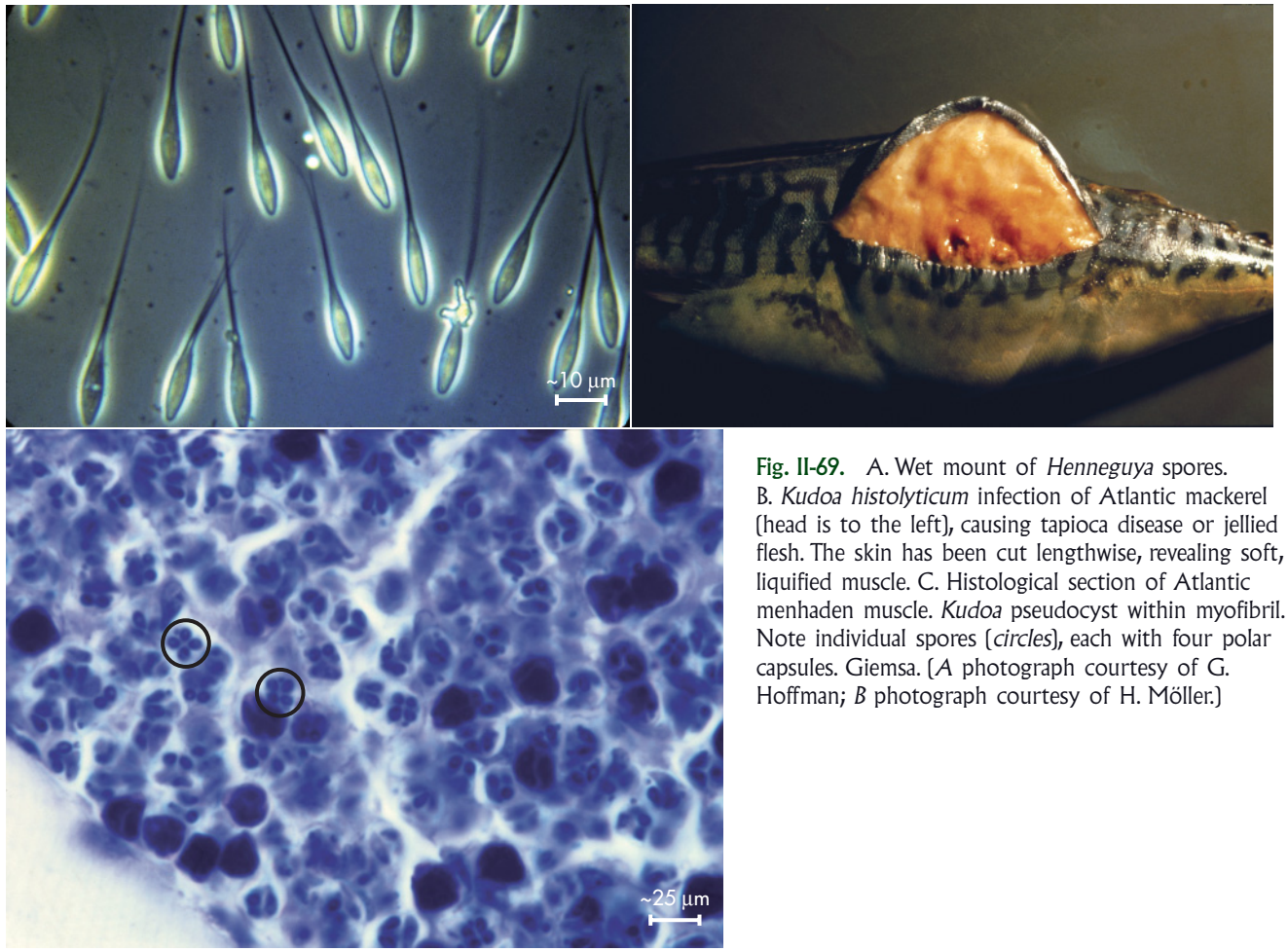

Fig. II-69. A. Wet mount of *Henneguya* spores. B. *Kudoa histolyticum* infection of Atlantic mackerel (head is to the left), causing tapioca disease or jellied flesh. The skin has been cut lengthwise, revealing soft, liquified muscle. C. Histological section of Atlantic menhaden muscle. *Kudoa* pseudocyst within myofibril. Note individual spores (*circles*), each with four polar capsules. Giemsa. (*A* photograph courtesy of G. Hoffman; *B* photograph courtesy of H. Möller.)

PROBLEM 70
Microsporidian Infection

Prevalence Index
WF - 3, WM - 4, CF - 3, CM - 4
Method of Diagnosis
1. Wet mount of affected tissue having typical spores
2. Histology of affected tissue having typical spores

History
Usually wild-caught or pond-raised fish; variously sized nodules that enlarge slowly, if at all
Physical Examination
Usually white or yellowish, variously sized nodules having firm to soft material; other clinical signs depend on the organ system(s) affected
Treatment
1. Disinfect and quarantine
2. Toltrazuril bath
3. Fumagillin oral

COMMENTS
Life Cycle
Microsporidians (class Microsporidia) are not as common as myxozoans, but they are responsible for a number of serious diseases in cultured fish, mostly because of fish morbidity/mortality, but some due to reduced market value from damaged muscle (Lom 2002) (Table II-70). They are often taxonomically specific, infecting only one fish species or a closely related group. However, some species (e.g., *Pleistophora hyphessobryconis*, *Ovipleistophora mirandellae*, *Glugea stephani*, *Glugea anomala*, *Loma salmonae*) can infect a broad range of fish.

While microsporidians have typically been considered protozoa, recent studies suggest that they are a separate phylum (Zygomycota) in the kingdom Fungi (Mathis 2000; Dyková 2006). All microsporidians of fish are intracellular parasites with a direct life cycle. They form a characteristic, thick-walled spore, which contains a sporoplasm. When a host ingests the spore, the sporoplasm is discharged through the channel of a polar tube that is stored coiled within the spore. The sporoplasm then migrates to the target organ and starts a proliferative phase (merogony), producing a large number of cells (meronts) by binary or multiple fission. In the final stages of development, meronts give rise to sporonts, which undergo sporogony, producing mature spores (Fig. II-70, L). Mature spores may be released from lesions on body surfaces (e.g., skin, gills, intestine) or after death of the host.
Epidemiology
Depending on the parasite species and the particular tissue predilection, microsporidian infections may be widely disseminated throughout various organs. They are obligately intracellular, and appear to be cell-specific, infecting only certain cell types in a host but may infect many organs if that cell is widespread throughout the body. How infections spread within a host is unknown; possibilities include migration of meronts and autoinfection, where spores hatch in the individual where they were formed, beginning another propagation cycle. Low temperature significantly slows parasite growth. Vertical transmission occurs in microsporidia of other animals and has been suspected in some fish microsporidia, such as *Loma salmonae* and *Pleistophora ovariae* (e.g., inside the egg). *Pseudoloma neurophila* appears to be transmitted with sexual products (outside the egg).
Pathogenesis
Clinical signs depend on the organ(s) infected (Table II-70) and can range from asymptomatic lesions to mortality. While mild infections may be innocuous, mechanical displacement and tissue disruption caused by parasite growth can lead to serious organ dysfunction (e.g., intestinal blockage, parasitic castration, muscle mass loss) with severe morbidity and/or mortality.

All microsporidians infect a host cell, but some (e.g., *Glugea*) also induce the formation of a tremendously hypertrophied cell that, together with the parasite, forms a xenoma, or xenoparasitic complex. Xenomas appear as whitish, cyst-like structures up to several millimeters in diameter (Fig. II-70, A and B). Some species (e.g., *Ichthyosporidium giganteum*) may form large (up to 2 cm or more) pseudotumors, consisting of many individual xenomas. In some species, mature spores can develop as soon as 3–4 weeks after infection (E. Noga, unpublished data). Infections can provoke a pronounced inflammatory response but there may be little host reaction in the early stages.
Taxonomy
Classification of the Microsporidia has been based on the life cycle, type of spore formation (sporogony), and spore morphology. This has divided microsporidians into two major groups: the "Pansporoblastina" (e.g., *Glugea*, *Pleistophora*, *Thelohania*, *Loma*, *Heterosporis*), where spores develop in membrane-bound packets known as sporophorous vesicles (SPV, pansporoblast membranes), which may be seen in wet mounts of lesions (Fig. II-70, C, J). The number of spores per vesicle is diagnostic. In the other group "Apansporoblastina" (e.g., *Nosemoides*, *Ichthyosporidium*, *Spraguea*, *Microfilum*, *Enterocytozoon*, *Tetramicra*, *Microgemma*), spores are free within the host cell cytoplasm. Recent molecular genetics studies indicate that these two groups are heterogeneous and actually consist of a number of separate groups (Lom and Nilsen 2003). However, this is not important from the standpoint of clinical diagnosis.
Diagnosis
Microsporidian lesions may grossly resemble other pathogens that cause masses, including myxozoans (see PROBLEM 63), ich (see PROBLEM 20), lymphocystis (see PROBLEM 40), dermal metacercariae (see PROBLEM 58), granulomas (see Fig. II-55, C), and

Table II-70. Important microsporidian infections of fish. See Canning and Lom (1986), Lom and Dyková (1992), and Dyková (2006) for more details on microsporidians. Lom (2002) provides a synopsis of the hosts, infection sites, and geographic ranges for all fish microsporidia.

Pathogen / disease	Host(s)	Site(s)	Geographic / ecological range	Diagnostic features	References
Glugea stephani (Fig. II-70, B)	Flatfish (European flounder; plaice; English sole; turbot; 7 other spp.)	Gastrointestinal tract	North Atlantic (M)	Xenomas in connective tissue of gastro-intestinal tract; may be important cause of natural mortality	Cali et al. (1986)
Tetramicra brevifilum	Turbot	Muscle	England Spain (M)	0+ fish with muscle nodules that show through skin; degenerate muscle fibers impair swimming	Matthews and Matthews (1980) Figueras et al. (1992)
Ichthyosporidium giganteum	Corkwing wrasse; ocellatus wrasse spot		France Black Sea; Eastern United States (M, E)	Large masses in subcutaneous and adipose tissues; can produce large ventral body swelling with xenomas	Sprague and Hussey (1980)
Glugea hertwigi (Fig. II-70, A)	Smelts (*Osmerus*)	Viscera	Holarctic (F, E)	Xenomas mainly in intestine; intestinal obstruction; lower fecundity; fish kills in late spring after spawning	Nepszy et al. (1978)
Glugea luciopercae	Pike-perch	Intestine	Asia Europe (F, E)	Intestinal damage in pike-perch	Dogel and Bykhovski (1939)
Heterosporis (= *Pleistophora*) *anguillarum* (Beko disease)	Japanese eel	Muscle	Japan Taiwan	Yellowish nodules on body surface, forming irregular indentations; chronic mortality; slow growth; decreased market value	T'sui and Wang (1988)
Glugea plecoglossi	Ayu; rainbow trout (EX)	Most tissues	Japan (F)	Xenomas may bulge from body surface	Takahashi and Egusa (1977)
Loma salmonae	Salmonids (*Oncorhynchus* spp.)	Gill	North America Japan France (F)	Xenomas on gill; heavy infections cause high mortalities; can assay via gene test	Putz (1964) Shaw et al. (2000) Brown and Kent (2002)
Nucleospora (= Enterocytozoon) salmonis	Chinook salmon; rainbow trout	Kidney, spleen	Northwest United States British Columbia, Canada France	Associated with anemia, leukemia; infects nuclei of leukocytes in blood, spleen, kidney; can assay via gene test	Hedrick et al. (1990b, 1991a) Brown and Kent (2002)
Kabatana (= Microsporidium) takedai	Salmonids	Muscle	Japan	Very common; only infects heart in chronic form (low temperature); also in skeletal muscle in acute form with high mortality	Awakura (1974) Urawa (1989)
Kabatana (= Microsporidium) seriolae (Beko disease)	Yellowtail	Muscle	Japan (M)	Depressions on skin in areas of degenerated muscle; caseous consistency to muscle	Egusa (1982)

Heterosporis finki	Freshwater angelfish	Esophagus, muscle	Germany (F)	Infects connective tissue of esophagus, forming nodules; infected muscles milky white with creamy consistency; emaciation; up to 5 mm necrotic foci on body surface	Michel et al. (1989)
Heterosporis schuberti	Egyptian mouthbrooder; bristlenose catfish (aquarium fish)	Muscle	(F)	Emaciation; myocytes not very hypertrophied	Lom et al. (1989)
Pleistophora hyphessobryconis (neon tetra disease) (Fig II-70, I, J)	16 species (mostly tetras); striped barb; zebrafish; goldfish	Muscle	Worldwide (F)	Focal color loss/fading with white patches under skin; body contorted from muscle damage; emaciation; in heavy infections, may spread to connective tissue of intestine, ovary, skin: sometimes can detect spores in skin scrapings; clinical signs for 2–4 weeks	Nigrelli (1953) Dyková and Lom (1980)
Glugea anomala	Threespine stickleback; ninespine stickleback; tropical killies	Most tissues	United States Europe Asia (F)	Xenomas in virtually any tissue; may bulge from surface. Uncertain if killie parasite is same species	Canning et al. (1982) Lom et al. (1995) Kurtz et al. (2004)
Glugea (=*Nosema*) *pimephales*	Fathead minnow	Viscera	United States (F)	Xenomas in viscera of fry; high mortality	Morrison et al. (1985)
Pleistophora ovariae	Golden shiner; fathead minnow	Ovary	United States (F)	Ovary mottled with white spots and streaks; parasitic castration; very common, especially spawning season (May–June)	Nagel and Summerfelt (1977)
Glugea (=*Pleistophora*) *cepedianae*	Gizzard shad	Viscera	Ohio, United States (F)	Xenomas in peritoneal cavity; protrude from 0+ fish; only 1 xenoma/fish	Price (1981)
Glugea heraldi	Lined seahorse	Skin	Florida, United States (M)	Xenomas protrude from subcutis of skin	Blasiola (1979)
Pseudoloma neurophila (skinny disease)	Zebrafish; neon tetras	Central nervous system (spinal cord and hindbrain)	United States (zebrafish research laboratories) (F)	The most common pathogen in zebrafish research facilities; emaciation, spinal curvature (e.g., scoliosis); a few spores also in muscle; also found in clinically normal fish; xenomas in CNS; probably can be transmitted vertically; PCR assay under development	Matthews et al. (2001) Kent and Fournie (2007)

F = freshwater; M = marine; E = estuarine; EX = experimental host.

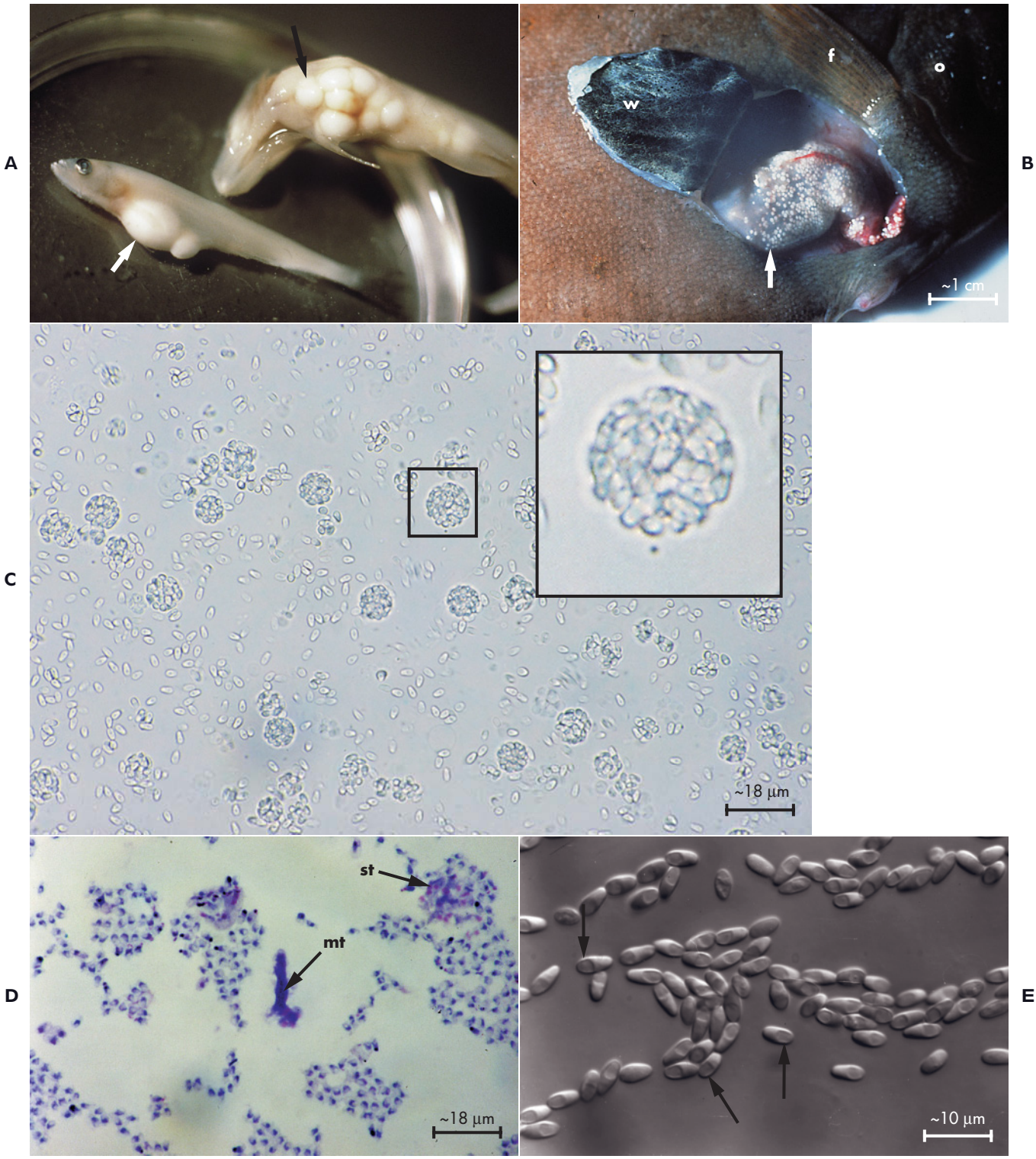

Fig. II-70. A. *Glugea hertwigi* infection that produces large pseudotumors (*arrows*) in the viscera of European smelt. B. *Glugea stephani* infection of dab. The abdominal wall (*w*) has been cut away, revealing the intestine with numerous xenomas (*arrow*). Head is to the right; *f* = pectoral fin; *o* = gill operculum. C. Wet mount of sporophorous vesicles (SPVs; *inset*) of a *Glugea* sp. Some SPVs are breaking up, releasing individual spores. D. Stained smear of a *Glugea* xenoma with developmental stages; *mt* = meront; *st* = sporoblast. Modified Wright's. E. Individual microsporidian spores. Note egg shape and vacuole (*arrows*) at posterior end of spore.

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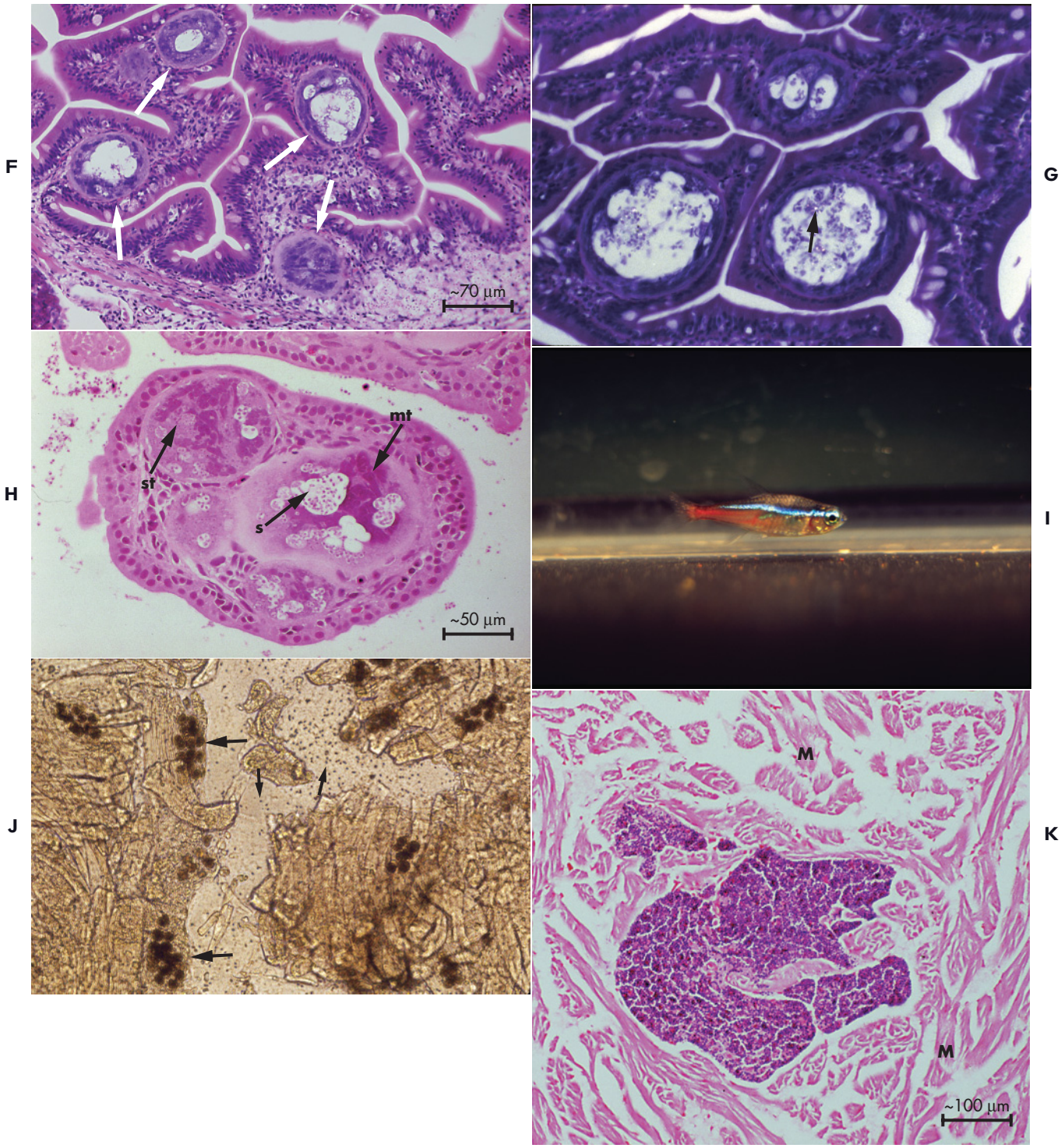

Fig. II-70.—cont'd. F. Histological section through xenomas (*arrows*) in the intestine of a killifish. Hematoxylin and eosin. G. Histological section in *F* viewed under polarized light. Note the birefringent (glowing) spores (*arrows*). Hematoxylin and eosin. H. Grazing histological section through wall of a xenoma; *mt* = meronts; *st* = sporonts; *s* = spores. Hematoxyin and eosin. I. Neon tetra disease (*Pleistophora hyphessobryconis* infection) in a neon tetra. Note the depigmentation along the dorsum due to infection of underlying muscle. J. Wet mount of sporophorous vesicles of *Pleistophora hyphessobryconis* (*large arrows*). Some SPVs are breaking up, releasing individual spores (*small arrows*). K. Histological section through muscle (*m*) of Atlantic menhaden, with Gram-positive spores of a *Pleistophora* sp. Gram's.

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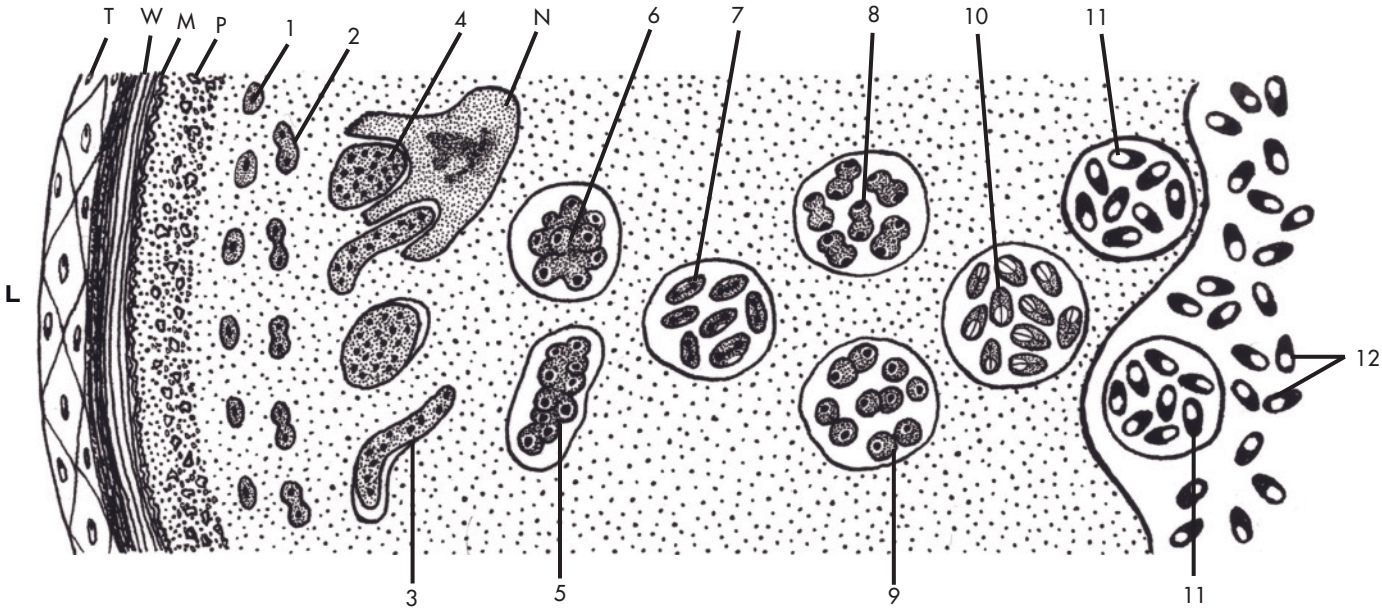


Fig. II-70.—cont'd. L. Diagram of a xenoma (*Glugea anomala* type), showing sequential development to form mature spores. Various developmental stages are similar in all microsporidians; *1* = uninucleate meront; *2* = dividing meront, forming multinucleated meront (*3*); *4* = multinucleated meront rounding up; *5* = elongate sporogonial plasmodium beginning to segment into sporoblast mother cells within a sporophorous vesicle (SPV; indicated by clear space); *6* = sporogonial plasmodium segmenting into sporoblast mother cells; *7* = sporoblast mother cells, which divide (*8* and *9*), producing sporoblasts (*10*); *11* = SPV with spores (*12*); spores are also free within the center of the xenoma. Note the spore's typical egg shape and prominent posterior vacuole; *T* = host connective tissue; *W* = xenoma cell wall; *M* = cell membrane of xenoma; *P* = periphery of xenoma, with increased pinocytotic activity; *N* = host cell nucleus. (*A* and *B* photographs courtesy of H. Möller; *C, D, F, G,* and *H* photographs by L. Khoo and E. Noga; *E* photograph courtesy of J. Lom; *I* from Lom and Dyková 1992.)

neoplasia (see PROBLEM 76); they are easily distinguished by examining wet mounts or histological material for spores.

Diagnosis of microsporidian disease is based on the identification of microsporidian spores in target tissues that have appropriate clinical signs (Fig. II-70, A through K). The presence of spores that are small (2–10 µm, usually 7 µm or less), egg-shaped to elliptical, and have a prominent posterior vacuole (Fig. II-70, E) is diagnostic for microsporidia.

Spores have a polar tube (typically not seen when using routine light microscopy) coiled inside the anterior part of the cell; unlike the Myxozoa, they have no polar capsule. Microsporidian spores are the only "protozoan" spores that are Gram-positive (Fig. II-70, K). Not all spores within a lesion may be Gram-positive. Birefringence also differentiates them from other protozoan spores (Tiner 1988) (Fig. II-70, G). Some spores are acid-fast. Microsporidia do not have mitochondria. Chitin-binding fluorochromes (e.g., Fungi-Fluor, Calcofluor [Polysciences]) are highly sensitive means of detecting spores in smears or histological sections, since microsporidian spores contain chitin (Weber et al. 1999).

Morphological criteria for definitive identification to genus and species is based upon spore morphology, especially developmental stages and their interaction with host cells. These can only be determined via electron microscopy. Gene probes have also been developed for some microsporidia.

Treatment

Spores are typically resistant to environmental conditions and can often survive for over 1 year at low temperatures. Spore inactivation may require high germicide doses. *Pseudoloma neurophilia*, and *Glugea anomala* require >100 or 1,500 ppm chlorine, respectively, to achieve >95% spore death (Ferguson et al. 2007). Aside from disinfection and quarantine, there are no proven remedies for microsporidian infections and drugs have not been tested against most species. Toltrazuril has shown some efficacy experimentally against *Glugea anomala* (Schmahl et al. 1990). Fumagillin has successfully treated several experimental microsporidian infec-

tions. Albendazole also has potential for treating *Loma salmonae*, and quinine hydrochloride delays xenoma formation (Speare et al. 1999). Monensin can prevent infection of *Loma salmonae* (Becker et al. 2002).

Lowering the temperature can slow and in some cases completely halt disease progression (Dyková 2006), but is rarely feasible. Because of possible vertical transmission in at least some microsporidia, it is probably best to avoid use of infected fish as broodstock.

PROBLEM 71
Ichthyophonosis (Swinging Disease)

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

1. Culture of *Ichthyophonus*
2. Wet mount of lesion (skin or viscera) with sporulating organism
3. Histopathology of pathogen

History/Physical Examination

Emaciation; usually shallow skin ulcers; sandpaper-like texture to skin; vertebral curvature

Treatment

Avoid exposure to contaminated feed

COMMENTS

Epidemiology

Ichthyophonus is a fungus-like agent that causes a chronic, systemic, granulomatous disease. It is endemic in many feral, cold water marine fish populations and has been reported in over 80 species of marine fish (McVicar 1982, 1999; Hershberger et al. 2002). Epidemics have occurred in Atlantic herring and yellowtail flounder in the northwest Atlantic Ocean, haddock and plaice in the northeast Atlantic, Pacific herring and rockfish in the eastern Pacific, and cod in the Baltic Sea (Noga 1993c; Yanong 2003). It is probably a significant cause of chronic mortality in some feral marine fish populations (McVicar 1999).

While it is rarely a problem in cultured fish, *Ichthyophonus* has infected freshwater fish that were fed contaminated marine offal (Wood 1974). The source of all epidemics in freshwater fish appears to be some form of contact with a marine species (McVicar 1999). Even though wild stocks are a significant reservoir, it is not a serious problem in marine cage-cultured fish (McVicar 1999). It is considered by some to be common in aquarium fish (Reichenbach-Klinke 1973), but there are no recent published reports substantiating this claim. Its supposedly high prevalence may be due to the misidentification of mycobacteriosis (see PROBLEM 55). Most isolates have been identified as *I. hoferi*, although genetic analysis indicated that an isolate from yellowtail flounder was a different species, named *I. irregularis* (Mendoza et al. 2002).

Life Cycle

The life cycle of *I. hoferi* is complicated, involving production of multinucleated spores (Fig. II-71, B), which produce endospores. Hyphae may or may not be produced before endospore formation. Endospores are disseminated to new hosts or to other parts of the same host. The endospores then produce multinucleated spores. McVicar (1982, 1999) discusses the life cycle in detail. *Ichthyophonus* is an obligate pathogen, but resting spores can survive in seawater for 2 years (McVicar 1999). *Ichthyophonus* is a member of a newly created group of microorganisms at the boundary between animals and fungi. This novel phylogenetic group has been referred to as the DRIP clade (an acronym of the original members: *Dermocystidium* [PROBLEM 42], rosette agent [PROBLEM 75], *Ichthyophonus*, and *Psorospermium*), forming the class Mesomycetozoea, kingdom Protista (Protoctista) (Arkush et al. 2003).

Pathogenesis

Lesions of ichthyophonosis are most common in highly vascularized organs, such as heart, spleen, kidney, and liver. The acute form, which takes several weeks to develop, involves invasion of tissue with little inflammatory response. In the chronic form, there is a strong, chronic inflammatory response to invasion (Fig. II-71, B). White or dark (pigmented) nodules may be present on various organs (Fig. II-71, A). Lesions on the skin may be rough ("sandpaper-like") or ulcerated. Neurological signs (swinging disease) are common in freshwater salmonids because of central nervous system involvement (Wood 1974). Fish may also have spinal curvature and darkening of the skin.

Diagnosis

Ichthyophonus can often be identified from fresh lesion material. Typical, thick-walled spores surrounded by granulomatous inflammation are seen on fresh wet mounts; in tissue of some affected species, spores readily germinate within 30 minutes but are best re-examined at least 5 hours after preparation if germination is not detected (McVicar 1982, 1999). The germinating spore is flask-shaped, with a neck that consists of a hypha that breaks through the outer wall (Fig. II-71, C). The germinating spore is pathognomonic. Characteristic life stages can also be identified in histological sections, including the spore (usually the most common stage; Fig. II-71, B), germinating spore with hyphae, and hyphae. *Ichthyophonus* is PAS- and silver-positive. Cultures from lesions can also be established using Sabouraud dextrose agar with 1% serum (McVicar 1982).

Treatment

There is no treatment. Avoidance or pasteurization of contaminated feed should be advocated. Ichthyophonosis can render fillets unmarketable, with a foul odor and poor flesh texture (e.g., muscle liquefaction, nodules in

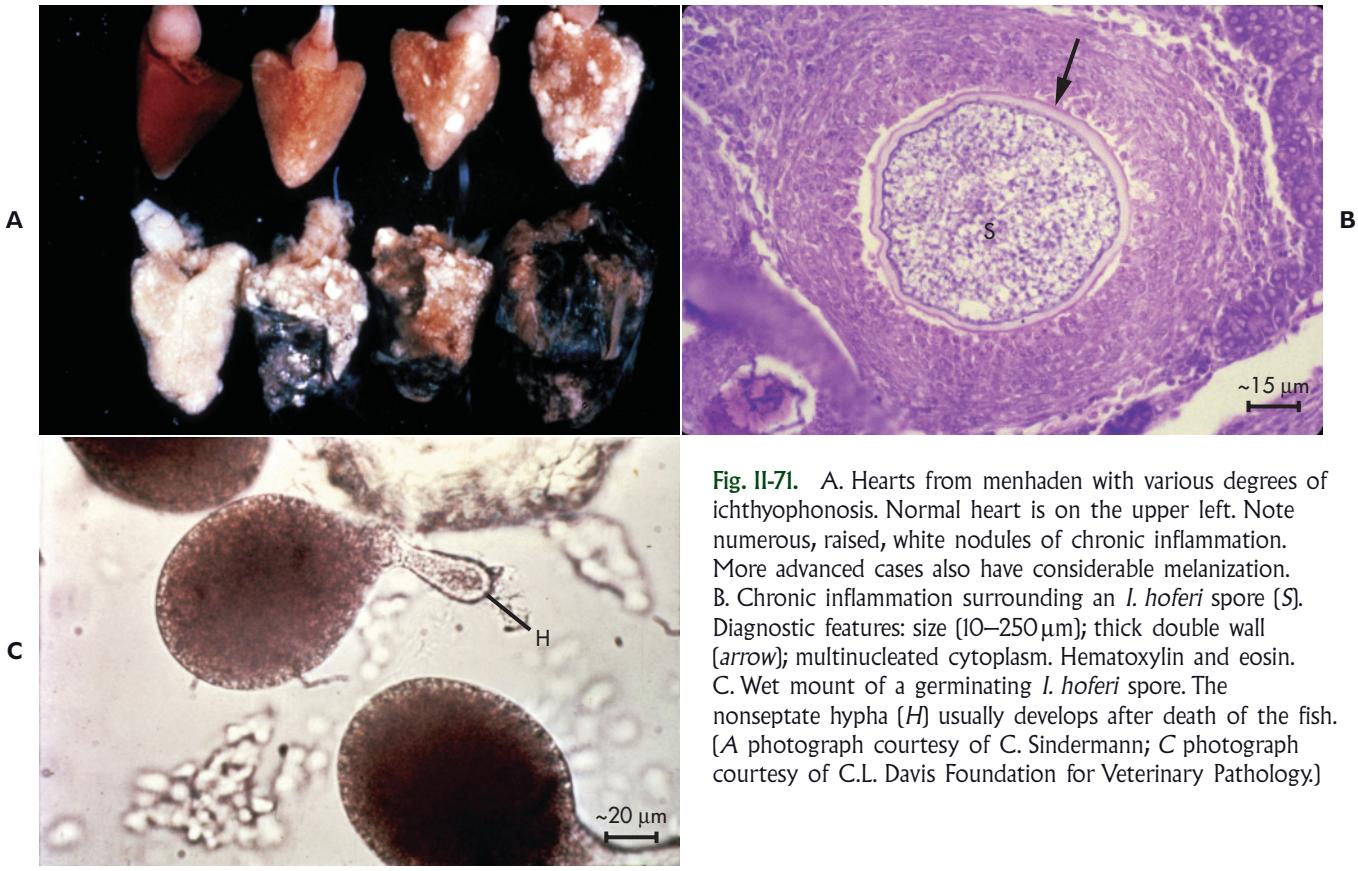


Fig. II-71. A. Hearts from menhaden with various degrees of ichthyophonosis. Normal heart is on the upper left. Note numerous, raised, white nodules of chronic inflammation. More advanced cases also have considerable melanization. B. Chronic inflammation surrounding an *I. hoferi* spore (*S*). Diagnostic features: size (10–250 µm); thick double wall (*arrow*); multinucleated cytoplasm. Hematoxylin and eosin. C. Wet mount of a germinating *I. hoferi* spore. The nonseptate hypha (*H*) usually develops after death of the fish. (*A* photograph courtesy of C. Sindermann; *C* photograph courtesy of C.L. Davis Foundation for Veterinary Pathology.)

muscle). Infected fillets should be culled, since they can contaminate normal fillets by contact.

PROBLEM 72
True Fungal Infections

Prevalence Index
WF - 4, WM - 4, CF - 4, CM - 4
Method of Diagnosis
1. Wet mounts or histology with fungus
2. Culture of fungus
History
Varies with organ affected
Physical Examination
Varies with organ affected
Treatment
None proven

COMMENTS

Compared to water mold infections (PROBLEMS 34, 35), true fungal infections (Table II-72) are rare; most have been encountered as sporadic cases, although some have caused localized epidemics. Virtually all of these diseases are chronic infections, although some can eventually cause high mortalities. Most are probably taking advantage of a stressed host (Noga 1990; Rand 1996; Yanong 2003). Presumptive diagnosis of at least the major group responsible (e.g., yeast, dematiaceous fungus) can often be discerned from histology or wet mounts of lesions. For example, *Ochroconis* and *Exophiala* are dematiaceous fungi, which have pigmented hyphae (chromomycoses) (Fig. II-72). Thus, a wet mount with

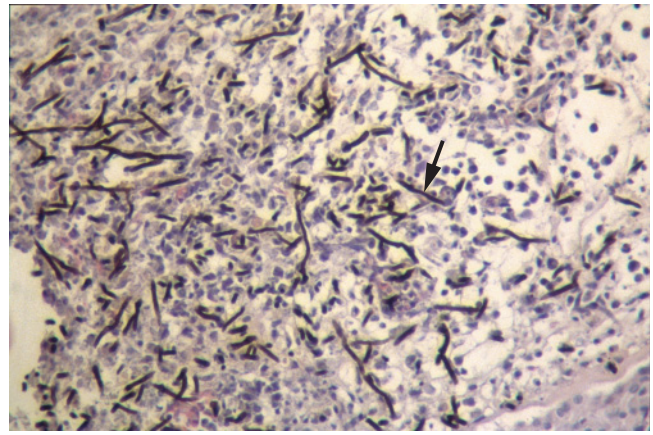


Fig. II-72. Histological section of oral region of a false percula clownfish with *Cladosporium* infection. Note the black-pigmented hyphae (*arrow*). Hematoxylin and eosin. (Photograph by U. Silphaduang and E. Noga.)

Table II-72. True fungal infections of fish. All of these infections are usually associated with chronic morbidity/mortality. Alternate classification is from Barnett and Hunter (1998).

Pathogen	Alternate classification	Hosts	Geographic range	Key diagnostic features	References
HYPHOMYCETES					
Fusarium solani	M	Vidua triggerfish scrawled filefish, marine angelfish, bonnethead shark, scalloped hammerhead shark	Canada Maryland, United States (aquaria) marine	Deep mycosis with chronic inflammation	Ostland et al. (1987) Muhvich et al. (1989) Crow et al. (1995) Yanong (2003)
Fusarium culmorum	M	Common carp	Europe freshwater	Infection of eyes and skin	Horter (1960)
Fusarium oxysporum	M	Red sea bream	Japan marine	Deep mycosis	Hatai et al. (1986b)
Fusarium moniliforme *Fusarium udum*	M	Rohu, tire track eel, tengra pool barb, tapah catfish green snakehead	India freshwater	Depigmentation; scale loss; fin erosion; dermal hemorrhage; necrosis; mass mortalities	Deepa et al. (2000)
Paecilomyces farinosus	M	Atlantic salmon	Scotland marine	Reddened vent; swollen abdomen; may be infected from insect larvae; infects swim bladder	Bruno (1989)
Paecilomyces marquandii	M	Hybrid red tilapia	United States freshwater	Infects kidney	Lightner et al. (1988)
Paeciliomyces lilacinus	M	Blue tilapia Mozambique tilapia	Puerto Rico, United States freshwater	"Tilapia wasting disease"; skin erosion and hemorrhage; granulomas in viscera	Rand et al. (2000)
Penicillium corylophilum	M	Red snapper	Gulf of Mexico, United States marine	Swim bladder infection, possibly due to contamination after deflating swim bladder with needle	Blaylock et al. (2001)
Aspergillus flavus	M	Tilapia (*Sarotherodon* sp.)	Kenya freshwater	?	Olufemi et al. (1983)
Aspergillus niger	M	Tilapia (*Sarotherodon* sp.)	Kenya freshwater	?	Olufemi et al. (1983)
Candida sake	M	Amago salmon	Japan freshwater	Distended stomach with viscid, turbid, fluid having many yeast cells	Hatai and Egusa (1975)
Candida albicans	M	Grey mullet	Italy marine	Isolated from skin lesions and muscle	Macri et al. (1984)
Cryptococcus sp.	M	Tench	Italy freshwater	Bilateral exophthalmos	Pierotti (1971)
Lecythophora mutabilis	M	Zebra danio	Massachusetts, United States freshwater	Fungal hyphae protruding from mouth, anus, and operculum of fry; associated with low hardness	Dykstrá et al. (2001)
Exophiala salmonis ("cerebral mycetoma")	D	Atlantic salmon, lake trout, cutthroat trout	Alberta, Canada Scotland North Carolina, United States freshwater and marine	Ataxia; erratic swimming; exophthalmos; cranial ulcers; chronic inflammation with many giant cells, especially in posterior kidney	Carmichael (1966) Richards et al. (1978) Alderman (1982)
Exophiala piscifila	D	Saltwater catfish, dogfish shark, channel catfish	New York, United States (aquarium) Alabama, United States freshwater and marine	Skin ulcers and focal necrosis of viscera often with chronic inflammation	Gaskins and Cheung (1986) Fijan (1969)
Exophiala angulspora	D	Atlantic wolfish, lumpfish, thornyback ray	United States marine	Head ulcers and grossly black, granulomas in viscera	Yanong (2003)

Continued.

Table. II-72. True fungal infections of fish. All of these infections are usually associated with chronic morbidity/mortality. Alternate classification is from Barnett and Hunter (1998), cont'd.

Pathogen	Alternate classification	Hosts	Geographic range	Key diagnostic features	References
Exophiala jeanselmei-like	D	Rainbow trout	England freshwater	Kidney infection	Alderman and Feist (1985)
Exophiala sp.	D	Atlantic salmon	Norway		Langvad et al. (1985)
Exophiala-like	D	Atlantic cod Seahorse Xanthichthys triggerfish Tautog (E) Flatfish (E) Fundulus (E)	Connecticut, United States marine (aquarium)	Nonulcerated dermal masses; raised white to yellow foci on viscera; acute necrosis or chronic inflammation in response to fungus	Blazer and Wolke (1979)
Cladosporium sphaerospermum	D	Red snapper	Gulf of Mexico, United States marine	Swim bladder infection, possibly due to contamination after deflating swim bladder with needle	Blaylock et al. (2001)
Cladosporium sp.	D	Atlantic cod	? marine	?	Reichenbach-Klinke (1956)
Cladosporium sp.	D	Tomato clownfish	North Carolina, United States (aquarium) marine	Deep dermal ulcer	Silphaduang et al. (2000)
Ochroconis humicola	D	Silver salmon, coho salmon, rainbow trout	United States freshwater	Low, chronic mortality with occasional skin ulcers; fluid in peritoneal cavity; adhesions; kidney often affected; necrosis with lymphocytic infiltrate	Ross et al. (1975) Ajello et al. (1977)
Ochroconis tshawytschae	D	Chinook salmon	California, United States freshwater	Infects posterior kidney	Doty and Slater (1946)
Ochroconis sp.	D	Yamame salmon, masu salmon	Japan freshwater	Kidney infection that may spread to other organs (visceral mycosis); chronic inflammation	Kuroda et al. (1986) Hatai and Kubota (1989)
Phialophora sp.	D	Atlantic salmon	Scotland		Ellis et al. (1983b)
Aureobasidium sp. (?)	D	Stingray	Germany marine (aquarium)	Hepatomegaly and fluid in peritoneal cavity; disease experimentally reproduced in common carp	Otte (1964)
COELOMYCETES					
Phoma herbarum	S	Silver salmon, chinook salmon, rainbow trout	Northwest United States England freshwater	Chronic infection of swim bladder that may extend to other tissues, causing necrosis and chronic inflammation	Ross et al. (1975)
Phoma sp.	S	Ayu	Japan freshwater	Infects swim bladder	Hatai et al. (1986a)
UNCERTAIN TAXONOMY					
Sarcinomyces crustaceus	—	Black sea bream	Italy marine	Exophthalmic eye	Todaro et al. (1983)

M = Moniliaceae, D = Dematiaceae, S = Sphaeropsidales.

pigmented hyphae would suggest that one of these agents may be involved.

Definitive identification of the specific fungus responsible requires fungal isolation. A relatively nutrient-poor, plant-based medium (e.g., potato flake agar, CM+) is a good general-purpose medium for isolation, since almost all pathogenic fungi are opportunists which would prefer to grow in soil or on plant tissue. Media such as Sabouraud's dextrose agar were designed to mimic mammalian tissue. Thus, they are nutrient-rich, which can suppress the induction of sporulation needed for identification and encourage bacterial growth (M.J. Dykstra, personal communication). See **p. 55** for details on culture. Since many of these fungi are common soil saprophytes, multiple samples, preferably from aseptically cultured internal lesions, should be done to reduce the chance that contaminants are cultured instead of the pathogen. Histological confirmation of tissue damage by specific fungi is also advisable.

PROBLEM 73
Diplomonad Flagellate Infection (Spironucleosis, Hexamitosis)

Prevalence Index
WF - 2, CF - 3, CM - 4
Method of Diagnosis
1. Wet mount of skin, feces, or viscera with parasites
2. Histopathology of lesion with parasites

History
Anorexia, chronic mortalities
Physical Examination
Abdominal swelling, exophthalmos, cachexia
Treatment
1. Metronidazole oral
2. Metronidazole prolonged immersion
3. Magnesium sulfate oral
4. Raise temperature to 35°C (95°F) for 7 days

COMMENTS
Epidemiology/Pathogenesis
Diplomonad flagellates, comprised of various *Spironucleus* species, have long been associated with gastrointestinal disease in salmonids and aquarium fish. Predisposing stress appears to play an important role in initiating disease, since these and similar flagellates (*Chilomastix*, *Trimitus*, *Tritrichomonas*, *Protrichomonas*, *Monocercomonas*) often reside in the gastrointestinal tract of clinically normal fish, including many other fish species (Noble and Noble 1966; Brugerolle 1980; Lom and Dyková 1992; Woo 2006). While diplomonad infections in fish have traditionally been attributed to *Hexamita*, more careful taxonomic studies have shown that these infections in fish appear to be exclusively due to *Spironucleus* species (Poynton et al. 2004).

Salmonid Infections
Spironucleus salmonis (formerly *Hexamita* [= *Octomitus*] *salmonis*) infects debilitated or stressed freshwater salmonids and has also been reported from seawater-cultured salmon (Mo et al. 1990; Kent et al. 1992; Poynton 2003). It primarily infects the anterior intestine and pyloric ceca, but, in advanced cases, it can spread to the gall bladder and other organs, causing high mortality (Wood 1976). Fish may have abdominal distension caused by fluid accumulation in the gut or may have exophthalmos. Fish may be emaciated and thus the head may appear relatively large (pinheads) (Wooten 1989). Histologically, gastrointestinal lesions may range from no visible damage to severe enteritis. *Spironucleus barkhanus* affects cage-cultured salmonids (Sterud et al. 2003). It does not infect the gut but rather, after proliferating in the blood, it localizes in internal organs, muscle and skin (Guo and Woo 2004).

Aquarium Fish Infections
In aquarium fish, related parasites of the genus *Spironucleus* (*S. vortens*, *S. elegans*) infect primarily cichlids and anabantids, causing cachexia, gastroenteritis, and peritonitis (Lom and Dyková 1992; Poynton and Sterud 2002). Parasites may eventually spread to other organs. Many cases of spironucleosis in aquarium fish are mixed infections that involve other parasites or bacterial opportunists (e.g., *Capillaria* nematode infections in angelfish; Ferguson and Moccia 1980). *Spironucleus* also commonly infects grass carp and other cyprinids (Molnár 1974). There is evidence that some amphibians can act as vectors (Lom and Dyková 1992; Poynton 2003).

Spironucleosis/hexamitosis has also been associated (mainly in the aquarium literature) with an idiopathic problem known as lateral line depigmentation (see PROBLEM 100). However, a role for diplomonad flagellates in causing this problem is not strongly supported.

Infections in Other Fish
Diplomonad flagellates have been identified in the gut of many other fish, including members of the families Acipenseridae, Anguillidae, Catostomidae, Centrarchidae, Cyprinidae, Cyprinodontidae, Gadidae, Gasterosteidae, Mugilidae, Percichthyidae, Percidae, Siganidae, and Sparidae. They are usually incidental findings in these species.

Diagnosis
Determining the infection intensity is important for both treatment and prognosis. Fecal exam may reveal the presence of typical trophozoites, but necropsy will give a more accurate indication of the degree of infection, since trophozoites are often localized in the anterior intestine. Determining degree of infection is important, as diplomonad flagellates are often present subclinically. Post (1987) has the following recommendations for grading severity of infections in salmonids when observed in the low power field of a microscope:

1. Occasional field with 1–5 organisms: no treatment needed
2. Average of 5–15 organisms in the field: no treatment needed unless no other cause of poor health is identified; watch closely for more serious infections
3. Average of 15–30 organisms in the field: treatment needed
4. Average of 30–100 organisms in the field: severe infection and therapy essential

Note that numbers in this scoring protocol will probably vary with other fish species. It is also advisable to examine fresh preparations of blood, internal organs (spleen, liver), muscle and skin lesions since these are targets of systemic infections.

A protozoan can be identified as a diplomonad flagellate based upon the typical morphological criteria present in all members of this group (Fig. II-73). Trophozoites are active, swimming rapidly forward; therefore, preparing fixed smears may facilitate identification. Alternatively, fresh preparations can be treated with a thickening agent (e.g., Protoslo, Carolina Biological) to allow more detailed examination. Identification to genus and to species requires the use of electron microscopy (Poynton 2003). However, this is not done in routine clinical diagnoses, which are typically based on the characteristic diplomonad morphology (Fig. II-73) and hyperactive motility in live preparations. Whether there are species differences in response to treatment is unknown. A cyst is produced by some species but is more difficult to identify in clinical specimens than the trophozoite. Diplomonad cysts are ~7 × 10 µm and filled with glycogen (turn brown when treated with iodine).

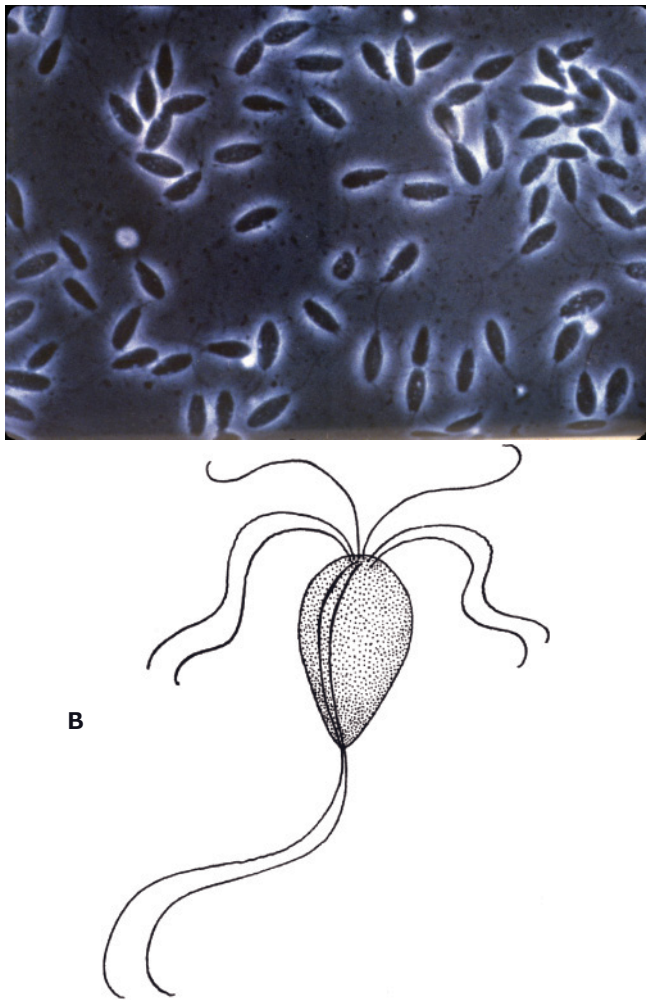


Fig. II-73. A. Wet mount of diplomonads. B. Diagram of a typical diplomonad flagellate with diagnostic features: size (from 5 to 20 µm long, excluding the flagella); eight flagella (three pairs anteriorly, one pair posteriorly); pyriform to ellipsoidal to egg-shape to tapering body. (*A* from Hoffman and Meyer 1974.)

In discus, do not confuse diplomonad infection with *Protoopalina* (see PROBLEM 75), which is easily differentiated by its larger size and slower, ciliate-like movement.

Treatment

Metronidazole is usually effective as a bath. Metronidazole, as well as the related secnidazole and triclabendazole also appear to be highly effective as oral medications but are too expensive for most commercial fish producers (Tojo and Santamarina 1998a). Magnesium sulfate has successfully treated freshwater salmonids, presumably acting as a cathartic. Raising the temperature has also been suggested for aquarium fish tolerant of this treatment. Many diplomonad flagellates are probably capable of a free-living existence. Treatment should always include improving environmental problems.

PROBLEM 74
Tissue Coccidiosis

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

1. Wet mount of affected tissue having oocysts
2. Histological section of affected tissue with parasite life stages

History

Varies with organs affected; may be acute or chronic

Physical Examination

Varies with organs affected (Table II-74)

Treatment

Monensin oral

COMMENTS

Epidemiology

Some coccidia are hemoparasites (see PROBLEM 44), but the most important fish pathogens affect solid tissues. Virtually all tissue coccidians that infect fish belong to the family Eimeriidae (*Eimeria*, *Goussia*, *Crystallospora*, and *Calyptospora*) (Lom and Dyková 1992; Perkins et al. 2000). The family Cryptosporidiidae includes several

Table II-74. Common and/or pathogenic coccidian infections of fish. See Lom and Dyková (1992) and Molnár (2006) for details on other coccidia.

Pathogen/ disease	Host(s)	Site(s)	Geographic/ ecological range	Diagnostic features	References
INTESTINAL FORMS					
Eimeria truttae	Brown trout, brook trout, masu salmon	Intestine	Europe Canada Japan (F)	No reported pathogenicity, but very common	Molnár and Hanek (1974)
Eimeria (= *Epieimeria*) *anguillae*	*Anguilla* sp. eel		Europe	Epithelial infection causes erosion, ulceration; can lead to emaciation and death	Hine (1975)
Goussia subepithelialis	Common carp	Intestine	Europe (F)	Common cause of nodular coccidiosis; usually 1+ carp; sporulation at >14°C	Marincek (1973) Studnicka and Siwicki (1990)
Goussia carpelli (dwindles)	Cyprinids	Intestine	Europe United States (F)	Very common in carp and crucian carp; most severe with overwintering stress; can cause high mortalities in goldfish fry after transport stress; can transmit directly and via tubificids or small crustaceans; infects epithelial cells, causing necrosis, ulceration; diffuse enteritis; *Goussia cheni* and *G. mylophanyngodoni* are similar, from east Asian herbivorous cyprinids	Steinhagen et al. (1989)
Goussia iroquoina	*Notropis* sp., *Pimephales* sp., other minnows	Intestine	Canada (F)	Primarily affects fry	Paterson and Desser (1982)
Goussia vanasi	Tilapia spp., *Pseudocrenilabrus*	Intestine	Israel South Africa (F)	Emaciation, slow growth, occasional mass mortalities in fry	Landsberg and Paperna (1987)
BOTH INTESTINAL AND EXTRAINTESTINAL					
Calyptospora funduli	Topminnow (*Fundulus*) silverside (*Menidia*)	Viscera, skin	U.S. Atlantic and Gulf of Mexico (M)	In heavy infections, white or black foci of oocysts in liver; also infects pancreatic acini, adipose tissue, mesentery, ovary, gall bladder and dermis; can be fatal; recovered fish may be immune; requires shrimp (*Palaemonetes*) intermediate host	Fournie and Overstreet (1983) Solangi and Overstreet (1980) Fournie et al. (2000)
EXTRAINTESTINAL FORMS					
Eimeria rutili	Roach	Kidney	Eurasia (F)	Infects tubules and interstitium, causing tubular necrosis	Dogel and Bykhovski (1939)
Eimeria sardinae	Herring, sardines (*Engraulis* sp.)	Testes	North Sea North Atlantic North Pacific (M)	High prevalence; damage (hemorrhage and fibrosis) to seminiferous tubules can cause parasitic castration	Kabata (1963)
Goussia clupearum	Clupeids	Liver	North Atlantic North Pacific Mediterranean Sea North Sea (M)	Oocysts associated with necrosis, inflammation, and fibrosis	Kabata (1963)
Goussia gadi	Atlantic Cod	Swim bladder	North Atlantic North Sea Baltic Sea (M)	Yellow, creamy, or waxy material (mass of parasites, fibrous debris and lipid); may eventually fill entire swim bladder, making nonfunctional; might cause death; most prevalent in fall	Odense and Logan (1976)
Goussia spragui	Codfish *Melanogrammus*	Kidney	Canada (M)	Tubular epithelial necrosis; granulomas around infected tubules	Morrison and Poynton (1989)
Goussia metchnikovi	*Gobio* sp.	Spleen, liver, kidney	Europe (F)	Heavy infections have white foci on spleen surface; inflammation and fibrosis around oocysts	Pellerdy and Molnár (1968)
Goussia cichlidarum	Tilapia	Swim bladder	Israel Uganda (F)	Epithelium covered by mass of gamonts	Paperna et al. (1986)

F = freshwater; M = marine.

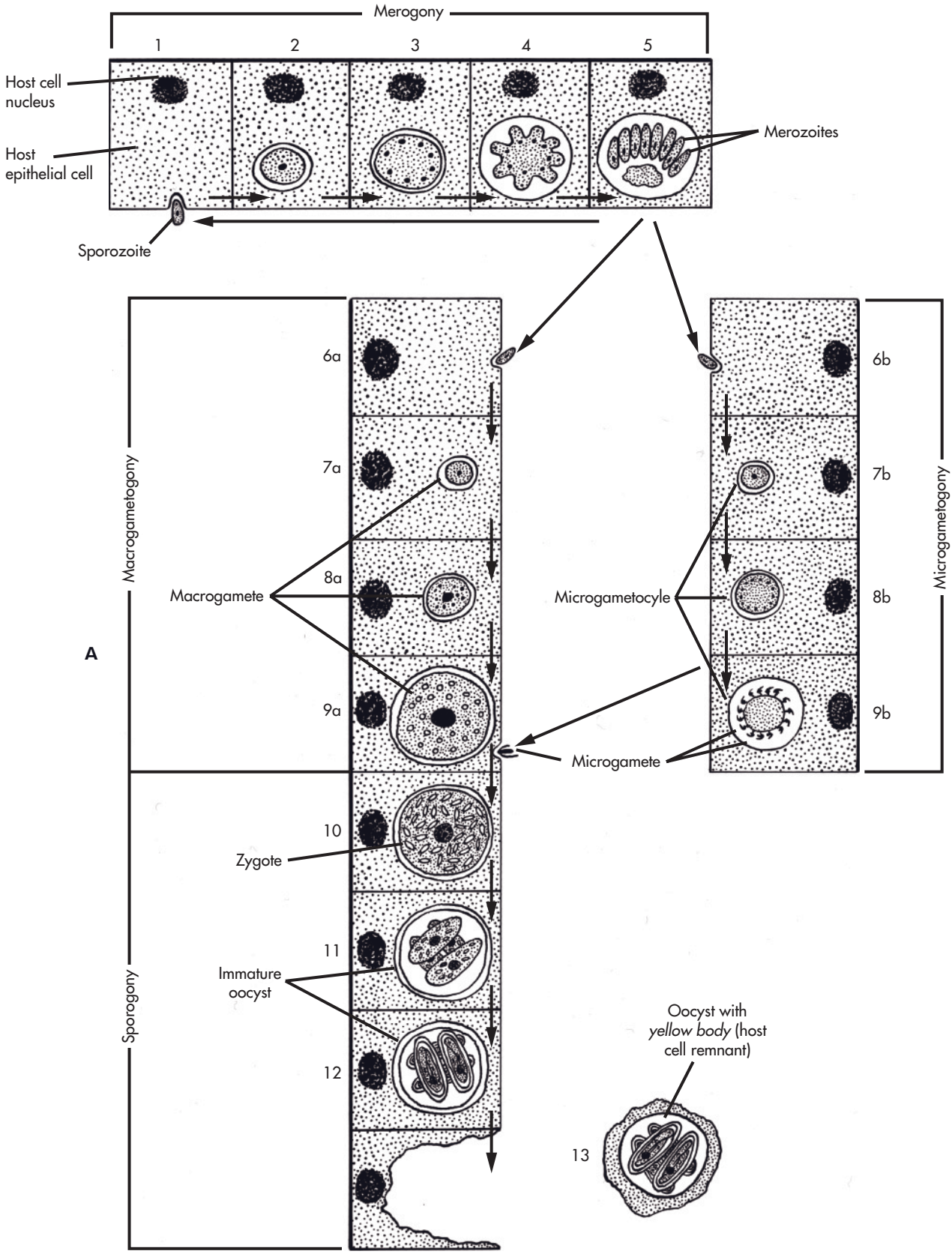


Fig. II-74. A. Life cycle of a typical fish-pathogenic coccidia of the family Eimeriidae (endocytoplasmic, intestinal species) (adapted from Lom and Dyková 1992). The sporozoite invades an epithelial cell (*1*) and grows within a parasitophorous vacuole (*2*), forming a multinucleated stage (*3* and *4*), which produces merozoites (*5*) by asexual reproduction. The merozoite then infects another host cell and either produces more merozoites or undergoes sexual reproduction, producing a single macrogamete (*6a* through *9a*) or many microgametes (*6b* through *9b*). A microgamete fertilizes a macrogamete, producing a zygote (*10*), which forms an oocyst (*11* and *12*). The oocyst may leave the host cell unsporulated, or it may undergo intracellular sporulation (as shown in *11* and *12*). A degraded remnant of host cell (*yellow body*, which is ceroid or lipofuscin [Kent and Hedrick 1985a) is often present (*13*).

Continued.

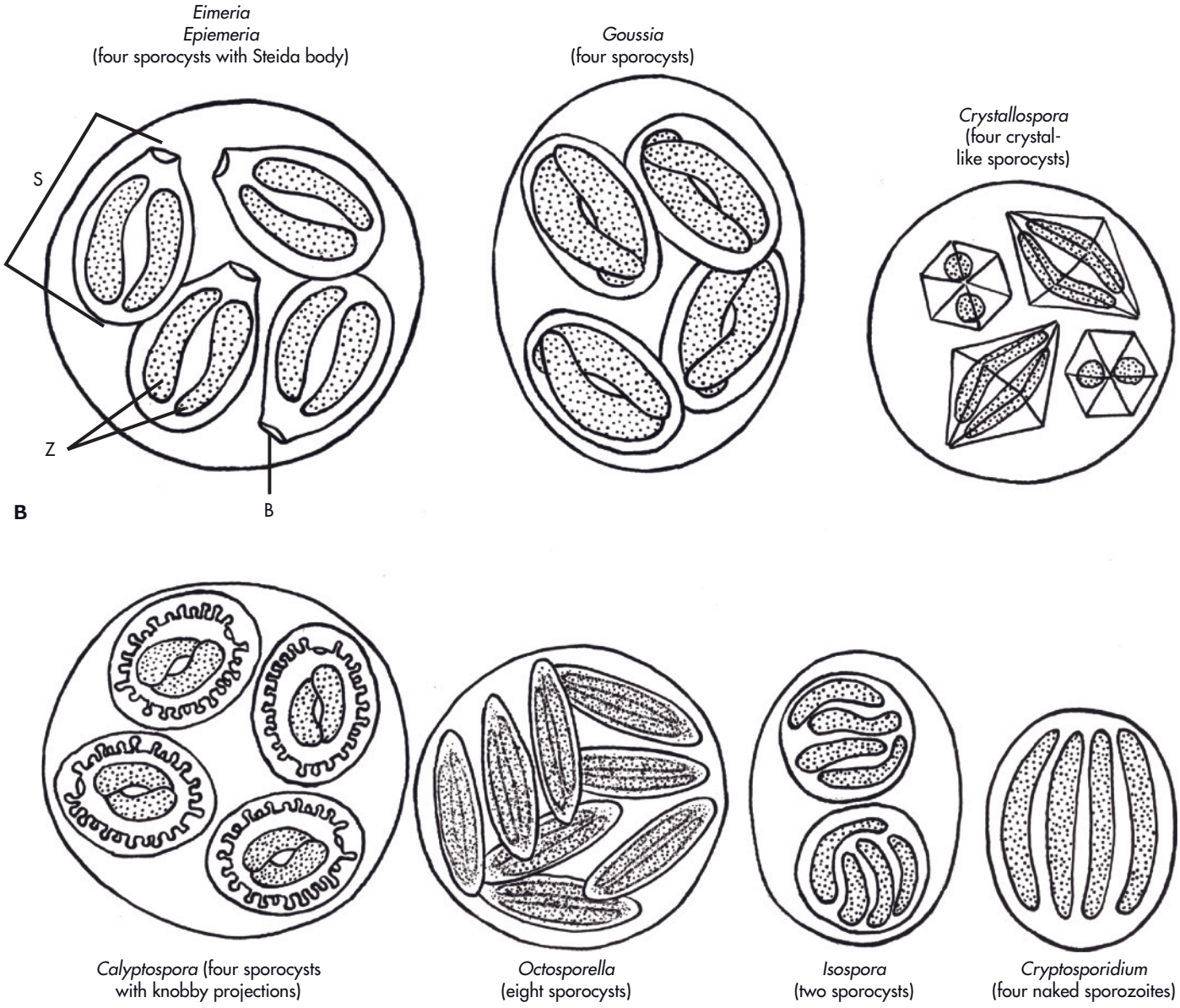
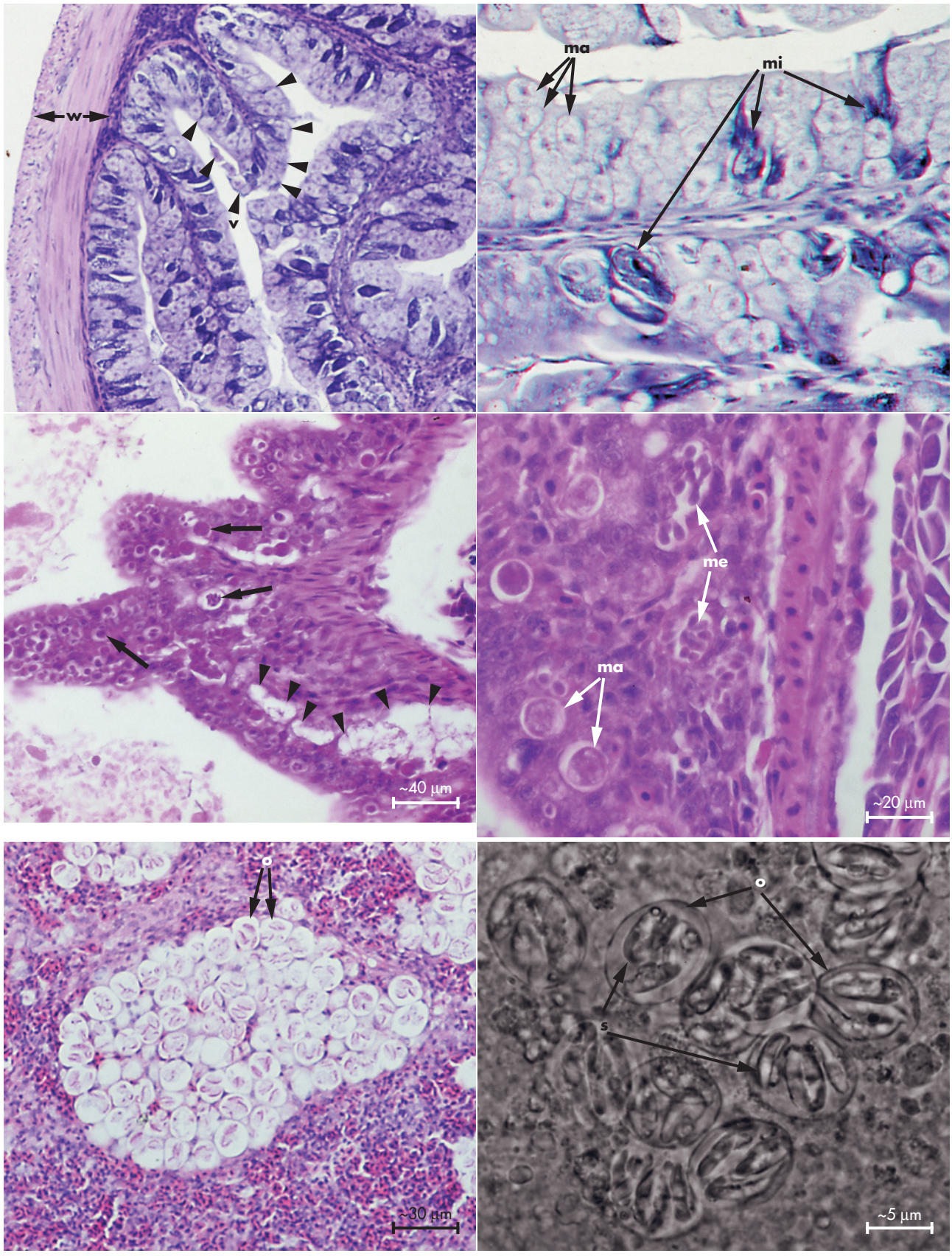


Fig. II-74—cont'd. B. Oocysts of various coccidian genera infecting fish. *S* = sporocysts; *Z* = sporozoites; *B* = Steida body.

Continued.

species of *Cryptosporidium*, but none are serious fish pathogens. All are intracellular parasites (Fig. II-74, A). Over 200 species of eimeriids have been identified from fish (Duszynski et al. 1999), and their prevalence is probably underestimated. They are uncommon problems in most cultured fish but have caused serious disease in some (e.g., common carp). Piscine coccidia tend to be less species-specific than mammalian coccidia, often infecting several closely related fish species (i.e., in the same genus).

The infective stage (sporozoite) is formed within an oocyst (Fig. II-74, A). After ingestion by the host, the sporozoite penetrates the intestinal wall to reach the final site of infection. In the host cell, the parasite forms a schizont, which produces many merozoites by asexual reproduction (merogony). Merozoites produce flagellated microgametes and oocyte-like macrogametes, which mate, producing a zygote (gametogony). The zygote then forms an oocyst (Fig. II-74, B), containing sporocysts with the sporozoites (sporogony). Most intestinal species produce oocysts continuously throughout the year but some in the northern hemisphere are shed only in spring. Unlike those of mammals, oocysts of fish-parasitic intestinal species that are shed in the gut are very short-lived and lose infectivity in several days. Oocysts of extraintestinal parasites (Table II-74) typically remain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viable for a long period after sporulation in the host and are not released until death of the host. The life cycle is usually direct, but an intermediate host is required in at least one species, *Calyptospora funduli* (Solangi and Overstreet 1980; Fournie et al. 2000). Paratenic hosts occur in several species (Molnár 2006).

The rarely encountered family Cryptosporidiidae (several *Cryptosporidium* species) is similar to the Eimeriidae, but infection occurs in a parasitophorous vacuole of the epithelial microvillus (Hoover et al. 1981; Landsberg and Paperna 1986; Alvarez-Pellitero and Sitja-Bobadilla 2002). Also, microgametes are not flagellated. The group is now considered to be closely related to the gregarines.

Pathogenesis

The pathogenesis of tissue coccidia infections is poorly studied, but there is increasing evidence that they can be serious pathogens. Intestinal infections are often asymptomatic but can cause epithelial necrosis and enteritis (Fig. II-74, C through F). Abundant mucoid material in the gut is characteristic of severe intestinal infections. Inflammation may encapsulate oocysts. Extraintestinal parasites can also cause lesions (Dyková and Lom 1981; Lom 1984), with characteristic destruction of target cells, followed by inflammation. Common infection sites include reproductive organs, liver, spleen (Fig. II-74, G), and swim bladder (Table II-74). Grossly visible nodules may be present in spleen, liver or gut.

Diagnosis

A definitive diagnosis of tissue coccidia is based on identification of oocysts (Fig. II-74, B and H). In contrast to coccidia infecting mammals, oocysts of fish parasites are thin walled, the walls are of host origin, and they are usually sporulated (i.e., sporozoites are present) when shed; in such cases they sporulate while still in the host cell. The fragile oocyst wall prevents the use of concentration procedures that are used for mammalian coccidia, requiring use of fresh smears or histopathology.

Unsporulated oocysts in fresh preparations are often mistaken for granulocytes or algae, both common in gut contents. Sporulated oocysts are much easier to identify and can be induced by incubating the sample in a small amount of water with frequent water changes to prevent bacterial overgrowth. Oocysts in mucus or feces can also be preserved for a short period in 4% formalin (Molnár 2006).

Coccidia that infect fish typically have oocysts with four sporocysts, each with two sporozoites. An exception is *Cryptosporidium* (sporozoites free). Genera are also differentiated by using sporocyst structure. For histopathology, Mallory's stain is especially useful: mature oocysts stain yellow in sharp contract to fish tissues. Oocysts also autofluoresce, appearing blue under fluorescence microsopy (Davies and Stewart 2000). Presumptive diagnosis of coccidiosis is based on histopathological identification of developmental stages (meronts, macro- and microgamonts) (Fig. II-74, C through F) in target tissues.

Treatment

There are few published studies of drug control of fish coccidiosis. The coccidiostat monensin significantly reduces infection burdens of *Calyptospora*. Toltrazuril, another coccidiostat used in mammals, has also shown efficacy experimentally (Melhorn et al. 1988). The coccidiostat sulfadimidine (33%, 1 ml/3 l water; repeat weekly) (Langdon 1990; Gratzek et al. 1992) has also been advocated, but there are no published clinical trials on the latter. *Calyptospora funduli* has been successfully treated with either amprolium (0.63 ml/l of a 9.6% solution given over 2 days) in water or the antibiotic narasin (<5 mg/moderate-sized *Fundulus*) in the food. Treatment has been successful when initiated soon after the fish are infected (Overstreet 1988). Maintaining a proper environment and reducing stress appear to be important in preventing outbreaks in cultured fish (Lom and Dyková 1992).

Fig. II-74—cont'd. C. Histological cross-section of yellow perch intestine infected with *Goussia* sp. The entire intestine is occupied by macrogametes and microgametocytes. *v* = intestinal villus; *w* = intestinal wall. D. Close-up view of a single villus in Fig. II-70, *C*, showing macrogametes (*ma*) and microgametocytes (*mi*); the latter is filled with microgametes. E. Histological section of blue tilapia intestine infected with *Goussia vanasi*. Various parasite stages are present (*arrows*). Note the detachment (*arrowheads*) of infected epithelium, before it sloughs into the lumen. Hematoxylin and eosin. F. Close-up of infection in Fig. II-70, *E*, showing meronts (*me*) and macrogametes (*ma*). Hematoxylin and eosin. G. Histological section of goby spleen infected with *Goussia metchnikovi*. Light-colored area is an aggregation of oocysts (*o*). H. Wet mount of *Eimeria* oocysts. Each oocyst (*o*) has four sporocysts (*S*). Each sporocyst has two sporozoites. (*A* from Lom and Dyková 1992; *C*, *D*, *G*, and *H* photographs courtesy of J. Lom; *E* and *F* photographs by L. Khoo and E. Noga.)

PROBLEM 75
Miscellaneous Endoparasitic Infections

Prevalence Index
WF - 4, CM - 4
Method of Diagnosis
1. Wet mount of viscera with parasite
2. Histology of viscera with parasite

Systemic Cryptobiosis
Cryptobia iubilans causes submucosal granulomas in cichlids. It is the only one among several *Cryptobia* species inhabiting the digestive tract that is severely pathogenic (see PROBLEM 30 for morphology of the genus). Cichlids (*Heterichthys* and *Cichlasoma* species) are infected, with parasites both inter- and intra- (macrophages) cellular. They induce the formation of granulomas (spleen, liver) and peritonitis (Dyková and Lom 1979a). This organism is primarily responsible for Malawi bloat reported in African rift lake cichlids (Ferguson et al. 1985).

Granulomatous Amoebic Disease
This disease affects various internal organs, causing chronic granulomatous lesions in goldfish, especially in the kidney and spleen. The organism responsible has not been cultured (Voelker et al. 1977).

Miscellaneous Amoebae
Valkamphia, *Naegleria*, *Acanthamoeba*, *Hartmanella*, *Schizamoeba*, and *Entamoeba* occasionally have been isolated from the internal organs of fish. In most cases these have been asymptomatic infections (Lom and Dyková 1992). However, Nash et al. (1988) described a severe systemic amoebiasis in cultured European catfish.

Intestinal Protozoa
Protoopalina is a large (60–100 μm), ciliate-like protozoan that is a common, nonpathogenic commensal in discus (Lom and Dyková 1992). Some claim that it may cause debilitation in young fish (Untergasser 1991). A few other *Protoopalina* species infect other fish.

Rosette Agents
The rosette agents are members of the class Mesomycetozoea, a newly created group of organisms that have characteristics of both animals and fungi (see PROBLEM 71). *Sphaerothecum destruens* causes chronic, high mortality in marine-cultured chinook and Atlantic salmon in Washington state. The agent is Gram-positive, PAS-positive, and GMS-positive, forming clusters (rosettes) of spherical, 3–7 μm cells in macrophages (Harrell et al. 1986; Arkush et al. 2003). A rosette-like agent has recently been identified in sunbleak in Europe, where it causes chronic, high mortality and reproductive failure; it is carried asymptomatically and transmitted by an exotic species (Asian topmouth gudgeon) (Gozlan et al. 2005). Fathead minnow is experimentally susceptible.

PROBLEM 76
Idiopathic Epidermal Proliferation / Neoplasia

Prevalence Index
WF - 4, WM - 4, CF - 4, CM - 4
Method of Diagnosis
Histology of lesion
History
Various-sized mass that has often increased slowly in size
Physical Examination
Varies, depending on organ affected
Treatment
Surgery if superficial mass (i.e., on skin)

COMMENTS
Epidemiology/Pathogenesis
IDIOPATHIC EPIDERMAL PROLIFERATION (IEP)
Idiopathic epidermal proliferation has been reported in several fish, mainly feral individuals (Table II-76, A). IEP is usually a benign disease that typically presents as various-sized, flattened-to-papillary, epidermal thickenings (Fig. II-76, A and B). Lesions may be simply hyperplastic or show evidence of early neoplastic change. Some IEP lesions are viral-associated, but all are idiopathic. Some are pollution-associated, but others occur in fish from relatively pristine environments. Most idiopathic proliferative skin lesions reported from fish are benign. However, papillomas (Figs. II-76, C through F) have caused serious problems in European eels and Atlantic halibut (Ottesen et al. 2007). Stomatopapillomas in eels may be so large that they prevent eating, causing starvation, while Atlantic halibut papillomas significantly reduce carcass quality.

NEOPLASIA
Many types of neoplasms have been documented in fish (see Table II-76, B) (Mawdesley-Thomas 1972, 1975; Harshbarger and Clark 1990; Harshbarger et al. 1993), mainly from feral fish. Some neoplasms have been highly prevalent (>25%) in feral populations. Environmental contaminants have been strongly suspected as the cause in many cases, although a cause-and-effect relationship has not been proven (Mix 1985).

Virtually any tissue can be affected by neoplasia. Skin tumors are the most common neoplasms that affect fish (Wellings 1969), especially papillomas (see "**Idiopathic Epidermal Proliferation/Neoplasia**"). Liver tumors are also prevalent in polluted environments and were common in salmonids in the 1960s because of feed contamination by carcinogenic aflatoxins (see PROBLEM 89). Several types of tumors can be experimentally induced in fish by exposure to carcinogens.

Neoplasia is rare in cultured fish, especially food fish. Tumors are occasionally seen in aquarium species. Thyroid growths are probably the most common tumors

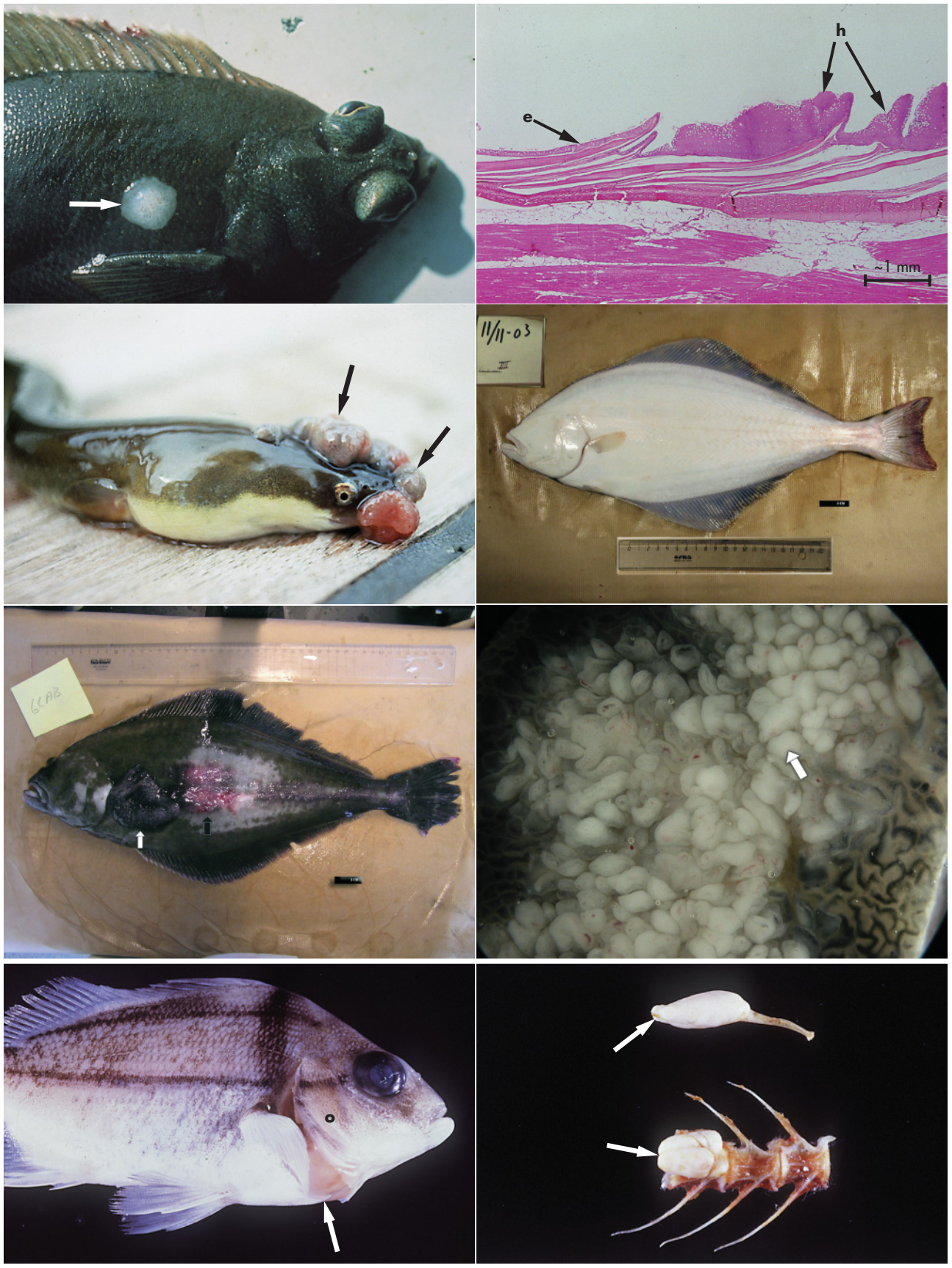
Table II-76, A. Idiopathic, proliferative, epidermal responses in fish. Clinically important diseases are indicated by an asterisk(*).

Disease	Species affected	Geographic range	Diagnostic features	References
HYPERPLASIA				
"Carp pox" (*Herpesvirus cyprini* disease)*	Common carp, crucian carp, barbel, bream, golden ide, rudd, smelt, carp × goldfish, aquarium fish	Europe Asia Russia, Israel Great Lakes, United States	Smooth to rough, milky white to grey plaques up to 2 mm thick This is not an idiopathic lesion (see PROBLEM 88)	
Lake trout epidermal hyperplasia	Lake trout	Lake Superior Lake Michigan	Gray-white, mucus-like, foci on body and fins	McAllister and Herman (1989)
Blue spot disease (Herpesvirus-associated)	Northern pike	Manitoba and Saskatchewan, Canada	3–10 mm × 0.25 mm raised foci	Yamamoto et al. (1984) Margenau et al. (1995)
Esox epidermal hyperplasia	Northern pike, muskellunge	Canada Sweden	5–10 mm × 1–3 mm plaques Undifferentiated cuboidal epithelial cells Retrovirus-associated	Yamamoto et al. (1984)
Discrete epidermal hyperplasia (Retrovirus-associated)	Walleye	Lake Oneida, New York Saskatchewan and Manitoba, Canada	Up to several cm plaque	Yamamoto et al. (1985)
Diffuse epidermal hyperplasia (*Herpesvirus vitreum*)	Walleye	North America	Thin epidermal plaque up to several cm in diameter Somewhat disorganized epithelium; herpesvirus-associated	Yamamoto et al. (1985)
Hybrid striped bass epidermal hyperplasia	Hybrid striped bass	North Carolina, United States	2–10 mm plaques (Fig. II-76, B)	E Noga (Unpublished Data)
Atlantic cod epidermal hyperplasia (adenovirus-associated)	Atlantic cod	Baltic Sea	3–20 mm plaques; only seen once	Jensen and Bloch (1980)
Dab epidermal hyperplasia (adenovirus-associated)	Dab	North Sea	2–10 mm plaques progressing to 5–15 mm papules (Fig. II-76, A)	Bloch et al. (1986)
PAPILLOMA				
Stomatopapilloma (cauliflower disease)*	European eel	Baltic, North, and Black Seas; England, Scotland rivers	Mostly mouth and head growths (Fig. II-76, C) can interfere with eating, breathing; virus suspected but not proven	Wolf (1988)
Atlantic salmon papillomatosis	Atlantic salmon	Russia (Arctic)	Blue-grey raised plaques	Shchelkunov et al. (1992)
White sucker papilloma	White sucker	Great Lakes, United States	Lesions on body, fins, eyes, lips; both raised papillomas and plaques	Premdas and Metcalf (1996)
Smelt papillomatosis (herpesvirus associated)	Smelt	Europe	Cowdry-type intranuclear inclusions	Anders and Moller (1985) Lee and Whitfield (1992)
Brown bullhead papilloma	Brown bullhead	United States		Grizzle et al. (1981)
Gilthead sea bream papilloma	Gilthead sea bream	Spain	Maxillary tumors; may interfere with feeding	Gutierrez et al. (1977)
Pleuronectid epidermal papilloma (X-cell disease)*	See PROBLEM 42			
Winter flounder papilloma (virus-suspected)	Winter flounder	Newfoundland, Canada	Blister-like swellings with spongiosis and hydropic degeneration	Emerson et al. (1985)

Table II-76, B. Common neoplasms in fish.

Organ or tissue	Species commonly affected
EPIDERMIS	
Hyperplasia	See Table II-76, A.
Papilloma	Yellow bullhead, American eel, Atlantic salmon, white sucker. Also see Table II-76, A.
Carcinoma	Yellow perch, brown bullhead
Sarcoma	Walleye
Pigment cell tumors	Goldfish, common carp, *Corydoras* catfish, platy × swordtail hybrids, melanomas most common
CONNECTIVE TISSUE	
Lipoma	Largemouth bass
Fibroma	Mullet, many salmonids—very common
Fibrosarcoma	Coho salmon, walleye, goldfish—very common
MUSCULOSKELETAL	
Chondroma ("osteoma")	Many species; "osteoma" (Fig. II-76, H) is often an idiopathic nonneoplastic, foreign body reaction.
NERVOUS TISSUE	
Schwannoma	Goldfish, bicolor damselfish
Neurofibroma	Gray snapper
Neurilemmoma/neurofibroma	Goldfish
HEMATOPOIETIC TISSUE	
Lymphoma	
Lymphosarcoma	Northern pike, muskellunge, rainbow trout
CARDIOVASCULAR	
Hemangioma	Salmonids, Atlantic cod, mackerel, pollock, plaice
RESPIRATORY	Gill neoplasia very rare.
THYROID	
Hyperplasia/Adenoma/Adenocarcinoma	Many salmonids, carp, koi, goldfish, many aquarium fish (Fig. II-76, G) yellow perch, coho salmon normal thyroid may be found in kidney, spleen, or epicardial surface.
GASTROINTESTINAL TRACT	
Ameloblastoma	Salmonids, Atlantic cunner
LIVER	
Hepatoma/Hepatocarcinoma	Many salmonids, brown bullhead, English sole, Atlantic tomcod, winter flounder; one of the few fish tumors that metastasize.
Cholangioma/Cholangicarcinoma	
KIDNEY	
Nephroblastoma	Rainbow trout—rare
REPRODUCTIVE TISSUE	
Testicular adenoma	Goldfish × carp

Fig. II-76. A. Idiopathic epidermal hyperplasia (*arrow*) in dab. B. Hybrid striped bass with idiopathic epidermal hyperplasia (*h*). Compare with normal epithelium (*e*). C. Stomatopapilloma (*arrows*) in a European eel. D. Abocular (ventral) side of a normal Atlantic halibut. E. Abocular (ventral) side of an Atlantic halibut with severe hyperpigmentation and epidermal papilloma formation (*arrow*). F. Closer view of *E* showing papillomas (*arrow*). G. Mass (*arrow*) in the throat region of a porkfish caused by a thyroid tumor. The operculum (*o*) cannot entirely close because of swelling. Preserved specimen. H. Osteomas (*arrows*) on the rib and vertebral column of an Atlantic croaker. (*A* and *C* photographs courtesy of H. Möller; *B* photograph by L. Khoo and E. Noga; D, E, and F photographs from Ottesen et al. 2007.)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affecting tropical marine aquarium fish, especially sharks (Crow et al. 1998). Thyroid growths (ranging from benign hyperplasia to adenomas to carcinomas) (Hoover 1984; Moccia et al. 1977) often result in grossly enlarged thyroid glands (Fig. II-76, G) that may interfere with breathing. Many of these may not be neoplastic but rather a hyperplastic response to iodine deficiency (Crow et al. 1998) (PROBLEM 89). Goldfish are one of the most common aquarium species affected by neoplasia (Fig. I-26), possibly because they are long-lived. Pigment cell tumors have also been seen in several aquarium species. Some proliferative lesions are apparently of no serious consequence (Fig. II-76, H).

Diagnosis

Histology is used for definitive diagnosis of idiopathic epidermal hyperplasia. Note that reactive hyperplasia is an extremely common response of fish epidermis to insults, such as parasite infestation or chronic trauma. Thus, chronic irritation caused by an exogenous agent must be ruled out.

Histology is also used for definitive diagnosis of neoplasia. Note that fish neoplasms do not always conform to mammalian criteria used to classify lesions. Hayes and Ferguson (1989) provide details on tumor biology and classification. Metastasis is rare, even for tumors that appear malignant histologically, although neoplasms may be locally invasive. Some proliferative, parasitic infections have been mistaken for neoplasia (Harshbarger 1984).

Treatment

Neoplastic growths, especially those on the fins or body surface but also those in the abdomen, are often amenable to surgical excision (Probasco et al. 1994) (Fig. I-26). Some may recur. Some fish can often live a long time with many cancers but others will kill the fish if not treated promptly.

CHAPTER 12

PROBLEMS 77 through 88

Rule-out diagnoses 1 (viral infections): *Presumptive* diagnosis is based on the absence of other etiologies combined with a diagnostically appropriate history, clinical signs, and/or pathology. *Definitive* diagnosis is based on presumptive diagnosis combined with confirmation of viral presence (e.g., antibody probe, gene probe)

77. Systemic viral diseases: general features
78. Channel catfish virus disease
79. Infectious pancreatic necrosis and other aquatic birnaviruses
80. Infectious hematopoietic necrosis
81. Viral hemorrhagic septicemia
82. Infectious salmon anemia
83. Spring viremia of carp
84. Iridoviral diseases
85. Nodaviral diseases
86. Koi herpesvirus disease
87. Alphavirus diseases
88. Miscellaneous systemic viral diseases and infections

PROBLEM 77
Systemic Viral Diseases: General Features

Prevalence Index

See group headings

Method of Diagnosis

Clinical signs characteristic of the disease combined with either or both:

1. Antibody or gene test of tissue or cultured virus
2. Histopathology of diagnostic lesions

History

Varies with etiological agent and environmental conditions (especially temperature)

Physical Examination

Varies with etiological agent and environment

Treatment

1. Disinfect and quarantine
2. Eliminate source of contamination (i.e., water or fish)

COMMENTS

Systemic viral infections are common and important diseases. Over 125 different viruses have been identified in fish (Essbauer and Ahne 2001) and new viruses are being discovered at an increasing rate.

General characteristics of viral diseases of fish include:

- Often temperature-dependent pathogenicity
- Host-specific (usually affecting only one species or a closely related group of species)
- Usually young fish get sick, while older fish become carriers

Common clinical signs include:

- Exophthalmos
- Abdominal distension
- Hemorrhage

Common microscopic lesions include:

- Organ necrosis
- Intracellular inclusions

Not all of these characteristics occur in all viral diseases. No medications are available to treat any fish viral disease. Various antiviral compounds have been tried with varying success, but none are commercially available for use in fish. Thus, one must rely upon disinfection and quarantine, or sometimes environmental manipulation (e.g., temperature) for management. Avoidance is the best method of control. This includes obtaining fish only from certified virus-free stocks and raising fish in virus-free water (spring, well, or disinfected). When exposure to virus-infected water is unavoidable (e.g., using contaminated surface waters), stocking fish that are past the age of greatest susceptibility is an option, since most important viral diseases are most damaging to young fish. Considerable experimental work has been done with vaccines, including inactivated and live attenuated preparations. DNA vaccines also hold promise (Walczak et al. 1981; Fryer et al. 1976; Vinitnantharat et al. 1999). Vaccines for some viral diseases are licensed for use in some countries.

Definitive diagnosis of systemic viral disease is based on observation of relevant history, clinical signs and/or pathology in combination with virus identification/detection via either an antibody or a gene test. Some viruses may be present in low numbers without causing disease (e.g., IPN [PROBLEM 79]), requiring quantification to determine its importance in causing disease. Some viruses may be shed from asymptomatic carriers during spawning time (e.g., IPN, IHN, VHS

[PROBLEMS 79, 80, 81]). See "**Sampling for Viruses**" (**p. 55**) for details on sampling for systemic viruses.

PROBLEM 78
Channel Catfish Virus Disease (CCVD)

Prevalence Index

WF - 2

Method of Diagnosis

Identification of channel catfish virus infection in fish displaying typical clinical signs and pathology

History

Acute to chronic morbidity/mortality; corkscrew spiral swimming

Physical Examination

Reddening on body and base of fins; depression; exophthalmos; swollen abdomen; equilibrium deficit

Treatment

1. Disinfect and quarantine
2. Reduce temperature to less than 15°C (59°F)
3. Treat secondary infections

COMMENTS

Epidemiology

Channel catfish virus is the most important viral disease affecting channel catfish. Except for its accidental introduction into Honduras, it is restricted to the channel catfish-producing areas of the United States (Wolf 1988). It is a highly species-specific herpesvirus and only naturally affects channel catfish, although it can experimentally infect some other ictalurids (blue catfish, channel × blue catfish hybrid) and possibly some clariid catfish (Galla and Hartmann 1974). Different strains of channel catfish vary in their susceptibility (Plumb and Chappell 1978).

During CCVD epidemics, the younger, more robust fish typically die first. While older fish can become clinically sick, epidemics occur almost exclusively in young (<1 year) and small (<15 cm) fish and most epidemics are in fish <4 months old. Mortalities are most rapid and severe with higher temperatures, being highest at 25–30°C (77–86°F). Clinical signs may be evident in as little as 1 day at 30°C (86°F), taking 10 days at 20°C (68°F). No mortalities occur at <15°C (59°F). There is also some evidence that young fish (Amend and McDowell 1983) or broodfish (Bowser et al. 1985) may develop a chronic infection.

During epidemics, virus is readily transmitted horizontally in the feces and urine of clinically affected fish. There is also evidence for vertical transmission (Wise et al. 1985). Virus can usually only be isolated during an active epidemic. However, virus can be isolated from clinically normal adult broodstock after injecting dexamethasone (an immunosuppresive synthetic steroid) (Bowser et al. 1985). There is evidence for recrudescence of latent infections.

Clinical Signs/Pathogenesis

GROSS LESIONS

Clinical signs include hanging head up in the water, disorientation (corkscrew spiral swimming), abdominal distension, exophthalmos, and hemorrhages on the body, gills, and at the bases of the fins (Fig. II-78, A). Internally, there is a yellowish fluid in the peritoneal cavity and punctate hemorrhage in the viscera.

HISTOPATHOLOGY

Channel catfish virus attacks all major organ systems. Focal necrosis begins in the posterior kidney and quickly develops into diffuse necrosis of both hematopoietic and excretory tissues, accompanied by hemorrhage and edema (Fig. II-78, B). Necrosis also affects the liver,

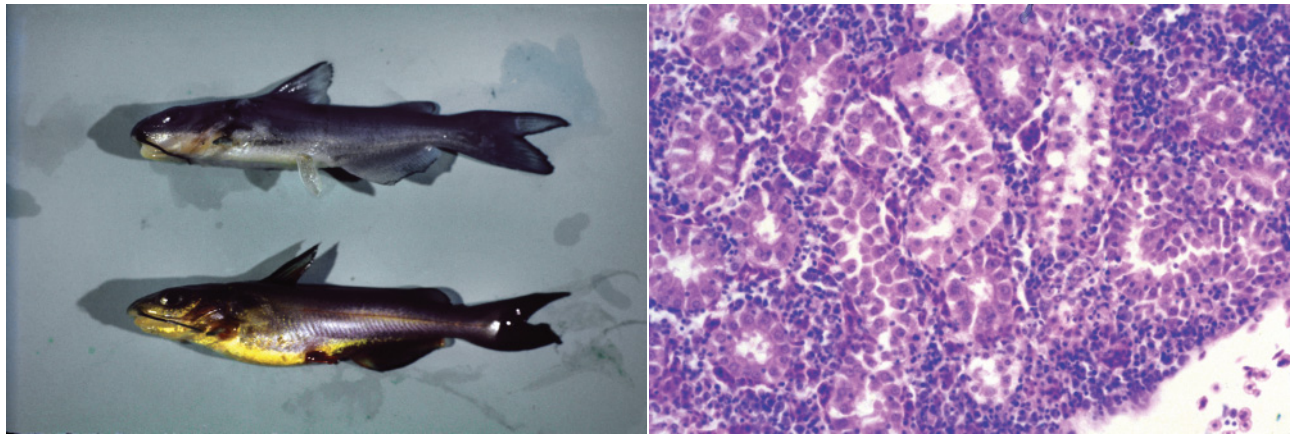


Fig. II-78. A. Channel catfish with CCV infection. Note hemorrhage in the fins (*bottom fish*) and abdominal swelling caused by fluid accumulation in the peritoneal cavity. Clinically normal fish above. B. Histological section showing acute necrosis of kidney caused by CCV infection. Hematoxylin and eosin.

spleen, gastrointestinal tract, pancreas, and skeletal muscle (Yasutake 1975). Neurological damage includes vacuolated neurons and edematous neurofibers (Major et al. 1975).

Diagnosis

The typical presentation of a CCVD epidemic is a rapid, abrupt increase in mortality in young-of-year channel catfish when the temperature is at least 25°C (77°F). Definitive diagnosis of clinical CCVD requires identification of virus from target tissues, with appropriate clinical signs. Peak viral titers correspond with the peak in tissue damage (Wolf 1988). Kidney is the best organ for isolation. Ictalurid cell lines (brown bullhead [BB] or channel catfish ovary [CCO]) are most commonly used for isolation; syncytia formation and presence of intranuclear Cowdry type A inclusion bodies are strong presumptive evidence for CCV. Serum neutralization of cell culture-isolated virus is the most widely used method for definitive diagnosis. Fluorescent antibody of frozen tissues (Plumb et al. 1981) or DNA probes (Wise et al. 1985) have also been developed. None of these reagents are commercially available. Identification of anti-CCV antibody titers (Crawford et al. 1999) in convalescent sera of recovering fish can also be used for presumptive diagnosis. Serum should probably be collected 1 or 2 months after exposure. False negatives are common.

Many CCVD epidemics are accompanied by secondary bacterial infections (*Aeromonas*, *Flavobacterium*, *Edwardsiella*), which can mask the primary diagnosis. Virus cannot be isolated from a fish population within days after an epidemic ends (Wolf 1988). The CCVD virus is also relatively unstable in the environment. There is a 50% loss of infectivity in fish stored for 100 days at approximately 20°C and 90% loss after 3 days on ice. It survives for less than 3 days in dead fish at room temperature (Plumb 1973). Freezing and thawing rapidly destroys activity.

There is no accepted method for detecting asymptomatic fish. While latent viral DNA can be detected in skin, gill, or kidney of experimental fish (Gray et al. 1999), the reliability of this test in commercial production has not been determined.

Treatment

Disinfection and quarantine is the most effective means of controlling CCVD epidemics. The virus can persist in water up to 1 or 2 months at 4°C (39°F) but less than 2 weeks at 25°C (77°F) (Plumb 1978). Treating ponds with 20–50 mg/l chlorine will ensure that the virus is eliminated. Thorough drying also inactivates it. All fish surviving an outbreak should be destroyed. Surviving fish are often stunted (McGlamery and Gratzek 1974). By reducing the temperature to less than 15°C (59°F) epidemics can be stopped, but this method is impractical and probably does not eliminate the carrier state. There is evidence that many commercial catfish broodstock carry CCV DNA in a latent carrier state (Wise et al. 1985; Gray et al. 1999), making it difficult to obtain virus-free broodstock. Only broodstock without anti-CCV serum neutralization titers and no history of prior exposure to CCV should be used for spawning. Note that some fish do not produce neutralizing antibody titers after exposure to CCV.

Stress reduction is considered to be important in managing the disease, including preventing overcrowding, adequate oxygen and nutrition, and not handling fish when temperatures exceed 20°C (68°F). However, the highly infectious nature of the virus suggests that stress is not essential for virus dissemination (Wolf 1988). Controlling concurrent secondary infections is mandatory. Vaccines experimentally provide protection (Walczak et al. 1981; Awad et al. 1989; Vanderheijden et al. 2001), but are not yet available commercially. Using resistant channel catfish strains may reduce severity of outbreaks (Plumb and Chappell 1978).

PROBLEM 79

Infectious Pancreatic Necrosis (IPN) and Other Aquatic Birnaviruses

Prevalence Index

CF - 1, CM - 3

Method of Diagnosis

Identification of birnavirus infection in fish displaying typical clinical signs and pathology

History

Usually acute, sometimes chronic, morbidity/mortality

Physical Examination

Neurological signs; trailing white feces; dorsal darkening; abdominal distension; exophthalmos; hemorrhage; pale gills; catarrhal exudate in stomach

Treatment

1. Disinfect and quarantine
2. Raise fish in virus-free water for first 6 months of life

COMMENTS: SALMONIDS

Epidemiology

Infectious pancreatic necrosis virus (IPNV), an aquabirnavirus, is a major cause of mortality in salmonids in freshwater and also can cause disease in seawater; it has recently become a major cause of mortality in marine salmon (Murray et al. 2003). Its geographic range is the United States, Canada, Chile, Japan, Taiwan, Korea, and Europe. It is not present in Oceania, although aquabirnaviruses from nonsalmonids have been isolated in Australia and New Zealand (McAllister 2007). It infects rainbow, brook, and cutthroat trout; Atlantic, coho, and Kokanee salmon; Arctic char; and other salmonids. Brook and rainbow trout are most susceptible. Only

young fish become clinically ill (mortality in fish >6 months old is rare), but any age fish can become infected, forming chronic carriers.

The time course of clinical disease varies with fish age, species, temperature, and other conditions, but clinical signs typically appear on day 3–5 (fry) or on day 8–10 (fingerlings) after exposure to the virus. Peak mortality usually occurs on day 12–18.

Mortality is most rapid and severe at high temperatures (e.g., 10–14°C [50–57°F]); at lower temperatures, mortality is prolonged and often reduced (Frantsi and Savan 1971). There is also usually less mortality above 14°C (57°F), possibly because of interferon production. In salmonids, resistance to clinical disease generally occurs at ~1,500 degree days, except in Atlantic salmon smolts, which can be affected after transfer from freshwater to seawater (Smail et al. 1989). Isolates show marked differences in virulence.

Even the most virulent outbreaks have at least a few survivors. Surviving fish often become stunted because of pancreatic fibrosis and up to 90% may become carriers. Survivors may shed virus in the feces and urine for over 2 years. Not all fish shed; some only shed intermittently (Billi and Wolf 1969). Chronic shedding does not occur with other salmonid viral diseases.

The virus is highly contagious. During epidemics, virus is readily transmitted horizontally by contact and by ingestion of infected tissue; the fecal pseudocast (see "**Clinical Signs/Pathology**") is a major source of virus. Virus can also be shed in the feces of piscivorous birds. Vertical transmission readily occurs via transport in reproductive fluids and on (or possibly in) the egg. IPNV is also suspected of contributing to embryo mortality (Wolf 1988).

Clinical Signs/Pathology

GROSS LESIONS

A typical presentation of IPN is a sudden increase in mortality of fry or fingerling trout, with larger, more robust fish dying first. Clinical signs include dorsal darkening, trailing white feces, abdominal distension (Fig. II-79, A), exophthalmos, hemorrhage on the ventrum, and pale gills. Neurological signs (corkscrew spiral swimming, whirling) can often be initiated by startling the fish. In older fingerling trout, there may be many petechial hemorrhages in the viscera (Fig. II-79, B). In contrast, fry have pale viscera with few petechiae. A catarrhal exudate in the stomach and intestine produces the mucoid, cohesive fecal pseudocast.

HISTOPATHOLOGY

The prime target of viral infection is the pancreatic acinar cells, which undergo acute necrosis (Fig. II-79, C) and have basophilic, intracytoplasmic "inclusions" ("inclusions" are actually products of cell degeneration). Adjacent adipose tissue may be damaged. Another diagnostic feature is the presence of McKnight cells, epithelial cells of the pyloric ceca, which swell and develop a fragmented nucleus; the eosinophilic cytoplasm is then shed into the lumen (McKnight and Roberts 1976). Renal tubular and hematopoietic tissue, as well as liver, may also be necrotic in terminal cases.

Diagnosis

CLINICAL IPN

Definitive diagnosis of clinical IPN requires isolation of high titers (~10^6–10^9 infective units/gram-of-tissue) of virus from target tissues, with appropriate clinical signs in susceptible species. High titers are needed for a definitive diagnosis because IPNV is often present in a subclinical carrier state. The best tissue for isolation from large (>6 cm [>2.3 in]) fish is posterior kidney; pyloric ceca, spleen and liver are also useful. For small fish (<4 cm [<1.6 in]), the entire fish should be sampled, while for 4–6 cm (1.6–2.3 in) fish, the entire viscera, including kidney, should be sampled (Anonymous 2006). If sampled within 24 hours, whole tissues are best stored on ice, while homogenates are best frozen. If samples must be stored longer, it is best to freeze at the lowest possible temperature.

Presumptive diagnosis of clinical IPN is based on the presence of typical clinical signs and pathology in susceptible species. Major differentials include other salmonid acute viral infections, including IHN (see PROBLEM 80), VHS (see PROBLEM 81), and HVS (see PROBLEM 88). IPN must also be differentiated from alphavirus infections (see PROBLEM 87). Diagnostic features especially include presence of white feces (which are more fragile than those seen with IHN or HVS) accompanied by the presence of clear to milky mucus in the stomach and anterior intestine (may be pathognomonic). Since the mucoid material does not coagulate in 10% neutral buffered formalin, it can also be detected in preserved specimens (Wolf 1988).

Key microscopic features include acute pancreatic necrosis and presence of McKnight cells (Fig. II-79, C and D). When the above lesions are present in salmonids, there is over a 90% probability of the disease being IPN. However, caution is warranted if there are lesions in other tissues, such as kidney and liver, since other viruses can cause similar lesions. There is also the possibility of more than one virus being present. IPNV may co-occur with other pathogens, so a clinical decision must be made as to whether the pathogens detected in the clinical workup can explain the severity and clinical signs of disease in the case. If not, it may be justifiable to examine fish for IPN, especially if the history suggests it. Immunological identification of IPNV in infected tissues is also used for presumptive diagnosis (Anonymous 2006).

SUBCLINICAL CARRIERS OF IPNV

The most reliable method for detecting carriers is to sacrifice a significantly relevant number of fish (Thoesen

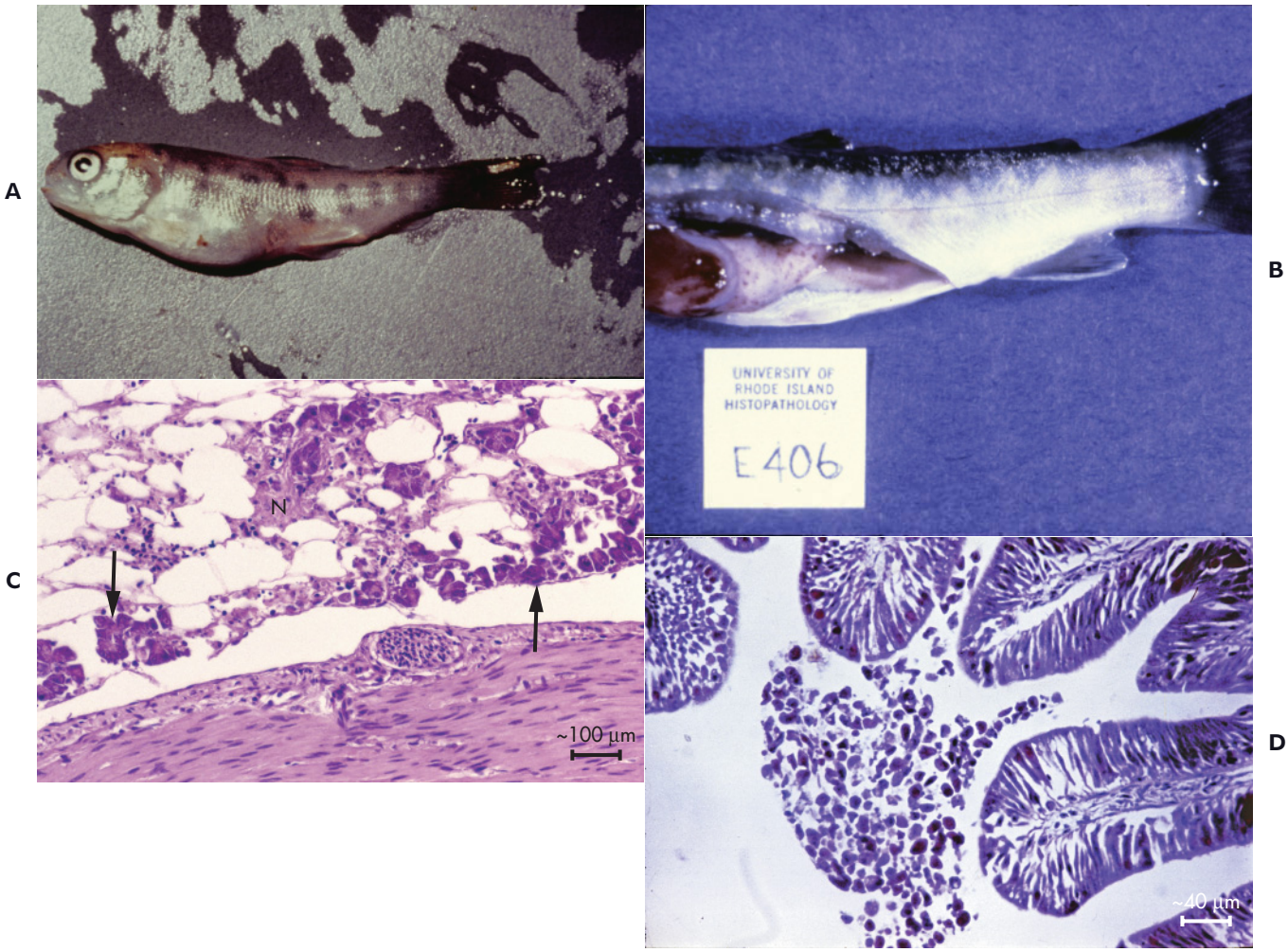


Fig. II-79. A. Rainbow trout with IPN. Note the swollen abdomen caused by accumulation of fluid in the peritoneal cavity. B. Rainbow trout with IPN. Note the punctate hemorrhages in the viscera. C. Histological section showing acute necrosis (*N*) of pancreatic acinar tissue caused by IPN infection. A few areas of more normal tissue remain (*arrows*). Hematoxylin and eosin. Compare with Fig. I-37, *D.* D. Histological section of pyloric ceca with McKnight cells sloughing into the lumen. Hematoxylin and eosin. (*A* and *D* photographs courtesy of R. Roberts; *B* photograph courtesy of R. Wolke; *C* photograph by L. Khoo and E. Noga.)

1994) and take a culture of the posterior kidney, which has the highest virus titers. Other viscera (liver, spleen especially) yield lower, but still significant, amounts of virus.

While nonlethal techniques for kidney sampling have been developed for some bacterial pathogens (Noga et al. 1988b, also see "**Clinical Techniques: Specialized Methods**"), this procedure has not yet been examined for diagnosing IPN carriers. Thus, nonlethal sampling is most reliable when sex products are examined, especially ovarian fluid sediment (McAllister et al. 1987). Blood, feces, and peritoneal washes are less sensitive (Yu et al. 1982). Adding 2% bovine serum albumin to body fluids helps to stabilize the virus, which can then be stored frozen. There is higher probability of virus recovery from stressed fish.

Treatment

Disinfection and quarantine are the only practical methods of controlling an IPN epidemic. Extreme caution should be taken to avoid spreading virus to uncontaminated areas, both on and outside the farm. The IPN virus is one of the most stable fish viruses. It can survive for months in frozen viscera. In freshwater, it can survive for 5 days at 15°C (59°F), for 10 days in a 4°C (39°F) stream, and for 3 months in sterile water (Toranzo et al. 1983). It is even more stable in brackish

water (Toranzo and Hetrick 1982). It survives air drying at 10°C (50°F) for over 1 month.

The virus is readily inactivated by 40 mg/l chlorine for 30 minutes, 20,000 ppm formalin for 5 minutes, 35 ppm iodine for 5 minutes, pH 12.5 for 10 minutes, or 90 ppm ozone for 0.5–10 minutes. However, it is resistant to ultraviolet irradiation (only partially inactivated by 330,000 mWs/cm^2), making this impractical for control (Wolf 1988).

Avoidance is the most useful prophylactic measure, but this may not be possible in many cases. Many watersheds have feral IPN-infected salmonids. Other fish (e.g., striped bass) that are known to harbor IPNV may also transmit the virus to salmonids (McAllister and McAllister 1988). To avoid clinical IPN in such cases, young fish can be raised in a virus-free water source (e.g., spring or well water) for the first 6 months of life, after which they may be stocked in IPNV-infected, grow-out waters. While the young fish may still become infected, they will not usually become sick. Lowering the temperature will also reduce the severity of outbreaks (Frantsi and Savan 1971), but this is usually impractical.

Aside from epidemics, the principal risk of IPNV infection is infected broodstock. Vertical transmission of IPNV cannot be controlled with antiseptic egg baths, possibly because the virus is carried within the egg or somehow sheltered on the egg's surface. Carriers that survive an outbreak are less commonly a risk. Populations that have recovered from IPN are susceptible to recrudescence of clinical disease if stressed. Maintaining a healthy environment can reduce the impact of IPN outbreaks.

IPNV is a potent immunogen and fish develop high titers of neutralizing antibody after exposure. However, the large amount of serological variation among various strains and apparent lack of cross-protection has hindered development of a practical vaccine. There are three major serotypes of IPNV (VR-299, Sp, and Ab) and many subtypes (Wolf 1988). There are two IPNV serogroups, with the majority of isolates belonging to the A serogroup, which comprises at least nine serotypes (Hill and Way 1995).

COMMENTS: NONSALMONID FISH

While freshwater salmonids are afflicted with the aquabirnavirus known as IPN, other aquabirnaviruses (IPN-like) have been isolated from many species of fish and aquatic invertebrates, including some marine species. Aquabirnaviruses can infect at least one member of 38 families of fish, including those of the lamprey, herring, salmon, whitefish, grayling, true eel, sucker, carp, loach, pike, poeciliid, lefteye flounder, bastard halibut, sole, silverside, cavalla, perch, percichthyid bass, drum, and cichlid families (Wolf 1988). A complete list of the families is provided in McAllister (2007). Aquabirnaviruses have also been isolated from five families of mollusks (including oysters and clams), five families of crustaceans (including *Daphnia*, shrimp, crayfish and crabs), rotifers and digenean trematodes (Wolf 1988; Isshiki et al. 2004; McAllister 2007). In the great majority of aquatic species, these isolates have not been proven to be pathogenic for the host species, although they have occasionally been pathogenic to trout. Thus, at present, these aquatic species are most often clinically important in acting as nonsusceptible viral reservoirs. However, some aquatic birnaviruses can cause clinical disease in nonsalmonid fish.

Clinical Disease in Nonsalmonid Fish

EELS

In young Japanese eels, aquabirnavirus causes muscle spasms, a retracted abdomen, congestion of the anal fin, and, in some fish, congestion of the abdomen and gills. Food is absent from the gut, and there can be ascites. The kidneys are hypertrophied, with exudative glomerulonephritis, congestion of renal interstitium, nephrosis with hyaline droplet degeneration, and sloughing of tubule cells into the lumens. There is focal necrosis of the liver and spleen. The disease can be reproduced experimentally (Sano et al. 1981).

YELLOWTAIL

In Japan, both spontaneous and experimentally infected fry and fingerlings develop an important, acute disease (pancreatic-hepatic necrosis) with ascites (Sorimachi and Hara 1985), caused by a marine aquabirnavirus (MABV) called yellowtail ascites virus (YTAV). Epidemics usually occur in May through June at 18–22°C (64–72°F; Kimura and Yoshimizu 1991). Co-occuring bacterial infection worsens the outcome of the disease (Pakingking et al 2003).

FLATFISH

Clinical disease associated with aquabirnavirus infection has been observed in cultured turbot (Novoa et al. 1993), dab (Olesen et al. 1988), and Atlantic halibut (Rodger and Frerichs 1997).

OTHER SPECIES

Other marine aquabirnavirus isolates are suspected to cause disease in red sea bream, tiger puffer, and other fish (Isshiki et al. 2004). Aquabirnavirus infection has also been suspected of causing disease in European sea bass (Bonami et al. 1983), Atlantic menhaden, and striped bass (Schultz et al. 1984), but the data are less convincing for these species.

PROBLEM 80

Infectious Hematopoietic Necrosis (IHN; Chinook Salmon Disease Virus, Sacramento River Chinook Disease, Columbia River Sockeye Disease, Oregon Sockeye Disease)

Notifiable to OIE
Prevalence Index
CF - 2, CM - 3
Method of Diagnosis
Identification of IHN virus infection in fish displaying typical clinical signs and pathology
History
Variable; acute to chronic morbidity/mortality
Physical Examination
Lethargy; sporadic hyperactivity; long, thick, trailing white feces; dorsal darkening; abdominal distension; exophthalmos; hemorrhage; pale gills; mucoid fluid in stomach
Treatment
1. Disinfect and quarantine
2. Raise temperature above 15°C (59°F)
3. Treat eggs with povidone iodine

COMMENTS

Epidemiology
Infectious hematopoietic necrosis virus, a rhabdovirus, is a major cause of mortality in salmonids (Bootland and Leong 1999). It is probably endemic to the Pacific northwest coast of North America but has been inadvertently introduced into and become established in Japan, Taiwan, Italy, France, and Germany, as well as other areas of the United States (Snake River Valley, Idaho).

In North America, natural IHN outbreaks have occurred in rainbow (steelhead) trout, Kamloops rainbow trout, and brown trout and in Atlantic, chinook, pink, and sockeye salmon. In Japan, epidemics have occurred in chum, amago, and yamame salmon (Wolf 1988). Viral strains vary in pathogenicity for different salmonids. Coho salmon and brook, brown, and cutthroat trout are considered refractory, although the virus has been isolated from asymptomatic coho salmon and from brook and cutthroat trout.

IHN is most serious as a disease of cultured rainbow trout in freshwater. However, Atlantic and Pacific salmon reared in seawater can be severely affected and large mortalities have occurred in some feral Pacific salmon populations (Anonymous 2006). During IHN epidemics, only young (<2 years old) fish become clinically ill. High mortality can occur in fish less than 6 months old, while older fish have lower mortality and may not show clinical signs (Yasutake 1978). Prodromal period is about 5–14 days. Temperature has an important influence on epidemics. Peak mortalities (to 100%) occur at 10°C (50°F); fewer and more chronic mortalities occur at less than 10°C, while fewer and more acute mortalities occur above 10°C. No disease occurs above 15°C (59°F; Amend 1970). Isolates vary in virulence.

During epidemics, virus is readily transmitted horizontally by ingestion of infected tissue, as well as by the feces, urine, and mucus of infected fish. Surviving fish release virus for about 45 days. Survivors have strong protective immunity and can become carriers, but the virus is not detectable until sexual maturity. Vertical transmission probably occurs via transport in reproductive fluids and on the outside of the egg. Virus is abundant in the water during spawning, and horizontal transmission between carriers and uninfected adults is also considered a distinct possibility. The gills are implicated as a major portal of entry and gill tissue has large amounts of virus just before spawning. Virus has also been isolated from leeches, copepods, and mayflies (Winton 1991).

Clinical Signs/Pathology

GROSS LESIONS

The typical presentation of IHN is increased mortality among fry or fingerlings of susceptible species at the appropriate temperature. Larger, more robust individuals die first. Fry are lethargic (swim feebly and avoid current by moving to the edge of the raceway) with sporadic hyperactivity. A long, thick, off-white fecal pseudocast trailing from the rectum (Fig. II-80, A) is diagnostic. Other clinical signs include darkening, abdominal distension, exophthalmos, and hemorrhage at the base of the fins. Gills are pale, and internally, there is visceral pallor, caused by anemia. There is no food in the gastrointestinal tract, which is distended with an off-white, translucent, mucoid, fluid. There may be petechiation of the visceral fat, mesenteries, peritoneum, swim bladder, meninges, and pericardium (Wolf 1988). In sockeye salmon, 5% or more of surviving fish may have spinal deformities (Amend et al. 1969). Clinical signs are less severe in older fish and may be absent or simply appear as lateral compression because of anorexia (Yasutake 1978).

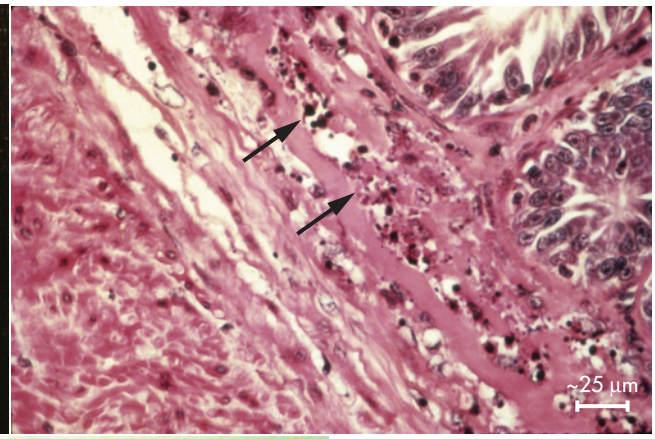
CLINICAL PATHOLOGY

IHN causes profound changes in cellular and chemical blood constituents, primarily because of renal damage. The most diagnostic change is the presence of remnants of necrotic cells ("necrobiotic bodies"), probably erythrocytes, in kidney smears (Fig. II-80, B) (Yasutake 1978). These cells are less frequent in peripheral blood (Yasutake 1978). Fish are anemic and leukopenic, and there is evidence of osmotic imbalance (hypoosmolality) (Amend and Smith 1974).

HISTOPATHOLOGY

In affected fry, major changes are necrosis of the kidney, hematopoetic tissue, pancreas, gastrointestinal tract, and interrenal tissue (adrenal cortex). Splenic and renal hematopoetic tissues are usually affected first and most severely (Fig. II-80, C); interrenal tissue may eventually be involved, as well as glomeruli and tubules. Pancreatic necrosis is common. Pleiomorphic intracytoplasmic and intranuclear inclusions are present in the pancreatic acinar and islet cells. Hepatic necrosis has been reported in some cases. Necrosis of the eosinophilic granule cells of the intestinal submucosa (Fig. II-80, D) is highly

A



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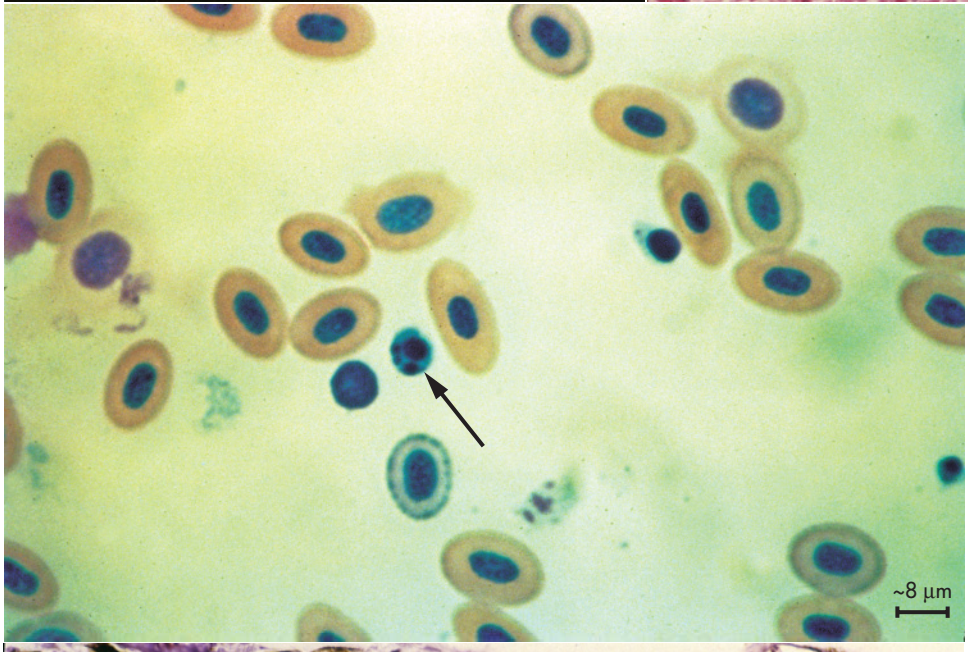

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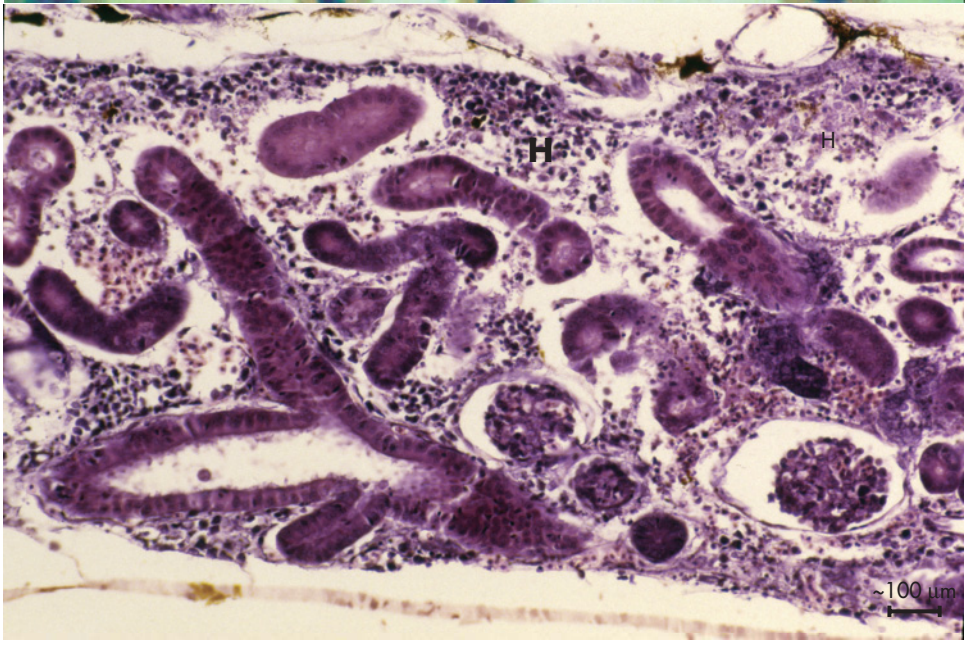


C

diagnostic but is only evident in fish at least 3–4 months old (Yasutake 1978).

In older fingerlings, lesions are similar (splenic and renal hematopoetic necrosis, moderate sloughing of intestinal mucosa, degeneration of pancreas) but more subtle. One distinguishing feature may be the presence of gill lesions (branchial hyperplasia and fusion) (Burke and Grischkowsky 1984).

Diagnosis

CLINICAL IHN

Definitive diagnosis of clinical IHN requires culture and identification of virus from target tissues with appropriate clinical signs and history in susceptible species. Note that there may be few clinical signs or histopathological changes in fish over 6 months old. Virus is usually abundant in organ homogenates from clinical cases. Virus can also be isolated from dead eggs and dead, partly developed, embryos. The IHN virus is stable in frozen viscera (months) and tissue samples can be stored at 4°C (39°F) before processing.

Presumptive diagnosis of clinical IHN is based on the presence of typical clinical signs and pathology in susceptible species kept at low temperature. Major differentials include other salmonid viral infections, including IPN (see PROBLEM 79), VHS (see PROBLEM 81), and herpesvirus salmonis disease (see PROBLEM 88). Especially diagnostic features include the presence of white feces (which are thicker and longer than those seen with IPN). Key microscopic features include renal and hematopoietic necrosis. Degeneration and necrosis of the granular cells of the stratum compactum and stratum granulosum are pathognomonic. The presence of necrobiotic bodies is also supportive, although such cells also are present to a lesser extent in IPN and VHS.

When the above lesions are present in salmonids, there is a high probability of the disease being IHN. However, caution is warranted if there are lesions in other tissues, such as pancreas, since other viruses can cause similar lesions; although rare, dual virus infection does occur (Mulcahy and Fryer 1976).

Histopathology should be supported by at least confirmation with immunological (Yamamoto et al. 1989; Anonymous 2006) or molecular (Winton and Einer-Jensen 2002) probes, when possible. Virus-infected cells can be immunologically identified in histological sections or tissue smears from target organs or blood (Yamamoto et al. 1989). IHNV is a relatively weak immunogen. However, there is little antigenic variation among various isolates, making serological identification of the virus relatively simple.

SUBCLINICAL CARRIERS OF IHNV

Adult carriers are asymptomatic. In female carriers the most sensitive tissues for virus isolation are ovarian fluid, gills, pyloric ceca, and kidney. Postspawning examination of a carrier's ovarian fluid is best, since no virus may be detectable for as little as 2 weeks before spawning. In spawning males, kidney and spleen are best. Sperm strongly adsorbs IHNV (Mulcahy and Pascho 1984) but appears to be inactivated by yolk components, reducing the chance of vertical transmission (Yoshimizu et al. 1989). While screening for carriers can also be done using immunological or molecular probes of tissue, this is not an approved method to obtain approved IHN-free status (Anonymous 2006).

Treatment

Disinfection and quarantine are the only proven means of controlling IHN epidemics. Extreme caution should be taken to avoid spreading virus to uncontaminated areas, both within and outside of the hatchery. Aside from epidemics, the principal risk of IHNV infection is infected broodstock. Carriers that survive an outbreak do not shed virus until immediately before and after spawning. Avoidance is the most useful prophylactic measure. Use of specific pathogen-free water (i.e., spring, well, or disinfected surface water) can be used for rearing susceptibles (Wedemeyer et al. 1979). However, a high percentage of many salmonid stocks are believed to carry latent IHNV infections, making avoidance of the virus virtually impossible in many cases, especially when propagating feral salmonids. Infection incidence among various feral American Pacific salmon stocks ranges from 5% to 94% (Grischkowsky and Amend 1976). Infection incidence is higher in females than males.

Infection incidence can be reduced with broodstock culling (Mulcahy 1983), where eggs and ovarian fluid of individuals or small groups (three to five fish) of females are sampled for virus. Each group's eggs are maintained under quarantine until virus status is determined. While males have a much lower incidence of infection, it is also advisable to screen them, as well. Infected lots are destroyed and only virus-negative progeny are combined for rearing. While it is labor intensive and does not totally

Fig. II-80. A. Salmonid with IHN. Note the characteristic, thick trailing fecal cast. B. Blood smear with necrobiotic body (*arrow*) caused by IHN. Giemsa. C. Histological section showing acute necrosis of kidney hematopoietic tissue (*H*) caused by IHN. Note the lack of damage to renal excretory tissue. Hematoxylin and eosin. D. Histological section showing acute necrosis of eosinophilic granular cells (*arrows*) of the intestinal submucosa. Hematoxylin and eosin. (*A* photograph courtesy of K. Wolf; *B* and *D* photographs courtesy of C. Smith; *C* photograph by L. Khoo and E. Noga.)

eliminate the virus, this procedure can dramatically reduce the incidence of viral infection and subsequently greatly increase fish yield (Mulcahy 1983). Some have suggested that salmonids might not become carriers of IHNV, but rather, might become infected with the virus immediately prior to spawning, via the presence of some reservoir of IHNV in the environment (Lewis and Leong 2004). If true, it would not justify culling, which is labor-intensive and risks the loss of valuable genetic material.

Elevating the temperature over 15°C (59°F) can stop epidemics but is only effective for some strains (Mulcahy et al. 1984). For example, the Buhl, Idaho, strain of IHNV is resistant to high temperature. Elevated temperature is also much less effective in fish that show clinical signs and does not eliminate the carrier state. Elevating temperature is only practical for small volumes of water (eggs or fry). Experimental vaccines look promising (Winton 1997; Lewis and Leong 2004), but none are yet commercialized.

The IHN virus can survive well in frozen viscera. Virus can remain infectious in water for months (Toranzo and Hetrick 1982). It is less stable in brackish or seawater compared to freshwater. The virus is readily inactivated by 25 ppm iodine for 5 minutes (Amend and Pietsch 1972). Treating eggs with iodophore will greatly reduce the chance of vertical transmission, but there have been cases where this treatment did not eliminate IHNV (Wolf 1988).

Ectoparasites (e.g., leeches) and insects are considered potential reservoirs for the virus (Mulcahy et al. 1990). Exposure to copper (Hetrick et al. 1979) or other stressors increases susceptibility.

PROBLEM 81

Viral Hemorrhagic Septicemia (VHS; EGTVED Disease)

Notifiable to OIE

Prevalence Index

CF - 2, CM - 4

Method of Diagnosis

Identification of VHS virus infection in fish displaying typical clinical signs and pathology

History

Acute to chronic morbidity/mortality

Physical Examination

Neurological signs; lethargy; darkening; exophthalmos; swollen abdomen; hemorrhage

Treatment

Disinfect and quarantine

COMMENTS: Salmonids

Epidemiology

Viral hemorrhagic septicemia, caused by a rhabdovirus of the genus *Novirhabdovirus*, is a major cause of mortality in salmonids in freshwater. It is primarily a disease of rainbow trout and brown trout. Atlantic salmon, brook trout, and golden trout are experimentally susceptible (Wolf 1988). Previously confined to Europe, VHSV has since been isolated from asymptomatic steelhead trout, coho and chinook salmon in Puget Sound and the Gulf of Alaska, United States. In addition, many nonsalmonid fish can also be infected with VHSV and some display clinical disease (see "**Comments: Nonsalmonid Fish**").

During VHS epidemics, any age salmonid can become clinically ill, but young fish are most severely affected. Mortality can be up to 100% in fry and often ~30–70% in older fish. Prodromal period is usually 1–2 weeks but may be 3–4 weeks at low (e.g., 2°C [36°F]) temperatures (Yasutake and Rasmussen 1968). Epidemics occur at 3–12°C (37–54°F), with highest mortalities at about 8–10°C (46–50°F). Outbreaks rarely occur above 15°C (59°F) and never above 18°C (64°F). Epidemics typically occur in spring when temperatures are fluctuating.

During epidemics, virus is readily transmitted horizontally by water; virus is shed in the urine and possibly from the gills but not from the feces. Ingestion of virus does not appear to be a route of transmission in salmonids (Wolf 1988), but rather gills and possibly skin wounds. Survivors can become carriers, shedding virus in urine or sex products, but virus is not consistently detectable in carriers until they are sexually mature. The surface of eggs released by latent carriers can carry virus, but it is lost within hours; thus, vertical transmission has not been demonstrated. Shedding only occurs in winter, when temperatures are low (Vestergard-Jorgensen 1982). Low temperature appears necessary to transfer the virus from one generation to the next (Wolf 1988). Individual fish vary widely in susceptibility. Wild strains vary in virulence. Four major genotypes of VHSV have been identified (See "**Comments: Nonsalmonid Fish**")

Clinical Signs/Pathology

GROSS LESIONS

The range of gross lesions seen with VHS in salmonids is great. There are three phases to VHS outbreaks, which reflect the severity of infection, not the chronological stages. The acute phase (typically at 15–18°C [59–64°F]) involves rapid, initially high mortality, but lower cumulative mortality. Fish are dark and lethargic (congregate away from the current on the edges of the pond or raceway, eventually massing near the outlet screen), with reddening at the base of the fins and gills caused by injection of vessels and punctate hemorrhage. There is also hemorrhage in the abdominal cavity and a leucopenia (Fig. II-81, A). In the chronic phase (typically at 1–5°C [34–41°F]), there are moderate, mainly protracted deaths that eventually result in high cumulative mortality. Fish are black, with anemia, exophthalmos, and a swollen abdomen (Fig. II-81, B). The organs are pale from severe

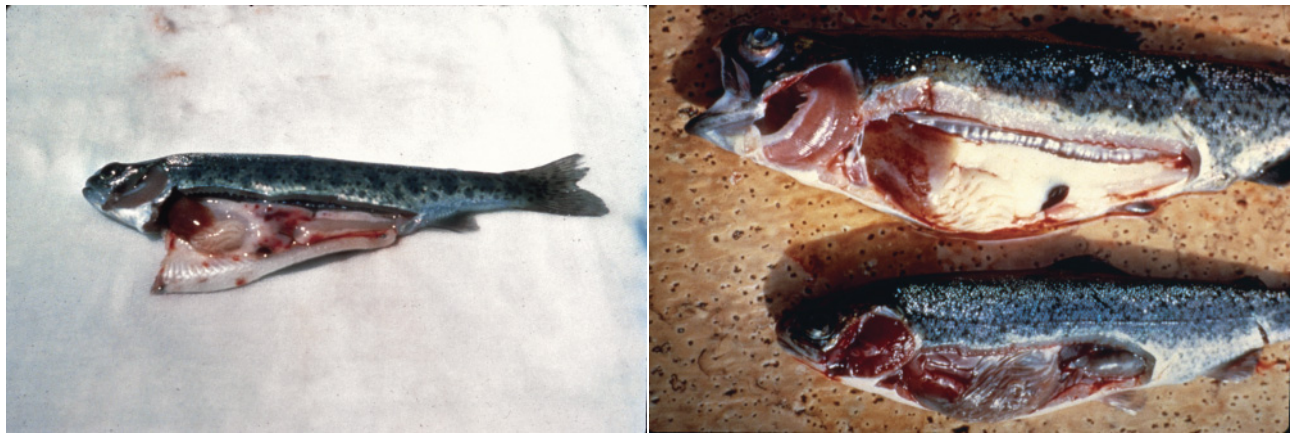


Fig. II-81. A. VHS in trout, showing punctate hemorrhage. B. Chronic VHS in trout (top fish), showing exophthalmos and anemia (pale gills and viscera). (*A* and *B* photographs courtesy of the National Fish Health Research Laboratory, USA.)

anemia, with organizing hemorrhages. The kidney and liver may be swollen. In the nervous phase, there are low mortalities. Fish do not have gross lesions but exhibit a looping swimming behavior, darting through the water and spiraling at the bottom of the pond.

HISTOPATHOLOGY

VHS virus infects endothelial cells, hematopoetic tissue and leukocytes and as with other rhabdoviruses, there is impairment of osmoregulation, resulting in edema and hemorrhage. While hemorrhage is a feature of VHS, degeneration and necrosis are the most common histopathological findings. Kidney is the prime target, with mostly damage to hematopoietic tissue. Liver necrosis and degeneration (vacuolation) is common. There is anemia, leukopenia, and thrombocytopenia.

In the acute phase there is focal hemorrhage, necrosis, and lymphocytic inflammation in all tissues, especially well-vascularized organs, such as spleen and kidney. Hemorrhage may be occasionally seen in skeletal muscle. In the chronic phase there is heavy hemosiderin deposition in melanomacrophages because of the anemia. There is also focal hyperplasia and degeneration of hematopoietic tissue; lesions that resemble membranous glomerulonephritis of mammals are also present. Exophthalmos is due to choroidal retrobulbar hemorrhage.

Diagnosis

CLINICAL VHS

Definitive diagnosis of clinical VHS requires isolation of virus from target tissues with appropriate clinical signs and history in susceptible species. Note that there may be few clinical signs or histopathological changes in salmonid fish over 6 months old. For small fish (<4 cm [<1.6 inches]), whole larvae should be examined, while for fish 4–6 cm (1.6–2.4 inches), entire viscera including kidney should be taken. For larger fish, individual organs should be sampled. Kidney and spleen have the highest titers in the acute or chronic phase. Brain should also be sampled in fish in the convalescent stage. The VHS virus is stable for months in frozen viscera and tissue samples can be stored before processing.

Presumptive diagnosis of clinical VHS is based on the presence of typical clinical signs and pathology in susceptible species kept at low temperatures. Major differentials include other salmonid viral infections, including IHN (see PROBLEM 80), IPN (see PROBLEM 79), and *Herpesvirus salmonis* disease (see PROBLEM 88). The previous restriction of VHS to Europe and absence of IHN and *Herpesvirus salmonis* disease from Europe made IPN the primary differential in Pacific coast salmonids in the United States and Canada. However, VHS must now be seriously considered as a possible differential in salmonid fish from the Pacific Northwest of the United States, although clinical disease from VHS in salmonids has not yet been reported in this area. However, the recent isolation of VHSV from diseased nonsalmonid fish in the Great Lakes region of North America and the pathogenicity of this strain for salmonids makes this an important differential for salmonid species in that area (see "**Comments: Nonsalmonid Fish**").

Especially diagnostic features include relative lack of pancreatic damage (compared with IHN or IPN), relatively normal intestine and gills, and lack of damage to eosinophilic granular cells of stratum compactum (compared with IHN).

For definitive diagnosis, histopathology or virus isolation should be supported by virus identification with an

antibody or gene probe, after submission of samples to a qualified reference laboratory. VHSV is serologically distinct from other rhabdoviruses, but there are three major serotypes, which only weakly cross-react. Polyvalent antiserum is needed for serological confirmation. Note that VHS may be complicated by concurrent infections of IPN or bacterial infections, which can confound the clinical presentation.

SUBCLINICAL CARRIERS OF VHSV

In carriers the most sensitive tissue for isolation is ovarian fluid, then pyloric ceca, then kidney. The brain should also be sampled. Postspawning examination of a carrier's ovarian fluid is best.

Treatment

Disinfection and quarantine is the most effective means of controlling VHS epidemics. VHSV is susceptible to most disinfectants but lime does not appear to be effective (Anonymous 2007b). VHSV is stable in water (over 1 week at 14°C [57°F]) and survives drying for up to 1 week at 4°C (39°F). There is no evidence for transmission by parasite vectors, but fish-eating birds can carry infected fish to other farms. The virus does not survive in the gut of homeotherms because of the low pH and high temperature.

Obtaining fish from certified VHSV-free stock is the surest method of avoiding the disease. Iodophore treatment will readily eliminate the virus from eggs of carriers, making it reasonably certain that the progeny will be free of VHS. Fish at risk because of environmental contamination should be raised in virus-free water (e.g., spring, well, or disinfected [Maisse et al. 1980]). The ability of VHSV to cause disease in fish of any age makes it a serious threat to salmonid culture and possibly other susceptible species. The virus has been successfully eradicated from some parts of Europe (Vestergard-Jorgensen 1974; Olesen 1998).

Because of the potentially devastating nature of this disease to North American salmonid stocks and its recent discovery off the west coast of the United States and in the Great Lakes region, all suspect cases of VHSV infection in the United States or Canada should be reported immediately to regional fish health authorities.

COMMENTS: NONSALMONID FISH

HOST AND GEOGRAPHIC RANGES

While freshwater salmonids are the group most commonly afflicted with clinical VHS, since the late 1970s, VHSV has been isolated from an increasingly large number of other fish that currently includes over 50 species (mostly marine), including at least 1 member of 11 orders of fish, including those of the salmoniform (7 species of salmon and trout), clupeiform (4 species of herring and anchovy), gadiform (11 species of cod), pleuronectiform (7 species of flatfish), osmeriform (3 species of smelt), perciform (6 species of perch), scorpaeniform (2 species of scorpionfish), anguilliform (1 species of eel), cyprinodontiform (1 species of topminnow), and gasterosteiform (2 species of stickleback) orders (Anonymous 2006). These nonsalmonid isolates are mainly from marine waters in North America, Asia and Europe (Skall et al. 2005) but some have recently been isolated from freshwater fish in North America (Anonymous 2007b; Table II-81).

Gene probes can distinguish four genetic strains of VHSV, which appear to group together according to geographic region, rather than host species. Genotype I includes European VHSV freshwater isolates and a group of isolates from northern European waters (Baltic Sea); Genotype II includes another group of marine isolates from the Baltic Sea; Genotype III includes isolates from the North Sea, Skagerrak and Kattegat; Genotype IV includes North American isolates and isolates from Japan and Korea. Genotype I includes the isolates that have traditionally caused disease in freshwater salmonids. The other three genotypes only include isolates from various nonsalmonid species, but the Genotype IV North American variant isolated from the Great Lakes is also experimentally pathogenic to salmonids.

CLINICALLY AFFECTED NONSALMONIDS

In the great majority of cases, there is no evidence that these nonsalmonid isolates are pathogenic for the host species but rather are only asymptomatic infections. However, VHSV can be highly pathogenic to some nonsalmonid species. Several European marine isolates cause significant losses to turbot fry in aquaculture (King et al. 2001). The Japanese flounder is highly susceptible to Japanese isolates (Isshiki et al. 2001). VHSV has also been linked to epidemics of wild Pacific herring, Pacific hake, and walleye pollock off the Pacific coast of the United States (Alaska and Washington), as well as pilchard, black cod, ratfish, and shiner perch off adjacent waters in Canada (Skall et al. 2005). Pacific isolates are experimentally very pathogenic to Pacific herring (Kocan et al. 1997).

In 2005, VHSV was isolated from diseased muskellunge and freshwater drum, as well as round goby, in the Great Lakes region (Lake Ontario, Lake Erie, Lake St. Clair, Conesus Lake [Finger Lakes, New York] and the St. Lawrence River and Niagara River) (Anonymous 2006a; Elsayed et al. 2006). It appears to have been introduced into the region in 2003 or earlier. This was the first documentation of VHSV in freshwater in North America (Anonymous 2007b). A very wide range of fish species have been associated with VHSV die-offs, including yellow perch, smallmouth bass, crappie, bluegill, gizzard shad, white bass, walleye, shorthead redhorse sucker and bluntnose sucker (Table II-81), including temperate warmwater fish (e.g., white bass, bluegill) that were never considered to be potentially susceptible to VHSV infection. It has also been isolated from asymp-

Table II-81. Fish species from which VHSV has been isolated, or which have been shown to be susceptible to VHSV by experimental infection (data from Skall et al. 2005 and Anonymous 2006). Note that a number of other poorly characterized rhabdoviruses have been isolated from various fish (Table II-88). The relationship of these viruses to VHSV is usually unclear.

Fish species	Year of first isolation
Wild-caught fish species	
North American Pacific Area	
Coho salmon	1988
Steelhead trout	1989
Pacific cod	1990
Pacific herring	1993
Tube-snout	
Shiner perch	
Pacific sandlance	1997
Pacific hake	1998
Walleye pollock	1998
Pacific tomcod	1998
Three-spined stickleback	
Pilchard	1998/99
Black cod	1998/99
English sole	
Eulachon	2001
Pacific mackerel	2001
Surf smelt	
North American Atlantic Area	
Greenland halibut	1994
Three-spined stickleback	2000
Mummichog	2000
*North American Great Lakes**	
Muskellunge	2005
Freshwater drum	2005
Round goby	2005
Yellow perch	2006
Smallmouth bass	2006
White bass	2006
Walleye	2006
Bluegill	2006
Black crappie	2006
Gizzard shad	2006
Shorthead redhorse sucker	2006
Bluntnose minnow	2006
Northern pike	2006
Brown bullhead	
Burbot	
Channel catfish	
Chinook salmon	
Emerald shiner	
Lake whitefish	
Largemouth bass	
Pumpkinseed	
Rock bass	
Spottail shiner	
Trout-perch	
White perch	
Wild caught-fish species	
Japan	
Japanese flounder	1999
Pacific sand eel	2001
Northern European Area	
Atlantic cod	1979, 1993
Haddock	1995
Atlantic herring	1996
Sprat	1996
Four-beard rockling	1996
Norway pout	1996
Whiting	1997
Blue whiting	1997
Lesser argentine	1997
Poor cod	1998
Plaice	1998
Dab	1998
Flesus flounder	1998
Sand goby	2001
Sand eel	2002
Farmed marine fish species	
Turbot	1991
Atlantic salmon	1986,1995
Japanese flounder	1996
Black rockfish	
Other fish species	
Rainbow trout	1962
Brown trout	1969
Northern pike	1978
Grayling	1979
Whitefish	1984
European eel	1987
Largemouth bass	1998
Experimentally susceptible fish	
Brook trout	
Golden trout	
Rainbow trout × coho salmon	
European sea bass	
Lake trout	
Atlantic halibut	
Schlegel's black sea bream	
Red spotted grouper	
Schlegel's black rockfish	
Pagrus sea bream	
Yellowtail	

*Rainbow trout and brown trout are also naturally infected from this region

tomatic walleye, white bass, silver redhorse sucker and short redhorse sucker. The very broad host range and high pathogenicity of this viral strain are quite striking, given that other North American isolates are low pathogenicity for all species that have been tested.

CLINICAL SIGNS IN NONSALMONID FISH

When VHSV causes disease in turbot and Japanese flounder, clinical signs and pathology are similar to those in salmonids and may include swollen abdomen with fluid and exophthalmos, as well as hemorrhages in the skin, eyes, muscles and serosal surfaces. Young fish are usually most severely affected but market size fish might also suffer mortalities, depending upon the species. In Japanese flounder, VHS is very similar to Japanese flounder rhabdovirus disease (see PROBLEM 88), but there is more prominent fluid accumulation in the peritoneal and pericardial cavities and necrotizing myocarditis is highly diagnostic (Isshiki et al. 2001). Clinical signs in other species are less typical of VHS in salmonids. For example, Pacific herring only display skin ulcers and reddening as gross lesions (Meyers et al. 1994).

RISKS TO AQUACULTURE

The high prevalence of VHSV in some marine fish species (as high as 17%) suggests that it is endemic in some marine waters (Skall et al. 2005). In areas such as Europe, the main concern for VHS control programs is protecting the freshwater rainbow trout industry. Since all VHSV isolates tested from wild marine fish have been found to have low or no mortality to rainbow trout or Atlantic salmon, from a regulatory standpoint, there is controversy over whether the presence of VHSV-infected free-living fish in an approved VHS-free area justifies withdrawal of that VHS-free status. In such cases, the main concern is how the presence of such VHSV-infected fish might affect the VHS status of adjacent rainbow trout populations and whether those aquatic species might act as nonsusceptible viral reservoirs. Further complicating this issue are data suggesting that VHSV might have originated in the marine environment, as well as circumstantial evidence that nonvirulent VHSV isolates can become pathogenic. Also, marine fish isolates are not serologically distinguishable from freshwater isolates.

For these same reasons, exposure of some cultured nonsalmonids to wild fish might be a risk factor in contracting VHS, making such wild fish a potential risk to mariculture. For example, many VHSV isolates from wild marine fish in European waters are pathogenic to turbot. For the same reasons, cultivation of rainbow trout and flatfish together in mariculture should be avoided, as well as introduction of farmed fish from seawater to freshwater (except for nonsusceptible species) (Skall et al. 2005).

In VHSV-affected regions in the Great Lakes, the methods for eventual management are even less clear, since the epidemic involves a wide array of wild species that have the potential to spread the disease well beyond its current boundaries (Anonymous 2006a). This VHSV isolate also causes moderate mortality in salmonids not affected by other VHSV isolates, including Chinook salmon, lake trout and steelhead trout, making it a threat to wild and farmed salmonids in this region and elsewhere.

PROBLEM 82

Infectious Salmon Anemia (ISA; Hemorrhagic Kidney Syndrome [HKS])

Notifiable to OIE

Prevalence Index

CM - 1

Method of Diagnosis

Identification of ISA virus infection in fish displaying typical clinical signs and pathology

History

Mainly chronic but sometimes acute morbidity/mortality

Physical Examination

Lethargy; hanging head up; dyspnea; abdominal distension; exophthalmos; skin hemorrhage; pale gills

Treatment

Disinfect and quarantine

COMMENTS

Epidemiology

Infectious salmon anemia (ISA), also called hemorrhagic kidney syndrome (HKS), is caused by an orthomyxovirus in the genus *Isavirus*. ISA is a major worldwide threat to Atlantic salmon farming. Initially observed in Norway in the mid-1980s and then identified in Norway in 1994, it has subsequently caused epidemics in Atlantic Canada (Bay of Fundy, New Brunswick) starting in 1996, Scotland in 1998, as well as the Faroe Islands and Denmark in 2000. In 2001, it was discovered in farms in Maine (Cobscook Bay) (Moneke et al. 2005). It has also been detected in Nova Scotia and the Shetland Islands (Keleher et al. 2001), and has most recently caused epidemics in Chile (Godoy et al. 2008).

Mortality is generally low but can be up to 100%. In Canadian epidemics, mean mortality can be 12% over a 60-day period, but has been as high as 3% per day in some cases. The disease only occurs in fish exposed to seawater and it usually affects fish after 1 year in seawater. The epidemic typically spreads slowly within a farm.

Transmission occurs from fish to fish by contact with infected fish (infectious virus is present in skin mucus, feces, urine, and blood), parts from infected fish (including viscera, trimmings, and muscle), or fomites. Vertical transmission has not been demonstrated. At the early

stages of infection, the virus is found only in the gill pillar cells and endocardial cells. Experimental application of skin mucus to gills is as efficient as injection; thus, the gills are the most likely port of entry. The infection is transmitted more than a week before fish show any clinical signs and long before the typical petechial hemorrhage occurs; thus, transmission from skin lesions is unlikely. However, the sea louse (*Lepeiophtheirus salmonis*) can experimentally transmit the virus. Coprophagy is ineffective. Virus remains infectious at 20 hours in seawater and 4 days in blood or kidney tissue kept at 6°C (43°F).

Infectious salmon anemia is only known to cause disease in Atlantic salmon, but virus has been identified in tissues of diseased, farmed coho salmon in Chile (Kibenge et al. 2001). Sea trout and rainbow trout are asymptomatic carriers (it is experimentally detectable for at least 1 month after challenge; there are mild lesions in liver and a slightly low hematocrit). The virus might also replicate in other *Oncorhynchus* or in *Salvelinus* species. ISAV genetic material is increasingly being detected in asymptomatic wild and cultured Atlantic salmon (Mjaaland et al. 2002). Isolates vary in virulence but this does not appear to be due to geographic source of the isolate (Moneke et al. 2005). Two genotypes are recognized, Genotype I (European and South American isolates) and Genotype II (North American isolates) (Godoy et al. 2008).

Few environmental factors are associated with epidemics but latent carriers that are stressed from treatment for sea lice or other infectious disease have experienced outbreaks 2–3 weeks later (Anonymous 2006).

Clinical Signs/Pathology

GROSS LESIONS

Pathology is highly variable. The uncommon, peracute form of the disease often presents with no clinical signs. Chronic disease is typical, and fish may display anorexia, dyspnea, and lethargy. Fish may congregate in the upper parts of a cage and hang motionless on the cage wall before sinking to the bottom.

ISAV primarily infects blood cells (e.g., kidney) and endothelial cells (such as those lining the liver and heart), typically causing severe anemia, vascular damage/leakage and hepatocellular degeneration. Thus, typical gross lesions include exophthalmos, distended abdomen, scale edema (vascular collapse), skin hemorrhage and pale gills (anemia) (Fig. II-82, A). Internally, there may be straw-colored or hemorrhagic ascites, hemmorhages in the peritoneal cavity (Fig. II-82, B), nephromegaly, and splenomegaly (Evensen et al 1991; Byrne et al 1998). Darkening of the liver is very characteristic, but some livers might be yellow or pale with petechiae (Anonymous 2006). Some outbreaks may be less obvious with only nonspecific mortality and poor growth.

ISA is most often diagnosed in spring. When first introduced into a population, there might be low mortality for months, until an "outbreak" occurs.

CLINICAL PATHOLOGY

In early stages, there may be a moderate to severe anemia (hematocrit 15–25), but in the later stages of the disease, there is a very severe anemia (often hematocrit <10). Blood smears show degenerate and vacuolated erythrocytes, as well as erthyroblasts (immature erythrocytes) with an irregular nucleus. There is a reduction in the proportion of leukocytes relative to erythrocytes, with the largest reduction being in lymphocytes and thrombocytes (Anonymous 2006).

HISTOPATHOLOGY

An early change is focal congestion and dilatation of hepatic sinusoids, with rupture of the sinusoidal epithelium and erythrocytes within the space of Disse. In late stages, there is multifocal hemorrhagic hepatic necrotic foci (Fig. II-82, C) that may coalesce to form a "zonal" appearance, leaving large areas around large veins intact. Kidney lesions include acute tubular necrosis with eosinophilic casts and/or moderate sinusoidal congestion with interstitial hemorrhage (Fig. II-82, D). The spleen may have moderate to severe sinusoidal congestion; there may be increased erythrophagia in the spleen and kidney (Fig. II-82, E). There may also be congestion and necrosis of the intestine and pyloric caecae. Gills may have congested branchial lamellar and filamental vessels.

Diagnosis

CLINICAL ISA

The appropriate local fish health authority should be notified if ISA is suspected. Three sets of features, including gross lesions (ascites, splenomegaly, petechiation of the visceral fat, dark or sometimes pale liver with petechiae), hematology (hematocrit <10, degenerate erythrocytes), and histopathology (hepatic necrosis and congestion) provide a strong presumptive diagnosis of ISA. All three sets of features must be present. Ascites and splenomegaly are early signs that are always present. Dark livers may not be present in all individuals but must be present in at least some fish, since this lesion is most specific to ISA (Anonymous 2006). A dark liver is typically seen when the hematocrit is <10.

A dark liver is also seen in cardiomyopathy syndrome (PROBLEM 102) and can be differentiated from ISA by its typical gross and microscopic heart lesions (e.g., lack of endothelium rupture). Pale livers with nonhemorrhagic necrosis have been observed in both ISA and winter ulcers (PROBLEM 50). Severe anemia can also be caused by ulcers or erythrocytic inclusion body syndrome (PROBLEM 44). Definitive diagnosis of ISA is via virus identification (isolation and/or molecular probe) from fish with typical pathology. Concurrent diseases may obscure the diagnosis. For virological examina-

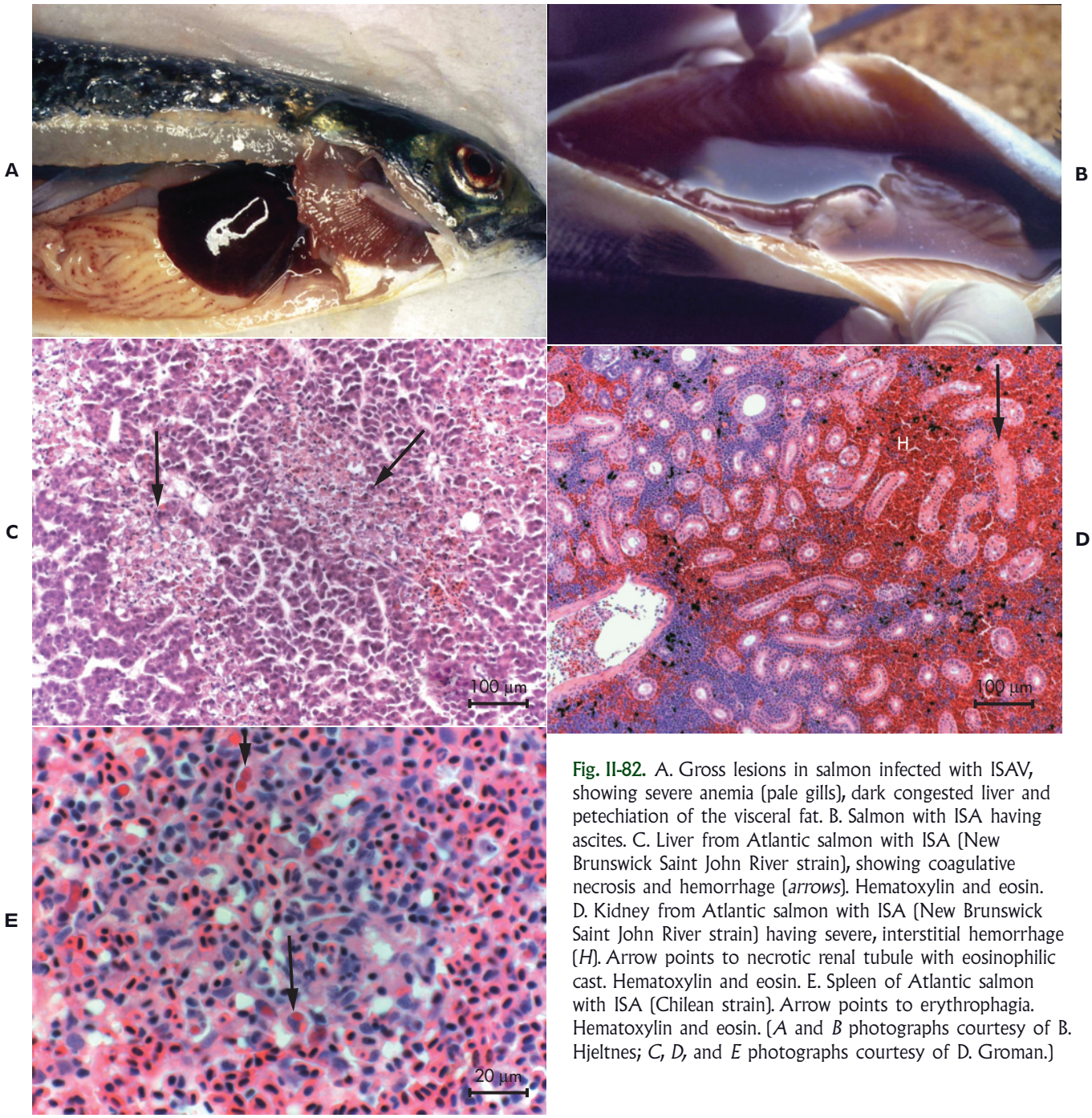


Fig. II-82. A. Gross lesions in salmon infected with ISAV, showing severe anemia (pale gills), dark congested liver and petechiation of the visceral fat. B. Salmon with ISA having ascites. C. Liver from Atlantic salmon with ISA (New Brunswick Saint John River strain), showing coagulative necrosis and hemorrhage (*arrows*). Hematoxylin and eosin. D. Kidney from Atlantic salmon with ISA (New Brunswick Saint John River strain) having severe, interstitial hemorrhage (*H*). Arrow points to necrotic renal tubule with eosinophilic cast. Hematoxylin and eosin. E. Spleen of Atlantic salmon with ISA (Chilean strain). Arrow points to erythrophagia. Hematoxylin and eosin. (*A* and *B* photographs courtesy of B. Hjeltnes; *C, D,* and *E* photographs courtesy of D. Groman.)

tion, kidney is best, with liver, spleen and heart also suitable.

CRITERIA FOR DIAGNOSING ISA ON A FARM

Because of the potentially severe economic consequences (e.g., slaughter, quarantine, etc.) resulting from a diagnosis of ISA on a farm, specific guidelines have been formulated by the OIE in making a determination of whether or not it is present (Anonymous 2006). This determination should be done in cooperation with the local regulatory agency responsible for fish health. A population is *suspected to be infected* with ISA if any one of these five criteria is met:

- Postmortem findings consistent with ISA
- Isolation and identification of ISA virus from a single sample or fish, even if without clinical signs
- Reasonable evidence for presence of ISA virus from two independent lab methods (e.g., PCR and antibody probe)

- Live fish originated from a farm where there were reasonable grounds to suspect that ISA was present at the time of transfer
- An investigation reveals other substantial epidemiological links to farms suspected to be infected with ISA.

Suspicion of *ISA can be officially excluded* if continual investigations of at least one inspection per month reveals no further significant evidence of ISA. On the other hand, the *presence of ISA is officially confirmed* if any one of these three criteria is met:

- Clinical and postmortem findings of ISA (as described in "**Clinical ISA**") are present, and ISAV is identified from either cell culture, antibody probe in tissue, or RT-PCR in tissue
- ISAV is cultured and identified in two separate samples from at least one fish per sample on two separate occasions
- ISAV is cultured and identified from at least one sample of fish, along with a positive test using an antibody or gene test

SUBCLINICAL CARRIERS OF ISAV

There is no validated method for screening healthy fish for virus but both a gene test and virus isolation have been used; a gene test appears to be much more sensitive (Anonymous 2006).

Treatment

Reservoirs of the virus are unknown. The disease can be spread from farm to farm via effluent from a slaughterhouse, or by purchase of subclinically infected Atlantic salmon smolts. A fish farm should not be closer than 5 km to the next closest farm; farms within 5 km (3.1 mi) of an infected farm might be at a 5–13 times higher risk of infection (Moneke et al. 2005). Disinfecting effluent (especially wastewater containing blood) from salmon processing plants seems to prevent transmission via this source.

Different countries have various national policies for managing ISA. Norway has "ISA-free zones," controls the sites of production and processing facilities, and restricts fish and equipment movement from infected areas to ISA-free zones. Scotland requires slaughter of all fish on infected facilities and has established zones surrounding infected farms within which all farms must fallow their pens for 6 months. In Canada, certain ISA-infected areas were fallowed and repopulated; however, this was felt to be inappropriate for the New Brunswick region, since many pens are geographically close to one another. Canadian aquaculturists in New Brunswick began vaccinating smolts with an autogenous vaccine in the winter of 1998.

Although as of 2006, ISA virus had spread no more than 15 miles from its initial site of infection in New Brunswick, the entire salmon farming industry in the Bay of Fundy area (including Maine) exists within a 25-mile radius of this focus of infection. With Maine salmon pen sites within a 3-mile radius of recently infected Canadian sites, in the event of an outbreak of ISA in the United States, the USDA (APHIS) will allow the preparation of autogenous ISAV vaccines for use in commercial U.S. salmon production facilities.

There are probably natural reservoirs in feral fish but rainbow trout is not the main reservoir in Norwegian salmon culture, where Atlantic salmon and sea trout are the only known susceptible species.

PROBLEM 83

Spring Viremia of Carp (SVC; *Rhabdovirus carpio* Infection, Swim Bladder Inflammation [SBI])

Notifiable to OIE

Prevalence Index

CF - 2

Method of Diagnosis

Identification of SVC virus infection in fish displaying typical clinical signs and pathology

History

Acute to chronic morbidity/mortality

Physical Examination

Lethargy; lying on bottom of pond; trailing mucus cast from anus

Treatment

Disinfect and quarantine

COMMENTS

Epidemiology

Spring viremia of carp is an acute rhabdoviral disease that naturally infects common carp and koi, as well as bighead, crucian, silver and grass carp. It also affects goldfish, golden ide, tench and sheatfish. Other cyprinids (roach, zebra danio, golden shiner) as well as fish in other families (northern pike, pumpkinseed, guppy), are experimentally susceptible. Common carp is the most susceptible species and the main host of spring viremia of carp virus (SVCV) (Ahne 2002; Fijan 1999).

SVCV causes major losses in cultured carp in eastern and western Europe as well as Israel. In the late 1990s, it was detected (in an unconfirmed report) in goldfish imported into Brazil (Goodwin and Winton 2004). Most recently, it has been detected in the United States in a North Carolina koi/goldfish farm and in wild common carp in Wisconsin and Illinois (Dikkeboom et al. 2004). It was subsequently isolated from common carp in the upper Mississippi River (Minnesota) and in Washington. In 2004, an outbreak was confirmed in cultured carp in China. An SVCV-like virus has also been isolated from diseased shrimp (*Penaeus stylirostris* and *P. vannamei*), but not fish, in Hawaii (Anonymous 2007a).

In typical outbreaks, SVCV spreads horizontally during the winter when water temperatures are low and host immunity is suppressed. In spring, as temperatures approach 10°C (50°F), fish develop clinical signs of SVC. Outbreaks are most severe within a very narrow temperature range, when temperatures begin to reach ~15–18°C (~59–64°F). Above (~18–26°C [64–79°F]) or below (~11–15°C [52–59°F]) this range, there are fewer, more chronic mortalities. At ~20–22°C (68–72°F) or higher, infection occurs but clinical disease is less likely to develop. When overwintering fish are in poor condition, they are more susceptible. When clinical disease is present, mortality ranges from 30% to 70%. Any age fish is susceptible (including broodstock), but disease is most severe in young fish. Horizontal transmission can occur via exposure to infected feces, urine, or skin/gill mucus. The gill is the most common portal of entry. While SVCV has been isolated from the fish louse (*Argulus foliaceus;* PROBLEM 15) and a leech (*Piscicola geometra;* PROBLEM 13) feeding on infected fish, there is no evidence that they can mechanically transmit the virus. Vertical transmission ("egg-associated") is suspected to occur, but may not be a significant source of infection. Individuals in a population vary widely in susceptibility and virus isolates vary significantly in virulence. Fish that recover from clinical disease can become asymptomatic carriers. Infectious virus can persist in 10°C (50°F) water for over 1 month.

Clinical Signs/Pathology

GROSS LESIONS

Affected fish often seek slow moving water or lie on the bottom. As the disease progresses, fish become dark, sluggish, nonresponsive to external stimuli; they often swim on their side and rest in abnormal positions. There may be exophthalmos and abdominal distension, as well as skin, gill, vent, or ocular hemorrhages (Fig. II-83). The gills may be pale. There is also internal hemorrhage and inflammation, especially in the swim bladder, but also in the intestine, peritoneum and muscle. Secondary bacterial infections, especially with *Aeromonas hydrophila*, are very common; SVC comprises the acute form of the syndrome "infectious dropsy of carp" (Fijan 1972) (see PROBLEM 47). In peracute infections, gross lesions may be absent.

HISTOPATHOLOGY

The swim bladder is significantly affected, with the epithelial monolayer becoming multilayered and the submucosal blood vessels dilated and inflamed (Negele 1977). There is also necrosis in the liver, hematopoetic tissue (kidney, spleen) and intestine. There is necrotic debris in the renal tubules and sloughing epithelium. Infected Purkinje cells in the brain cortex may have eosinophilic inclusions, which is especially diagnostic (Hoole et al. 2001).

Fig. II-83. Gross presentation of spring viremia of carp in common carp. Note the punctuate hemorrhages in the gill. (Photograph courtesy of the National Fish Health Research Laboratory, USA.)

Diagnosis

CLINICAL SVC

The appropriate local fish health authority should be notified if SVC is suspected. Definitive diagnosis is best accomplished using a specific probe to confirm the identity of cultured virus (Anonymous 2006). There is only one serotype of SVCV (isolates are antigenically homogenous). For definitive diagnosis, histopathology or virus isolation should be supported by virus identification with an antibody or gene probe, after submission of samples to a qualified reference laboratory.

For virological examination, whole fish should be used for individuals <4 cm; the entire viscera including kidney and brain for fish 4–6 cm; and liver, kidney, spleen, and brain for fish >6 cm. When virus culture is not possible due to decomposition of the carcass, the presence of typical clinical signs along with identification of the SVCV antigen (via antibody probe) is sufficient to initiate control measures.

Differential diagnoses include other swim bladder infections (e.g., *Sphaerospora renicola* [PROBLEM 69]). Spring viremia of carp is responsible for the acute phase of a disease complex known as infectious dropsy of carp (the other phase, carp erythrodermatitis or CE, is caused by *Aeromonas salmonicida*). See PROBLEM 47 for a discussion of this syndrome.

SUBCLINICAL CARRIERS OF SVCV

Definitive diagnosis of infection is based upon culture of the virus followed by identification via a gene test. An antibody test can also be used (e.g., ELISA, serum neutralization, immunohistochemistry) but is not as reliable (Goodwin and Winton 2004). Kidney, spleen, gill and brain should be sampled (Anonymous 2006). While there is a strong neutralizing antibody response to prior

infection, using this to detect prior infection has not been validated.

Treatment

Disinfection and quarantine are the only proven means of controlling SVC epidemics. Successful treatment of infected fish has not been demonstrated. However, antibiotics can be used to control the bacterial component of the disease complex. There is no approved vaccine for SVC, but naturally infected fish have strong protective immunity. Control measures include povidone iodine antisepsis of eggs and periodic chemical and physical disinfection of ponds and equipment. Minimizing stress and overcrowding, and sanitary disposal of dead fish are also recommended. Reducing fish stocking density in winter and early spring can reduce virus spread. Raising fish at a water temperature of 19–20°C (66–68°F) has been suggested, but the cost of heating water in a temperate climate can be prohibitive. There are concerns about the possible effect of this virus on indigenous, wild cyprinids in North America, so all efforts should be made to prevent viral spread. Koi and goldfish hobbyists should only show their fish where each participant's fish are kept in separate aquaria. Anglers should avoid transferring fish (including bait fish) or fish parts between bodies of water.

PROBLEM 84
Iridoviral Diseases

Notifiable to OIE

Only epizootic hematopoetic necrosis and red sea bream iridovirus

Prevalence Index

WM - 2, CF - 3, CM - 3

Method of Diagnosis

Identification of specific iridovirus infection in fish displaying typical clinical signs and pathology

History

Acute to chronic morbidity/mortality

Physical Examination

Varies greatly with affected species: abnormal swimming, reddening of body, skin ulcers, anemia and/or abdominal distension

Treatment

Disinfect and quarantine

COMMENTS

Epidemiology

Iridoviral diseases (family Iridoviridae) can cause acute to chronic morbidity and mortality in many fish species (Chinchar et al. 2005), but not all iridoviruses have been proven to cause disease (see PROBLEM 88) and whether certain iridodvirus infections are pathogenic is uncertain. The first systemic iridovirus disease to be discovered in fish was epizootic hematopoetic necrosis (EHN, also known as perch iridovirus or Nillahcootie redfin virus), a member of the genus *Ranavirus*. EHN causes mass mortalities in two exotic species in Australia, redfin perch and rainbow trout. In Australia, EHN has only been reported in farmed rainbow trout in the Murrumbidgee and Shoalhaven catchments of New South Wales, while infected redfin perch occur in many areas of southern Australia. Native Australian fish (Macquarie perch, Australian silver perch, mountain galaxias) and Atlantic salmon are also experimentally susceptible. Outbreaks of EHN have also occasionally been reported in Pakistan, Kuwait and Peru (Anonymous 2007).

Redfin perch kills occur in late spring/summer and can cause 100% mortality, while rainbow trout have low mortality, but high morbidity in summer/fall (Langdon et al. 1988; Langdon 1992b). Reinfection of rainbow trout on a site may occur annually, possibly originating from infected redfin perch in the water supply. A wide age range, fry to adults, are susceptible via horizontal transmission. A carrier state in rainbow trout seems uncommon, but infected fish can be present at very low levels in a population; thus, clinically affected fish may easily go undetected.

Disease occurs at 11–17°C (52–63°F). Other environmental factors affecting epidemics are poorly understood, although outbreaks are associated with poor water quality. The virus is very persistent in the environment and it might possibly cycle through insects or amphibians.

The closely related European sheatfish virus (ESV), affecting sheatfish, and European catfish virus (ECV I-III), affecting sheatfish and black bullhead, are both endemic to Europe and cause high mortality. ESV can also infect channel catfish, goldfish and short finned eels. These two viruses are different from EHN. EHN, ESV and ECV are all closely related to frog virus 3 (FV-3). Rainbow trout can be infected with ESV and ECV, but do not develop disease.

Clinical Signs/Pathology

GROSS LESIONS

Clinical signs are nonspecific. In perch, sudden death is the most common sign. Perch may display nervous signs (ataxia, lethargy), as well as a darkened body, and reddening around the nostrils, gills and base of the fins. Rainbow trout may also display skin ulcers and abdominal distension. In both species, the kidney and spleen may be swollen, with petechial hemorrhage on the viscera.

HISTOPATHOLOGY

There is multifocal to diffuse necrosis in the viscera, especially kidney (hematopoetic), spleen and liver (baso-

philic, spherical, intracytoplasmic inclusions in hepatocytes). Necrotic foci in the gastrointestinal epithelium are characteristic.

Diagnosis

CLINICAL EHN

Sudden high mortality in redfin perch combined with necrosis of the renal hematopoetic tissue, spleen, and liver is strongly presumptive for EHN. Definitive diagnosis is based upon the presence of typical clinical signs combined with culture of the virus or its identification in tissue. For virological examination, whole fish should be used for individuals <4 cm, the entire viscera including kidney for fish 4–6 cm, and liver, kidney, and spleen for fish >6 cm. In rainbow trout, concurrent infections are common, complicating the diagnosis. The typically low mortality rate in rainbow trout also may cause a farmer to dismiss an EHN outbreak as due to normal losses.

SUBCLINICAL CARRIERS OF EHNV

For asymptomatic fish, the kidney, liver, spleen and heart, as well as milt and ovarian fluid at spawning, should be sampled for the virological exam.

Treatment

Good sanitation and biosecurity are required in EHN-endemic areas to prevent introduction of the virus onto a facility. EHNV is relatively resistant to drying and disinfection. Sodium hypochlorite is effective for equipment and surfaces and lime may be effective in ponds (Anonymous 2007). No commercial vaccines are available. Perch that recover appear to have immunity to reinfection.

Other Iridoviral Diseases

LARGEMOUTH BASS VIRUS INFECTION

Largemouth bass virus (LMBV, also called Santee-Cooper Ranavirus [SCRV]), another member of the genus *Ranavirus*, has been isolated during kills of wild, adult (usually >30 cm [>12 inches]), largemouth bass in the United States. First identified in Florida in 1991, it has subsequently been isolated from largemouth bass in other areas of the southeastern United States, as well as Indiana and Michigan (Grizzle and Brunner 2003). Kills associated with LMBV occur during summer and usually continue for several weeks. Its association with kills of large, trophy-size bass has made it a significant concern.

The only clinical signs associated with LMBV are related to the swim bladder. Affected fish lose equilibrium and float on the water surface. The swim bladder might have a thick, yellow or brown exudate, or might be slightly reddened or overinflated. However, it might also appear normal and there are conflicting reports on the ability to experimentally reproduce disease with LMBV (Plumb and Zilberg 1999; Grizzle and Brunner 2003). Fish might need to be stressed, such as by environmental hypoxia, to be susceptible, or some fish might have acquired resistance, but the possibility that this virus might not cause disease in largemouth bass has not been entirely ruled out.

Implicating LMBV in an epidemic requires the presence of typical clinical signs, gross swim bladder lesions, and the isolation of virus from affected fish. Isolation of LMBV from healthy fish in the same population is insufficient. The virus has also been isolated from hatchery stocks of largemouth bass; chain pickerel and several centrarchids can carry the virus. No vertical transmission has been documented. The LMBV is most closely related to two other iridoviruses, each from aquarium fish: guppy virus 6 and doctorfish virus. LMBV is fairly stable in water, maintaining 10% of its infectivity after two days and still being detectable after seven days. It is stable in frozen largemouth bass for several weeks. Swim bladder, spleen, and posterior kidney are best for viral isolation (Grizzle and Brunner 2003).

MEGALOCYTIVIRAL DISEASE

Epidemiology

The genus *Megalocytivirus* includes a number of closely related viruses that all produce a highly characteristic cytopathology. First identified in 1990, it is most commonly referred to as red seabream iridoviral disease (RSID), and includes viruses that cause significant mortality in many marine fish in Japan, China and southeast Asia (Table II-84). Red seabream iridovirus (RSIV) causes disease in pagrus seabream (also known as red seabream), as well as 30 other cultured marine fish species in Japan, including members of the orders Perciformes, Pleuronectiformes and Tetraodontiformes (Kawakami and Nakajima 2002). Red sea bream iridovirus can cause significant mortality in rock bream and Japanese flounder in Korea (Do et al. 2005). A related iridovirus has been identified from brownspotted grouper in Thailand. Also within this group is ISKNV (infectious spleen and kidney necrosis virus) from mandarin fish. Turbot iridovirus (TBIV) has caused mass mortality of cultured turbot in Korea (Oh et al. 2006). Fish vary in their susceptibility to different types of megalocytiviruses. For example, red seabream is resistant to ISKNV but susceptible to RSIV, while rock bream is resistant to TBIV but susceptible to RSIV. Megalocytivirus infections are also carried asymptomatically by some fish (e.g., TBIV in Japanese flounder and rock bream), and there is evidence for the subclinical presence of megalocytivirus in many wild marine fish (Oh et al. 2006).

Clinical Signs

Clinically affected fish (mainly juveniles, but sometimes even market-size fish) are lethargic, severely anemic with gill petechiae, and have splenomegaly. Horizontal transmission is via water. Disease typically occurs when water temperature is >20°C (>68°F).

Diagnosis

A key feature of *megalocytivirus* infection is the formation of inclusion body-bearing cells (IBC). IBCs may be

Table II-84. Host range of megalocytivuses in fish (data mainly from Kawakami and Nakajima 2002).

Order Perciformes	Order Perciformes (cont'd)
Lateolabrax seabass	Threeline grunt
Redspotted grouper	Threeband sweetlips
Malabar grouper	Adjutant
Sevenband grouper	Spangled emperor
Kelp grouper	Pagrus red seabream
Orangespotted grouper	Crimson red seabream
Banded grouper	Schlegel's black seabream
Brownspotted grouper	Rock bream
Mandarin fish	Japanese parrotfish
Cobia	Spotted parrotfish
Yellowtail	Largescale blackfish
Amberjack	
Goldstriped amberjack	**Order Pleuronectiformes**
Albacore	Japanese flounder
Japanese Spanish mackerel	Spotted halibut
Pacific mackerel	Turbot
Buri-hira	
Japanese horse mackerel	**Order Tetraodontiformes**
Snubnose dart	Tiger puffer

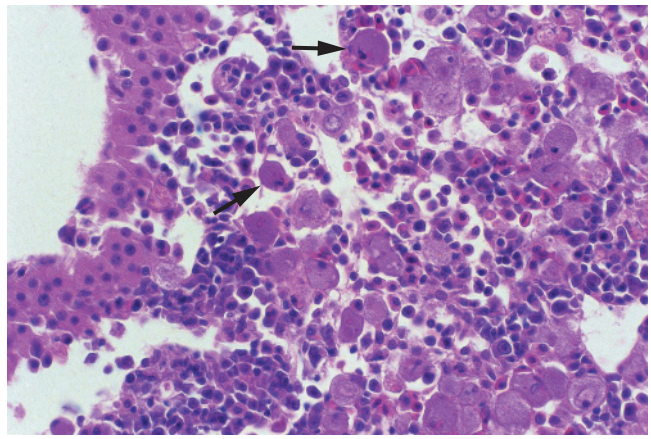


Fig. II-84. Iridovirus (*megalocytivirus*) infection of the anterior kidney of a chromide cichlid showing large, spherical, basophilic inclusions (*arrows*) in the hypertrophied, infected cells (IBC; inclusion body-bearing cells). (Photograph courtesy of H. Ferguson.)

derived from virus-infected macrophages and enlarge via the growth of a unique inclusion body that may be sharply delineated from the host cytoplasm by a limiting membrane (Fig. II-84). IBCs frequently appear in the spleen, hematopoetic tissue, gills and digestive tract. Necrotic splenocytes are also common. Along with clinical signs in the target species, the IBCs justify a strong presumptive diagnosis. Definitive diagnosis is via virus identification either from cell culture or diseased tissue using a specific antibody or gene probe. Kidney and spleen are used for both clinically affected fish and asymptomatic carriers (Anonymous 2006).

Treatment

A vaccine is under development for RSIV. When feasible, decreasing temperature might control the infection.

WHITE STURGEON IRIDOVIRAL DISEASE

White sturgeon iridovirus (WSIV) is an unclassified iridovirus that causes serious disease (up to 95% mortality) in juvenile (<1 year old), farm-raised white sturgeon in North America. First discovered in sturgeon hatcheries, it might be endemic in white sturgeon populations of the Pacific Northwest of North America. WSIV can be transmitted vertically as well as horizontally. Lake sturgeon is experimentally susceptible. Horizontal transmission is via water. The virus is also suspected to be transmitted vertically but has never been isolated from adults. A similar virus causes disease in Russian sturgeon in Europe (Anonymous 2006).

The key clinical sign is emaciation, presumably due to the cessation of feeding that accompanies infection and damage of the alimentary mucosa and olfactory epithelium. Skin and gill epithelium are also damaged and secondary infections are common. No internal lesions are diagnostic as the virus does not infect internal organs. Histological sections of skin show focal to diffuse hyperplastic epidermis with hypertrophied, amphiphilic to basophilic, Malphigian cells (Watson et al. 1998).

LYMPHOCYSTIS VIRUS DISEASE

Lymphocystis virus disease is almost exclusively a dermal disease and very rarely causes systemic infection. See PROBLEM 40.

OTHER IRIDOVIRUS INFECTIONS

See PROBLEM 88 for other iridoviruses that have been isolated from fish but for which there is little evidence of pathogenicity.

PROBLEM 85

Nodaviral Diseases (Viral Nervous Necrosis [VNN]; Vacuolating Encephalopathy and Retinopathy [VER])

Prevalence Index

WM - 2, CM - 3

Method of Diagnosis

Identification of nodavirus infection in fish displaying typical clinical signs and pathology

History

Usually acute but sometimes chronic morbidity/mortality

Physical Examination

Abnormal swimming (whirling, belly-up); abnormally dark or light body color

Treatment

1. Apply appropriate biosecurity
   a. Disinfect and quarantine
   b. Screen broodstock to eliminate carriers
   c. Ozone antisepsis of eggs

COMMENTS

Epidemiology

The piscine nodaviruses are members of the genus *Betanodavirus* in the family Nodaviridae (Munday et al. 2002). They cause mostly acute but sometimes chronic disease in at least 30 species of marine fish (Table II-85). Groupers, sea bass, and flatfish are especially common hosts. The first piscine nodavirus was described in 1990. Since then, nodavirus infections have been reported worldwide, except Africa. They have caused epidemics in Japan, Europe (Norway, Mediterranean Sea, and probably Irish Sea and Isle of Man), the Caribbean Sea (Martinique), North America, and much of the southern tropical Pacific Ocean. The unrestricted movement of fish stocks to various geographic locations has been a major reason for the spread of nodavirus infections in the marine environment (Munday et al. 2002).

There are four genetic groups of piscine nodaviruses: striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV) and red grouper nervous necrosis virus (GGNNV). Fish nodaviruses exhibit close relatedness to each other and most are relatively nonspecific in host range (Table II-85). Temperature might be more important than host specificity for the distribution of these various subtypes (Korsnes et al. 2005).

Clinical disease is usually seen in larvae and less commonly in juveniles. The earliest onset of disease also varies among viral strains, but may occur as soon as 1 day posthatch (Anonymous 2006). Lesions are usually more severe and mortalities highest in younger fish, but some nodavirus diseases can affect even market-size fish, especially European sea bass, groupers and Atlantic halibut. Horizontal transmission has only been demonstrated by co-habitation, but these viruses are very environmentally resistant and may persist in seawater for months (Frerichs et al. 2000). However, there is no evidence for fomites being a major source of contamination. Also, rotifers and brine shrimp, major feeds for larval marine fish, are resistant to infection. Vertical transmission has been demonstrated for VNN in striped jack (Muroga et al. 1998) and there is also some evidence for it in European seabass, Japanese flounder, barfin flounder and Atlantic halibut. The relative importance of horizontal versus vertical transmission seems to vary among fish species.

Clinical Signs/Pathology

GROSS LESIONS

There are high mortalities in larval or juvenile fish in hatcheries showing abnormal swimming, especially darting or whirling at the surface or bottom of the tank. Flatfish typically have a looping swimming motion and are belly-up at rest. Affected fish may also display inappetance, blindness, and abnormally dark or light body color (Munday and Nakai 1997). Swim bladder hyperinflation may also occur (Munday et al. 2002).

HISTOPATHOLOGY

Nodavirus disease causes highly consistent histopathological lesions, typified by vacuolation and necrosis of the central nervous tissue (Munday et al. 2002). Most characteristic is presence of vacuoles in the grey matter of the brain. The vacuolating encephalopathy may especially involve optic tectum and cerebellum as well as spinal cord, in some species. Gliosis, may also occur. There may be intracytoplasmic, ~1–5 μm inclusions in brain cells of some fish species, but these are difficult to see with light microscopy. Neuronal necrosis is present in most species. Retinal damage has been observed in all species where the eyes have been examined, but does not occur in all individuals. All layers of the retina might be involved, but vacuolation primarily involves the cellular layers of the retina. In some cases, retinitis (increased protein content in the posterior chamber and foamy macrophages in the outer layers of the retina) has been observed (Starkey et al. 2001). In some species, there are also lesions in other tissues, such as the endocardium or pillar cells in the gill (Grotmol et al. 1997), but these are of minor diagnostic importance compared to the neurological lesions.

Diagnosis

CLINICAL NODAVIRAL DISEASE

When high mortalities occur in susceptible larval or juvenile marine fish in hatcheries without the presence of pathogens in the clinical workup, nodavirus infection should be ruled out. For virological exam, whole larvae or small juveniles should be sampled. From larger fish, the brain, spinal cord and eyes should be sampled. Presumptive diagnosis can be made by histopathology of the brain and/or retina showing the typical vacuolating encephalopathy, necrosis, and retinopathy (Fig. II-85). However, fish may sometimes only have a few vacuoles in the brain, making diagnosis challenging (Anonymous 2006). Viral particles can be detected in brain tissue via electron microscopy, but this is only a presumptive diagnosis. Definitive diagnosis can be accomplished via identification of the virus using a specific probe (antibody or gene) (Anonymous 2006). The main method of definitive diagnosis is using a gene probe (PCR) to identify virus in either the tissue or in cell culture. Alternatively, polyclonal anti-VNN serum will detect all nodavirus strains and can be used to recognize infected cells in either tissue sections or tissue smears (Munday et al. 2002). In some fish, tiger puffer virus disease (PROBLEM 88) can mimic nodaviral infection.

SUBCLINICAL CARRIERS OF NODAVIRUS

Whole larvae or small juveniles should be sampled from asymptomatic fish. From larger fish, the brain, spinal

Table II-85. Fish reported to be susceptible to nodaviral infections (data mainly from Munday et al. 2002).

Fish species	Countries / regions
Order Anguilliformes	
Family Anguillidae	
European eel	Taiwan
Order Gadiformes	
Family Gadidae	
Atlantic cod	United Kingdom, Canada
Order Perciformes	
Family Centropomatidae	
Barramundi	Australia, China, Indonesia, Israel, Malaysia, Philippines, Singapore, Tahiti, Taiwan, Thailand
Japanese sea bass *Lateolabrax japonicus* (Cuvier)	Japan
Family Percicthydae	
European sea bass	Caribbean, France, Greece, Italy, Malta, Portugal, Spain
Family Serranidae	
Redspotted grouper	Japan, Taiwan
Yellow grouper	Taiwan
Blackspotted grouper	Taiwan
Brownspotted grouper	Thailand, China
Orangespotted grouper	China
Tauvina grouper	China
Banded grouper	China
Dusky grouper	Mediterranean
Kelp grouper	Japan
Sevenband grouper	Japan, Korea
Greasy grouper	Malaysia, Philippines, Singapore
Humpback grouper	Indonesia
Family Latridae	
Striped trumpeter	Australia
Family Carangidae	
Striped jack	Japan
Amberjack	Japan
Snubnose pompano	Taiwan
Family Sparidae	
Gilthead sea bream	Italy
Family Sciaenidae	
Red drum	Korea
Shi drum	France, Italy
White seabass	United States
Family Oplegnathidae	
Japanese parrotfish	Japan
Rock porgy	Japan
Family Eleotridae	
Sleepy cod	Australia
Family Rachycentridae	
Cobia	Taiwan
Order Pleuronectiformes	
Family Pleuronectidae	
Barfin flounder	Japan
Atlantic halibut	Norway, United Kingdom
Family Bothidae	
Japanese flounder	Japan
Turbot	Norway
Family Soleidae	
Dover sole	United Kingdom
Order Tetradontiformes	
Family Triodontidae	
Tiger puffer	Japan

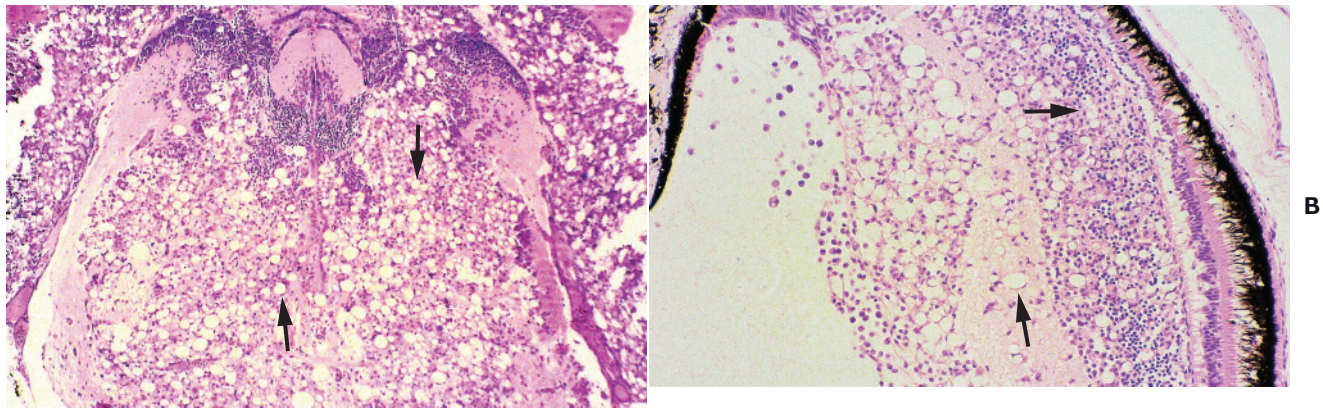


Fig. II-85. Typical histological lesions of viral nervous necrosis. A. Brain from a European seabass showing severe neuronal vacuolation (*arrows*). Hematoxylin and eosin. B. Retina of Atlantic cod showing vacuolation (*arrows*) in all cell layers. Hematoxylin and eosin. (*A* and *B* photographs courtesy of H. Ferguson.)

cord, and eyes should be sampled, and/or the ovarian fluid at spawning.

Treatment

Disinfection and quarantine are the only proven means of controlling most nodaviral epidemics.

Elimination of the infection has been achieved in some instances. VNN in striped jack was successfully controlled by ozonation of fertilized eggs combined with detection and elimination of virus-carrying broodstock (Mushiake et al. 1994). Ovarian products of broodstock are screened with a gene probe. The fish are also bled to determine if serum antibodies to nodavirus are present, since the virus is not always detectable in the gonads. For ozonation, eggs are washed in seawater with residual ozone levels of either 0.2 µg/ml (striped jack) or 4.0 µg/ml (Atlantic halibut). In fish species where vertical transmission appears to only occur at a low level, stocking larvae at a low density (<10 per liter in ponds) has been successful. Broodfish should not be stressed by too frequent spawning. No vaccine is commercially available for any nodavirus disease.

PROBLEM 86

Koi Herpesvirus Disease (KHVD; Carp Nephritis and Gill Necrosis Virus [CNGV])

Notifiable to OIE

Prevalence Index

WF - 2

Method of Diagnosis

Identification of KHV infection in fish displaying typical clinical signs and pathology

History

Acute morbidity/mortality

Physical Examination

Pale, swollen, mottled gills; abnormal coloration, skin lesions, enophthalmos, dyspnea, erratic swimming

Treatment

Disinfect and quarantine

COMMENTS

Epidemiology

Koi herpesvirus disease is a serious emerging disease caused by an agent considered a herpes virus (KHV, cyprinid herpesvirus 3 [CyHV-3]) (Pokorova et al. 2005). It has caused major losses in koi and mass mortalities in common carp (Grimmett et al. 2006). First identified in 1998 in Israel and the United States (Perelberg et al. 2003), it has subsequently been reported in an ever greater number of countries in Asia (Japan, China, Indonesia, Taiwan, probably Malaysia) and Europe (United Kingdom, Belgium, Denmark, the Netherlands, Germany, Italy, Austria, Switzerland, Luxembourg, and France) (http://www.koihealth.org). It has been identified in some Eastern European countries (Poland and the Czech Republic), where common carp culture is a major industry. The rapid spread of KHVD is probably due to the unrestricted movement of koi in the aquarium industry.

KHV is highly contagious and can be transmitted via water, feces, or direct contact. The virus is relatively short-lived in water (can remain viable in water for at least 4 hours), but appears to be much more stable in sediment or filter medium. There is some indication that infection can be spread by transfer of contaminated filter medium (Way et al. 2004). The gills and/or intestine might be portals of entry.

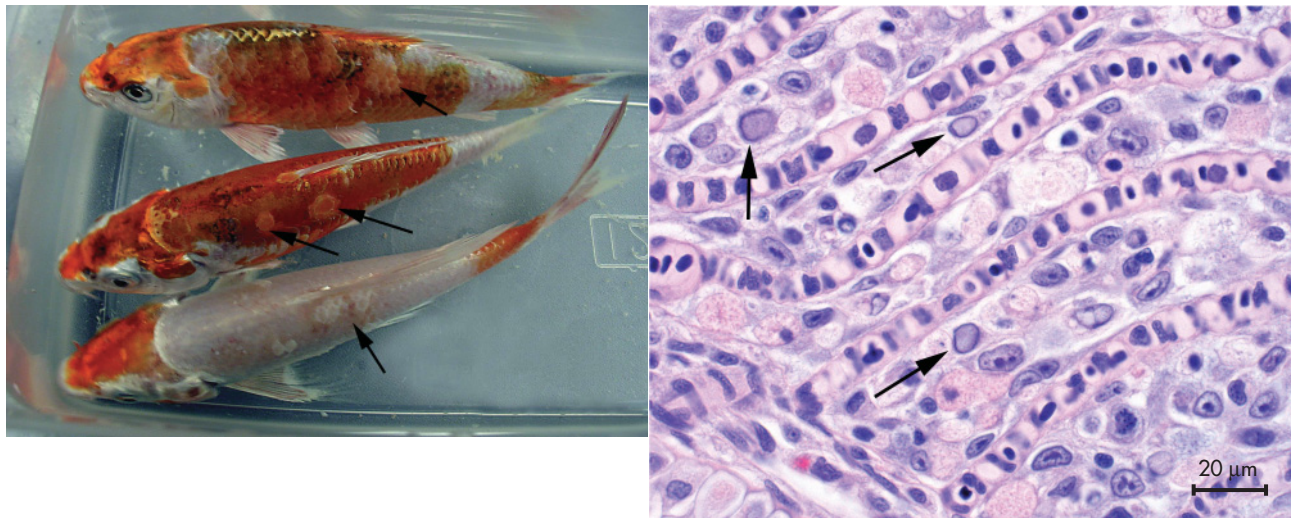


Fig. II-86. Typical lesions of koi herpesvirus (KHV). A. Koi from a naturally occurring outbreak of KHV. *Arrows* indicate skin lesions presenting as blanched, circular to diffuse areas, characteristically seen during acute episodes of KHV disease. B. Histological section of gill of KHV-infected fish showing cells with characteristic changes, including swollen cytoplasm and nucleus with the margination of chromatin prominent in the affected nuclei (*arrows*). Hematoxylin and eosin, bar = 20 µm. (*A* and *B* photographs courtesy of R. Hedrick.)

Affected populations can exhibit up to 100% mortality (Dishon et al. 2005). Temperature is the single most important determinant of pathogenicity. Outbreaks are most severe and fish are most susceptible at 18–28°C (64–82°F); disease does not develop at <13°C or >30°C (<55°F or >86°F). Epidemics typically occur in spring or summer. Moving infected fish from a cooler (13°C [55°F]) to a warmer (23°C [73°F]) temperature can rapidly induce mortality (Gilad et al. 2003). The permissive temperature range (i.e., the range in which clinical disease always occurs) has been generally considered to be 23–28°C (73–82°F), but some consider it to be a slightly lower range. Recovered fish develop high antibody titers (Ronen et al. 2003) and often become latent carriers. The site of latency is unknown but both the carrier state and virus shedding have been demonstrated (St.-Hilaire et al. 2005).

Clinical Signs/Pathology

GROSS LESIONS

Pale, swollen, mottled gills is the most common gross sign. Gill damage probably significantly contributes to morbidity. Other characteristic signs include abnormal coloration, skin lesions (Fig. II-86, A), enophthalmos and dyspnea (increased respiratory rate). Affected fish may also be anorectic and display erratic swimming.

HISTOPATHOLOGY

There is massive branchial epithelial hyperplasia with degenerative and necrotic changes. Infected cells develop intranuclear inclusions (Fig. II-86, B). Liver, spleen, and gastrointestinal tract have parenchymal necrosis and numerous macrophages with ingested cell debris. Neural tissue is not prominently involved.

Diagnosis

CLINICAL DISEASE

The presence of typical clinical signs and histopathology demonstrating massive proliferation, degeneration, and necrosis of gill epithelium in the absence of another etiological agent (parasite or bacterium) is strongly suggestive of KHVD. However, virus identification is required for confirmation. Cell culture of kidney and spleen can be used but PCR of gill, kidney and spleen is more sensitive. A rapid antibody kit for measuring KHV antigen in feces is available in Israel (Ko Vax, Ltd., Jerusalem).

It is important to rule out carp pox (cyprinid herpesvirus disease [CHVD]; PROBLEM 88). Carp pox can be lethal to many cyprinids when they are less than 2 months old, but in older koi and common carp, carp pox only causes skin lesions. Specific confirmation of KHV should be done by identifying the specific virus involved. A gene test for KHV is offered by Research Associates Laboratory. Several other commercial laboratories in various countries (United States, United Kingdom, Israel, and others) can also perform diagnostic tests for the diagnosis of clinical cases or identification of carriers. Laboratories are listed at: http://koiclubsandiego.org/library/khv.

SUBCLINICAL DISEASE

Antibodies can be detected via either ELISA or virus neutralization (more sensitive) (Anonymous 2008), with commercial testing labs (see above).

Treatment

If KHV is diagnosed, it is advisable to depopulate the facility and thoroughly disinfect the premises. If this is not feasible, temperature manipulation may control the disease, but will most likely result in a fish population with chronic carriers that can later infect other fish. If KHV-infected fish are held for a long period (~2 months) at low (13°C [55°F]) temperature, they do not develop disease when moved to the warmer, permissive temperature at which they would normally experience high mortality (Gilad et al. 2003). There may also be development of natural resistance after recovery from infection if fish are exposed to virus for 3–5 days at 23°C (73°F) and subsequently moved to 30°C, where disease does not develop (Ronen et al. 2003). Highly susceptible strains of koi that are outbred with wild-type carp display significant resistance (Shapira et al. 2002). KHV might also infect goldfish and crucian carp, so goldfish should not be co-habited with koi.

When fish have an unknown history of KHV exposure, quarantine is the best means of reducing the likelihood of KHV introduction into a facility. The level of biosecurity imposed will dictate the degree to which it is likely that virus-infected fish will be excluded. The most basic quarantine procedure is to hold fish at the permissive temperature (23–28°C [73–82°F]) for at least 4 weeks. Fish must be held within this temperature range for 4 weeks and thus quarantine will be longer if the fish must first be acclimated to this temperature. It is also advisable to test all fish for KHV antibodies. Antibodies might be detectable in fish that have been exposed to KHV 1 year previously. Note that a live, attenuated vaccine is being used in some countries (see below); and thus in fish from those countries, one cannot determine if an antibody response is due to natural infection or to the vaccine. If antibody screening is done prior to placing all fish at the permissive temperature, fish that are antibody-positive can first be removed, reducing the risk of infecting the KHV-free fish in the quarantine group. If done after the fish are in quarantine, fish should not be sampled until the end of the quarantine period. A third level of biosecurity is to include fish that are known to be free of KHV in the quarantine group; these sentinel fish increase the likelihood of detecting virus since asymptomatic fish in the test group might only shed virus and not display clinical signs at the permissive temperature (Anonymous 2008).

A live commercial vaccine is available in Israel (KV3, Kovax/IL, Ko Vax, Ltd., Jerusalem), but is not yet approved in other countries. A number of other groups are also working on development of a vaccine.

PROBLEM 87

Alphavirus Diseases (Pancreas Disease [PD]; Sudden Death Syndrome [SDS]; Sleeping Disease [SD])

Prevalence Index

CF - 3, CM - 3

Method of Diagnosis

Identification of alphavirus infection in fish displaying typical clinical signs and pathology

History/Physical Examination

Pancreas disease: anorexia; emaciation

Sleeping disease: fish recumbent on bottom

Treatment

None

COMMENTS: Pancreas Disease (PD)

Epidemiology

Two alphaviruses (*Alphavirus* spp., family Togaviridae) affect fish: salmon pancreas disease virus (SPDV; salmonid alphavirus [SAV] 1, 3, 4, 5, 6) and sleeping disease virus (SDV; SAV 2) (McLoughlin and Graham 2007). These are subtypes of the same virus (Weston et al. 2002) with further subtypes having been recently identified (Fringuelli et al. 2008).

Pancreas disease (PD), first described in Scotland in 1984, is a severe, usually chronic problem that affects sea-cultured salmonids in Europe (Norway, Scotland, Ireland, France) and the northeast Pacific coast (United States, Canada) (Lewis and Leong 2004). The SPD virus is most likely endemic in Atlantic salmon farming areas in Europe and possibly worldwide. PD has not been observed in freshwater.

Atlantic salmon are most susceptible and at any age (S0, S1/2, and S1 smolts); brown and rainbow trout are experimentally susceptible, but lesions tend to be much less severe. Sea reared rainbow trout are susceptible to Norwegian PDV (SAV 3) (Taksdal et al. 2007). Transmission is usually fish-to-fish contact, but possible involvement of vectors (e.g., sea lice) or a wild reservoir have not been ruled out. Fish usually develop the disease 6–12 weeks after transfer to seawater, but outbreaks have occurred after 2 years in seawater. Epidemics have occurred at any time of the year, but the temperature range most favorable for the virus is 9–12°C (48–54°F). The virus is inhibited at >15°C (>59°F).

There is up to 100% morbidity but usually low mortality, although it can range up to about 60%. Higher losses tend to occur in high energy sites (i.e., cages offshore or in strong tidal zones) due to exhaustion (see "**Histopathology**"). While mortalities are typically lower at colder winter temperatures (i.e., later in the produc-

tion cycle), such epidemics are more costly than ones that occur soon after the smolts are placed in cages because the disease is more insidious, spreading slowly though the sea cages, and thus the effect on growth rate is more prolonged and damaging (McLoughlin et al. 2002). While most fish usually recover, they may be "poor doers" (stunted) and susceptible to other diseases.

Clinical Signs/Pathogenesis

CLINICAL SIGNS

Epidemics of PD run through four phases: peracute, acute, sub-acute, and chronic. These phases are most readily identified via histopathology but the general course of epidemics is typically initiated by a rapid drop in feeding, in some cases preceded by voracious feeding. Fish become lethargic and swim around the edges and corners of the cage. There might be abnormal swimming (including whirling or circling), spitting out feed pellets, yellow, cast-like feces, and mortality in large fish (sudden death syndrome), with fish lying motionless on the bottom of the cage. Some fish may die in the early stages but most mortality occurs in the chronic stage (3–6 weeks after the acute phase at 12–14°C).

GROSS LESIONS

In the acute phase PD, the gut is empty of food, has yellow-white casts and has hemorrhage on the surface of the pyloric caecae (i.e., in the pancreas and in pancreatic fat between the pyloric caecae). In chronic PD, there is a large decrease in abdominal fat and poor body condition (Figs. II-87, A, B). In some cases, there may be tissue atrophy between the pyloric caecae. Fish with good condition may show no gross lesions but cardiac rupture is occasionally observed.

HISTOPATHOLOGY

The pancreas is invariably damaged, but concurrent severe cardiac and subsequent skeletal myopathies can also occur (Fig. II-87, C through E). Histological lesions in pancreas disease and sleeping disease are similar. The acute phase is rarely observed, but is characterized by acute, diffuse necrosis/apoptosis of pancreatic acinar tissue, with a rapid disappearance of the exocrine pancreas tissue. Inflammation in the peri-pancreatic fat is variable. There may also be acute necrosis of cardiac myocytes (eosinophilic cells with shrunken nuclei) in both the compact and spongy ventricular muscle and atrium. Epicarditis may also be a feature. In sub-acute PD, there is major loss of exocrine pancreas, and variable cardiac myopathy. There is also hyaline degeneration of both red and white skeletal muscle fibers.

In chronic PD, the pancreas is recovering but if severely damaged may undergo fibrosis. Recovering fish may have foci of regenerating acinar tissue among the fibrotic lesions (McLoughlin et al. 2002). Cardiac myocytes undergo rapid regeneration, especially in younger smolts, displaying numerous mitotic figures. Heart tissue is highly cellular, with large nuclei evident in the junction between the compact and spongy ventricular muscle. Skeletal and heart muscle damage peaks in the chronic phase, often corresponding to peak mortality in the cage. In severely affected fish, all red muscle bundles and many white muscle fibers are affected. Skeletal muscle lesions are similar to vitamin E-selenium deficiency (Ferguson et al. 1986) and PD was initially suspected to be a non-infectious disease (McVicar 1990).

Diagnosis

Definitive diagnosis of PD is based on the characteristic clinical signs and histopathological lesions combined with virus isolation, positive gene probe in serum or heart tissue, and the presence of specific neutralizing antibodies (Hodneland and Endresen 2006) (Table II-87, A, B). Careful interpretation of key histological lesions is important for a proper diagnosis and prognosis. Acute signs of PD are difficult to detect, especially in winter; subacute and chronic phases are most easily identified. However, after about 40 days into an epidemic, it becomes more difficult to make a definitive diagnosis as all severely affected fish might have died and all others are recovering.

Prognosis for survival is directly related to the severity of red and white skeletal muscle damage, with severe damage correlated with poor survival. If the damage to pancreas is minimal, full recovery of organ function can be expected, but with severe fibrosis, the fish will be stunted. Pancreas disease may be significantly underdiagnosed because of the presence of IPN (PROBLEM 79), which can mask the presence of PD, and the widespread presence of IPNV, that can interfere with isolation of SPDV (McLoughlin et al. 2002). Some key differences are:

- IPN tends to occur within 3 months of transfer to the sea
- Mortalities occur during the acute phase of IPN
- Inappetance is less with IPN
- With IPN, the acute pancreatic necrosis is often focal, there is a catarrhal enteritis, and no muscle lesions
- IPN can be identified in tissues using immunochemistry

Also, concurrent infections with both IPNV and SPDV can occur.

PD must also be differentiated from two idiopathic diseases causing muscle lesions in Atlantic salmon. Cardiomyopathy syndrome (CMS) tends to occur in the first sea winter or in fish close to harvest. Histological lesions are usually confined to the heart and liver and are typical of chronic, congestive heart failure (see PROBLEM 102). Heart and skeletal muscle inflammation (HSMI; PROBLEM 102) causes histopathological lesions very similar to PD and CMS, but careful examination shows them to be more inflammatory than those observed in

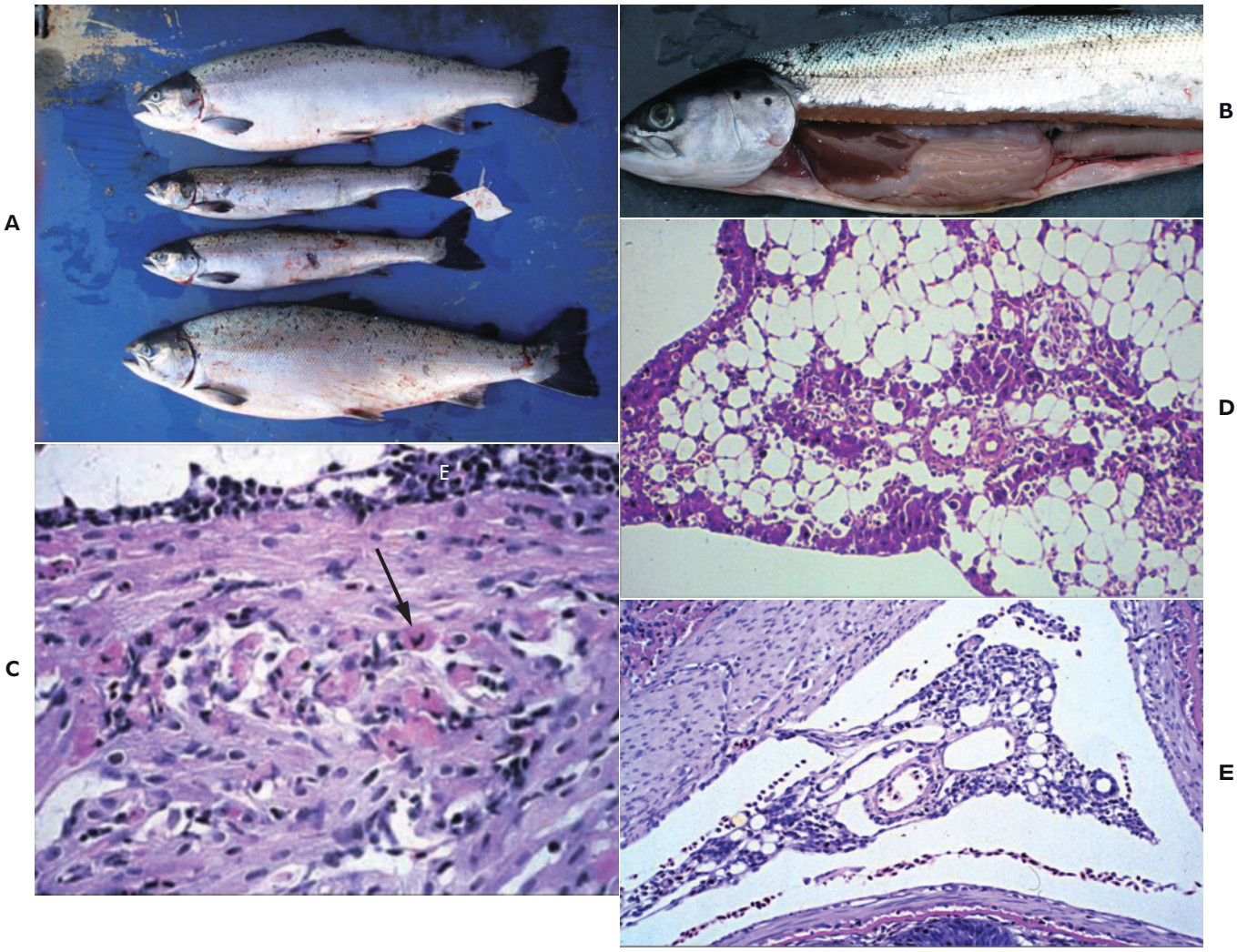


Fig. II-87. Pathology of pancreas disease. A. Severely underweight Atlantic salmon smolts (middle two fish). Compare to normal smolts (above and below). B. Runt due to PD. Note the greatly reduced amount of pericaecal fat. C. Early stage of PD. Acute, multifocal cardiac myocyte necrosis; note the shrunken and eosinophilic cytoplasm with pycnotic nuclei (arrow). There is also focal epicarditis (*E*). Hematoxylin and eosin. D. Early stage of PD. Note the acute pancreatic necrosis. Compare with Fig. I-37, *D*. The islet tissue is normal. Hematoxylin and eosin. E. Chronic stage of PD. Note the significant loss of pancreatic acinar tissue. Hematoxylin and eosin. (*A* photograph courtesy of M.F. McLoughlin; B photograph courtesy of T. Turnbull; *C* and *E* photographs from McLoughlin and Graham 2007; *D* photograph from McLoughlin et al. 2002.)

PD. Concurrent HSMI may also occur along with IPN (Kongtorp et al. 2004).

Treatment

Best management practices, such as all-in, all-out stocking and fallowing sites, have probably reduced the impact of PD (McLoughlin et al. 2002). Proactive monitoring of serum for viremia and antibody prior to risk periods for susceptible populations may also be used as early warning of infection (Graham et al. 2005). Reducing stress during the acute phase can lessen mortalities. Feeding fish smaller pellets may reduce the anorexia and overall mortality (Kent 1992). There is some evidence that withholding feed for 5–10 days upon suspecting the presence of PD can reduce losses; however, since it might take 3 months for all cages at a site to become infected, this strategy could cause significant production loses due to many days off feed. Dietary management should be specific for each cage. Vitamin C and vitamin E supplementation may aid recovery (McLoughlin et al. 2002). Fish that recover do not experience another outbreak,

Table II-87, A. Usefulness of various diagnostic features and tests for identifying pancreas disease during various stages of the disease (courtesy of M.F. McLoughlin).

Test	Peracute	Acute	Subacute	Chronic	Carrier
Days Postinfection*	0–7	0 + 7	7 > 21	21 > 42	42 > ?
Clinical signs	Absent	↓ appetite	Fecal casts	Mortalities	Runts
Virus isolation	Serum Heart	Serum Heart	Serum Heart	–	–
Histology	Absent	+ Pancreas Heart	+ Pancreas Heart	+ Heart Skeletal muscle	+ Heart Skeletal Muscle
IHC	Absent	+	–	–	–
Gene test	+ Serum Heart	+ Serum Heart	+ Serum Heart	+ Heart	+ Heart
Serology	–	–	–	+	+

*Timeline may be longer at lower winter temperature.
Virus isolation—tissues from which virus is most likely to be isolated.
Histology—tissues having the most diagnostic histopathological lesions.
IHC—immunohistochemistry (antibody test of tissue).
Gene test—RT-PCR.
Serology—detection in blood of antibody against virus.
+ diagnostic feature is present; – diagnostic feature is absent.

Table II-87, B. Differentiation of pancreas disease, infectious pancreatic necrosis (IPN), cardiomyopathy syndrome (CMS), and heart and skeletal muscle inflammation (HSMI) using histopathology (courtesy of M.F. McLou).

Lesion	Pancreas Disease						
	Acute	Subacute	Chronic	Recovery phase	IPN	CMS	HSMI
Multifocal cardiomyocytic necrosis	+	+	+	–	–	+	+
Focal or diffuse endocardial proliferation	+	+	+	+	+/–	+	+
Hypertrophy and hypercellularity of myocardial cells	–	+/–	+	+	–	+	+
Epicarditis	+/–	+	+	+	–	–*	+
Pancreas damage	+	+	+	+/–	+	–	–
Skeletal muscle damage	–	–	+	+	–	–**	+

*Key lesion is in the spongy ventricular myocardium; epicarditis may or may not be present and may be due to other causes.
**Skeletal muscle lesions are not associated with uncomplicated CMS.

suggesting that long-term immunity develops. An inactivated vaccine has been developed.

COMMENTS: SLEEPING DISEASE (SD)

Sleeping disease (SD) affects rainbow trout in freshwater. Epidemics have also been observed in coho salmon; Atlantic salmon are experimentally susceptible (Brown and Bruno 2003). Affected fish lie on their sides on the bottom in a typical "sleepy" behavior. When disturbed, fish swim for a short period but then return to the bottom. Mortality is very variable, but fish may stop feeding for several weeks, resulting in significantly decreased growth (Graham et al. 2007). First identified in France, it also occurs in the United Kingdom, Italy, Spain, and Germany (Bergmann et al. 2008).

Lesions first appear in exocrine pancreas (necrosis with rounding of acinar cells followed by lymphocyte and fibrocyte infiltration) and subsequently in heart muscle (focal hyalinization and loss of striation) and skeletal muscle (loss of fibers, increased cellularity). Diagnosis of SD is based on the characteristic clinical signs and histopathological lesions combined with identification of virus in affected tissue via gene test (RT-PCR) in serum or heart tissue and the presence of specific neutralizing antibodies (Boscher et al. 2006; Graham et al. 2007; Hodneland and Endresen 2006). Careful interpretation of key histological lesions is important.

PROBLEM 88

Miscellaneous Systemic Viral Diseases and Infections

Prevalence Index

See specific agents

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Gene or antibody identification of specific virus from typical lesions
2. Morphological (electron microscopy) identification of virus with typical histopathology

History

Variable; acute to chronic morbidity/mortality

Physical Examination

Varies with target organ(s)

Treatment

1. Disinfect and quarantine
2. Prophylactic therapies for secondary invaders

COMMENTS

This list (Table II-88) includes common to rare viral infections reported from fish. Clinically important diseases (e.g., Fig. II-88, A through C) are defined as those that have consistently been responsible for morbidity or mortality in wild or cultured fish. Not included in the group of clinically important diseases are viruses that have caused disease but have only been observed or isolated once or twice or are agents that have not been shown to cause any clinically obvious sickness in naturally infected fish (even if histopathological lesions can be seen in infected fish).

Note that for some important diseases, there is little evidence that the virus(es) isolated from affected fish is (are) responsible for that disease (e.g., EUS).

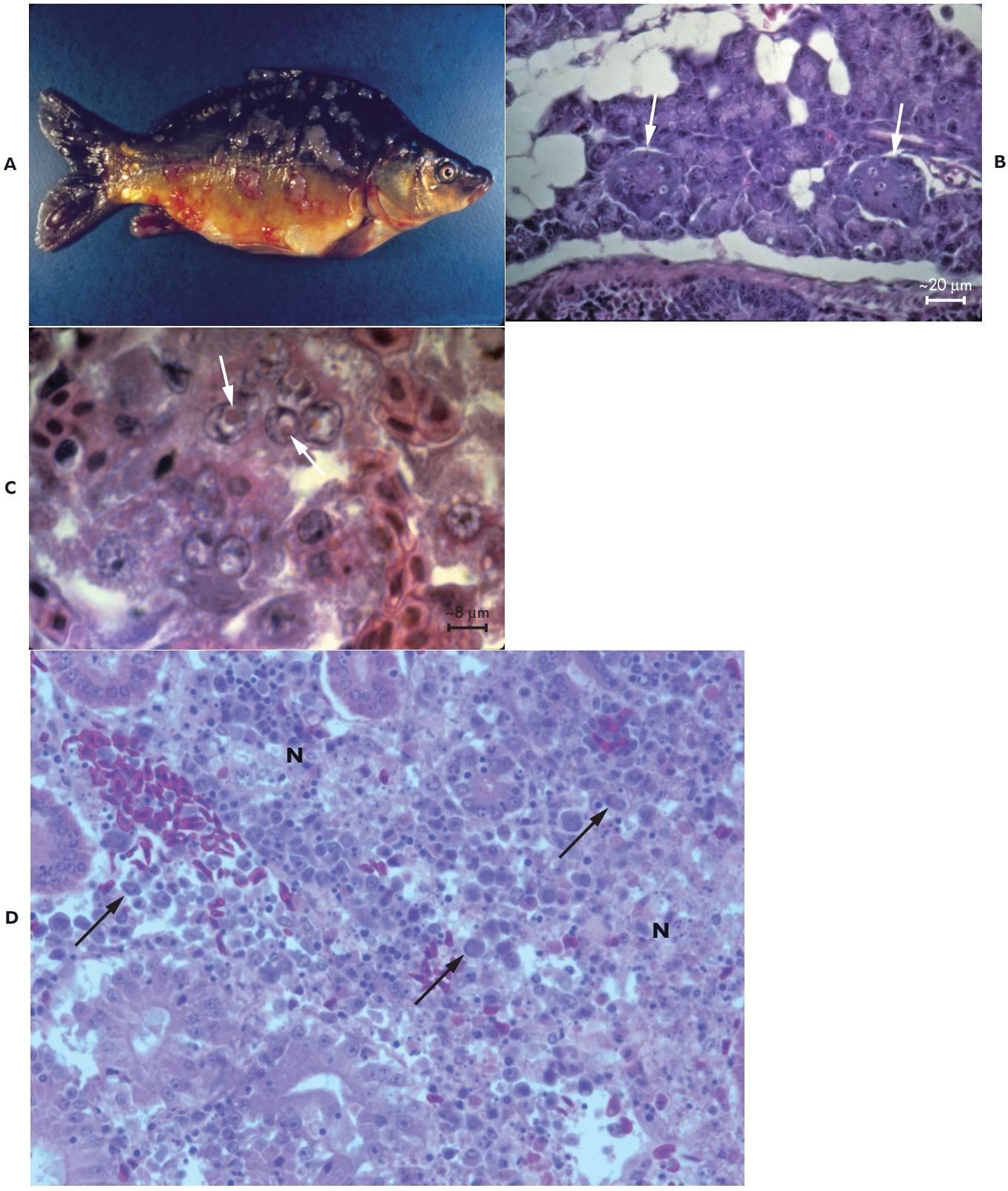


Fig. II-88. A. Carp pox lesions on common carp. B. Histological section of syncytia (fused cells; *arrows*) in the pancreatic acinar tissue of a salmonid with *Herpesvirus salmonis*. Hematoxylin and eosin. C. Intranuclear, Cowdry type A inclusions (*arrows*) in pancreatic cells infected with *Herpesvirus salmonis*. Hematoxylin and eosin. D. Histological section of goldfish kidney affected with herpesviral hematopoetic necrosis (cyprinid herpesvirus 2) showing severe, diffuse, interstitial hematopoetic necrosis (*N*). Some cells have enlarged nuclei with inclusion bodies (amphophilic centers and peripherally displaced chromatin, *arrows*). (*A*, *B*, and *C* photographs courtesy of National Fish Health Research Laboratory, USA; *D* photograph courtesy of L. Khoo.)

Table II-88. Miscellaneous systemic viral infections of fish. Clinically important diseases are indicated with an asterisk (*). For viruses associated with epithelial hyperplasia and neoplasia, see PROBLEM 76. For virus-associated hemopathies, see PROBLEM 44.

Disease / pathogen	Hosts	Geographic range	Morbidity / mortality; significance	Diagnostic features	References
Smooth dogfish herpesvirus	Smooth dogfish	Massachusetts, United States	Chronic 4	Oval, elongated, depigmented foci to 1cm on skin; degeneration of basal epithelial cells with edema and eosinophilic bodies	Leibovitz and Leibovitz (1985)
***Sturgeon wasting disease (adenovirus)**	White sturgeon	Sacramento River, California, United States	Chronic 1	Enlarged nuclei in intestinal and spiral valve epithelium; epithelial cells eventually rupture	Hedrick et al. (1985b) Benko et al. (2002)
***White sturgeon herpesvirus (WSHV)**	White sturgeon	California, United States	Acute to chronic 1	Epidermal and oral hyperplasia, hypertrophy, edema and necrosis	Watson et al. (1995) Kwak et al. (2006)
Eel rhabdoviruses (eel virus European X; eel virus American)	American eel (H) European eel (H) rainbow trout (E)	Japan France Cuba	None 2	Hemorrhage and necrosis of viscera; isolated from all ages of eels; clinical disease not yet proven experimentally for eels	Sano (1976) Wolf (1988) Vestergard-Jorgensen et al. (1994)
EV-102 (iridovirus; ICDV; icosahedral, cytoplasmic, deoxyribovirus)	Japanese eel (E)	Japan	Acute 1	Only isolated once; pathogenic to young eels: congested fins, increased mucus production; highest mortality at lower temperature (<24°C)	Sorimachi (1984) Sorimachi and Egusa (1987)
***Eel birnaviruses (eel virus European, branchionephritis, eel virus kidney disease)**	Japanese eel	Japan Taiwan	Acute 1	IPN-like. See PROBLEM 79.	Sano and Fukuda (1987)
Anguillid herpesvirus	Japanese eel European eel	Japan Taiwan	Acute 3	Reddened skin on ventrum; swollen kidney and spleen; hemorrhage of kidney, spleen necrosis, pancreatic atrophy	Ueno et al. (1996)
Rainbow smelt picorna-like virus	Rainbow smelt	New Brunswick, Canada	Acute 3	Isolated from sick fish but not yet proven pathogenic	Moore et al. (1988)
European smelt picorna-like virus	European smelt	North Sea	Chronic	Hyperplastic skin	Ahne et al. (1990)
***Pike fry rhabdovirus disease (hydrocephalus, red disease of pike, grass carp rhabdovirus)**	Northern pike, brown trout, grass carp, white bream, gudgeon, tench	Europe	Subacute to Acute 1	Spontaneous disease only in young pike; two syndromes: (a) swelling on skull (hydrocephalus) or (b) hemorrhagic mass between pelvic fins and hemorrhage on flanks; hemorrhagic necrosis of viscera	Ahne (1985) Lewis and Leong (2004)
***_Herpesvirus salmonis_ disease (HVS) (Fig. II-88, B, C)**	Atlantic salmon, brook, brown, and rainbow trout, chum salmon (E), chinook salmon (E)	Washington, United States California, United States	Subacute to Chronic 1	Natural disease only in young rainbow trout at < 10°C; exophthalmos, swollen abdomen; thick, white fecal casts; pale viscera; kidney is primary target (hematopoetic hyperplasia); Pathognomonic: syncytia in pancreas (not always present); little or no pancreatic necrosis (ddx from IPN, IHN VHS)	Eaton et al. (1989) Wolf and Smith (1981)
Lake trout herpesvirus	Lake trout	Great Lakes region, United States	Acute 4	Inflamed epithelial hyperplasia of skin	McAllister and Herman (1989)
Lake trout rhabdovirus	Brown trout	Finland	Acute 3	Typical systemic rhabdoviral signs (hemorrhage, etc.)	Koski et al. (1992)
Carpione rhabdovirus	Carpione	Lake Garda, Italy	Acute 1	High mortality in cultured fry	Bovoa et al. (1995)
Landlocked salmon virus disease	Masu salmon	Taiwan	Acute 1	None described	Hsu et al. (1989)
Focal necrotizing hepatitis (aquareovirus)	Masu, chum (I,H) kokanee (I) and chinook (I) salmon; rainbow trout (E)	Japan	None 3	No clinical disease; some focal hepatic necrosis	Winton et al. (1981)

Rhabdoviral salmonid hepatitis	Rainbow trout	Ukraine		Hyperactivity; splenomegaly; hepatitis	Osadchaya (1981)
Chinook salmon paramyxovirus	Chinook salmon (H)	Oregon	None 3	No clinical disease	Winton et al. (1985)
Atlantic salmon paramyxovirus (ASPV)	Atlantic salmon	Norway	Acute 3	Associated with gill pathology in post-smolts	Kvellestad et al. (2003)
Atlantic salmon picorna-like virus	Atlantic salmon	Washington	Chronic 3	Mild hematopoetic necrosis; focal hepatitis; only isolated once	McDowell et al. (1989)
Picorna-like virus of salmonids	Brook, brown, rainbow and cutthroat trout; kokanee salmon (E)	California	None 2	No clinical disease except in experimentally infected kokanee salmon	Yun et al. (1989)
***Oncorhynchus masu virus (OMV herpesvirus)**	Masu, coho, chum and kokanee salmon; rainbow trout	Japan	Acute to Chronic 1	Serious problem; to 100% mortality in fry (exophthalmos, petechiation); up to 60% of survivors develop epithelial tumors on mouth; Yamame tumor virus (YTV) causes similar disease	Kimura et al. (1981) Sano et al. (1983) Kimura and Yoshimizu (1989)
Nerka virus in Towada Lake Akita (NeVTA herpesvirus)	Kokanee, chum (E), pink (E), and yamame (E) salmon; rainbow trout	Japan	Acute 1	Mortality in fry; dark; anorexia; depression; secondary water mold infection	Sano (1976)
***Salmon leukemia virus (SLV retrovirus)**	Chinook salmon, sockeye salmon (E), Atlantic salmon (E)	British Columbia, Canada; California, United States?	Chronic 1	Sea-cultured fish, usually after 1 year at sea; infiltration/proliferation of immature plasma cells into viscera and retrobulbar tissue; anemia; exophthalmos; grossly resembles BKD (PROBLEM 54); often follows BKD epidemics; avoid using progeny of fish with history of SLV (may be vertically transmitted)	Eaton and Kent (1992) Kent (1992)
***Golden shiner virus (reovirus)**	Golden shiner	United States	Chronic 1	Hemorrhage, especially in dorsal muscles, ventrum, eyes, visceral fat; most mortality in older fish	Plumb et al. (1979)
Goldfish viruses 1 and 2 (GFV-1, GFV-2, iridovirus)	Goldfish	Massachusetts	None 3	Not associated with disease	Berry et al. (1983)
Rosy barb virus (birna-like virus)	Rosy barb	Australia	Acute 3	Visceral necrosis; only seen once	Langdon (1992b)
***Grass carp aquareovirus disease (hemorrhagic virus of grass carp)**	Grass carp, black carp, chebachek	China	Acute 1	Causes severe losses; exophthalmos, swollen abdomen, hemorrhage in gills, mouth, fins, viscera; focal hyperplasia of liver, intestinal damage; disease at 25–30°C; most severe in young fish. May be related to golden shiner virus.	Nie and Pan (1985) Jiang and Ahne (1989) Ke et al. (1990)
Grass carp virus CIVH 33/86	Grass carp	Hungary	None 3	No clinical disease	Ahne et al. (1987)
***"Carp pox" (*Herpesvirus cyprinid* disease, carp epithelioma, Cyprinid herpesvirus 1) (Fig. II-88, A)**	Common carp, crucian carp, barbel, bream, golden ide, rudd, smelt, carp × goldfish, aquarium fish	Europe; Asia; Russia; Great Lakes, United States; Israel	Acute to Chronic 1	Smooth to rough, milky white to grey plaques up to 2 mm thick; may cause scarring, retard growth, lead to skeletal deformities; hyperplastic epithelium (may be papillomatous) intracytoplasmic and intranuclear (Cowdry type A) inclusions; plaques up to several cm along longest dimension; lesions eventually slough but can last for months; lesions may become dark pigmented, reducing value; lesions develop in low temperatures (winter/spring) and regress with high temperature (summer) but latent infection remains; transmission probably from wounds; acute disease in young fish; experimentally virulent to carp fry; Ddx: koi herpesvirus (PROBLEM 86)	Sonstegard and Sonstegard (1978) Wolf (1988) Sano et al. (1990) Sano et al. (1993) Calle et al. (1999)

Continued.

Table. II-88. Miscellaneous systemic viral infections of fish. Clinically important diseases are indicated with an asterisk (*). For viruses associated with epithelial hyperplasia and neoplasia, see PROBLEM 76, cont'd.

Disease / pathogen	Hosts	Geographic range	Morbidity / mortality; significance	Diagnostic features	References
***Herpesviral hematopoetic necrosis (cyprinid herpesvirus 2) (Fig. II-88, D)**	Goldfish	Japan, Taiwan, United States, Australia	Acute 1	Listless with pale gills; pale liver, enlarged spleen with white nodules, abdominal fluid; hematopoetic necrosis, pancreatitis; disease at 15–25°C (59–77°F), none at > 25°C; virus probably widespread but only causes disease under certain conditions	Jung and Miyazaki (1995) Goodwin (2006)
Pilchard herpesvirus	Australasian pilchard	Australia, New Zealand	Acute 4	Associated with large epidemic in wild fish; lethargy, then die after being chased; dark gills; gill epithelial hypertrophy and hyperplasia	Hyatt et al. (1997)
Catfish aquareovirus	Channel catfish	California, United States	Chronic 3	Associated with gill lamellar hyperplasia and fusion; low mortality in naturally infected fish	Amend et al. (1984)
Black catfish virus (herpesvirus)	Black bullhead	Italy			Alborali et al. (1996)
Perch rhabdovirus infection	Eurasian perch, northern pike (E)	France	Chronic 1	Neurological signs; exophthalmos; only isolated once	Dorson et al. (1987)
Bluegill virus infection	Bluegill	West Virginia and Kentucky, United States	None 3	No clinical disease	Wolf (1988)
13p2 aquareovirus infection (bluegill hepatic necrosis reovirus)	Bluegill (E), golden shiner (E), rainbow trout (E)	Long Island Sound, New York, United States	Subacute 2	Only naturally isolated from oysters; clinical disease only in bluegill fry; focal necrotic hepatitis that can be lethal	Meyers (1983)
Gilthead seabream aquareovirus	Gilthead seabream	Spain	Subacute	Moderate mortality	Bandin et al. (1995)
Ramirez dwarf cichlid virus disease	Ramierez dwarf cichlid	Uncertain (South America?)	Chronic 4	Dyspnea, neurological signs, hemorrhage in eyes, skin; focal necrosis of viscera; splenomegaly; eosinophilic inclusions in splenocytes; to 80% mortality after 4 weeks	Leibovitz and Riis (1980)
Rio Grande cichlid rhabdovirus disease	Rio Grande cichlid, convict cichlid, zilli cichlid	Uncertain (Florida? Mexico?)	Acute 1	Lethargy	Malsberger and Lautenslager (1980)
Deep angelfish disease (herpesvirus-like)	Deep angelfish	Uncertain (Amazon basin?)	Acute 4	Loss of equilibrium, headstanding; hemorrhage on surface; only seen once	Mellergaard and Block (1988)
Chromide cichlid anemia (iridovirus-like)	Chromide cichlid	Uncertain (Malaysia?)	Acute 4	Pale; weak; cachexic; ballooned cells with virus particles in renal hematopoetic tissue and other organs	Armstrong and Ferguson (1989)
Striped bass aquareovirus-like virus	Striped bass	Potomac River, Maryland, United States	? 3	Large hemorrhages along flanks and on swim bladder; "membranous material" connecting liver to body wall; only isolated once	Baya et al. (1990b)
***Tiger puffer virus (kuchihiro-sho = white mouth disease)**	Tiger puffer, grass puffer (E), fine-patterned puffer (E), panther puffer (E), pagrus sea bream (E), Schlegel's black rockfish (E)	Japan	Chronic 1	Ulcers on mouth and snout; viral particles in brain; epidemics May–June (18–22°C) Ddx: Nodavirus infection (PROBLEM 85)	Wada et al. (1986) Miyadai et al. (2001)
LLD-associated virus (aquareovirus)	Semicirculatus angelfish	Uncertain	Chronic 3	Isolated from fish with LLD (PROBLEM 100); little evidence for viral involvement	Varner and Lewis (1991)
Turbot aquareovirus (TRV)	Turbot	Spain	Chronic 3	Associated with low-grade bacterial infections	Lupiani et al. (1989) Rivas et al. (1996)

***Turbot epithelial cell gigantism (*Herpesvirus scophthalmi* infection)**	Turbot	Scotland, Denmark	Acute or chronic 4	Hypertrophic (fused) epithelial cells in gills and skin	Richards and Buchanan (1978) Bloch and Larsen (1994)
***Japanese flounder rhabdovirus disease (*Rhabdovirus olivaceus*; hirame rhabdovirus, HIRRV)**	Japanese flounder, ayu; rainbow trout and other salmonids (E); black sea bream, red sea bream; black rockfish; redspotted grouper; spotbelly greenling, yellowfin goby; sunrise sculpin	Japan	Acute 1	Ascites, focal hemorrhage of muscles, fins, and viscera; exophthalmos; hematopoetic necrosis; highest mortality at low (<10°C) temperature (keep temperature >15°C)	Oseko et al. (1988) Kimura and Yoshimizu (1991) Oseko et al. (1998)
***Epidermal hyperplasia/ necrosis (herpesvirus-like)**	Japanese flounder	Japan	4	Larvae and juveniles affected with opaque fins due to epidermal hyperplasia; may be epidermal necrosis or ascites; disease at 18–20°C	Iida et al. (1989)
***Epidermal necrosis**	Fox jacopever	Japan	4	Larvae with necrotic epidermis having herpes-like particles	Kimura and Yoshimizu (1991)
***Epithelial necrosis (paramyxovirus-like)**	Schlegeli black sea bream	Japan	4	Larvae with rounded, necrotic, epithelial cells of skin, mouth, gill, and intestine; intracytoplasmic, enveloped virions	Miyazaki et al. (1989)
Opaleye calicivirus	Opaleye	Eastern Pacific (United States)	None 3	Transmitted to marine mammals via feeding; causes vesicular lesions in swine; very rarely skin lesions in humans	Smith et al. (1998)
Retroviruses				See PROBLEM 76.	
***EUS viruses (striped snakehead rhabdovirus [SHRV], ulcerative disease rhabdovirus [UDRV])**	Striped snakehead, swamp eel	Southeast Asia	Acute to Chronic 3	Virus isolated from viscera of fish with EUS (PROBLEM 35); little evidence for involvement in EUS	Frerichs (1995)

1—Virus proven to cause disease in spontaneously affected fish (River's postulates fulfilled).
2—Virus proven to cause disease only in experimentally affected fish (not the host from where the virus was originally isolated.)
3—Virus isolated from fish but not yet proven to cause any disease.
4—Virus particles seen in lesions of affected fish; virus not yet isolated.
H—In spontaneous cases, isolated only from clinically healthy individuals of this species.
E—Only shown to cause disease in experimentally challenged individuals of this species.
I—Fish can be experimentally infected with virus, but it does not show clinical signs of disease (histopathological lesions may be present in some cases, but virus does not cause gross morbidity/mortality).
Note that a *disease* may be clinically important even if the *virus* isolated from the lesions has not been proven to be clinically important in causing the disease.

CHAPTER 13

PROBLEMS 89 through 99

Rule-out diagnoses 2: ***Presumptive*** **diagnosis is based on the absence of other etiologies combined with a diagnostically appropriate history, clinical signs, and/or pathology.** ***Definitive*** **diagnosis is based on presumptive evidence combined with further, more extensive workup with a specific identification of the problem.**

89. Nutritional deficiency
90. Hypercarbia
91. Hydrogen sulfide poisoning
92. Chlorine/chloramine poisoning
93. Metal poisoning
94. Cyanide poisoning
95. Miscellaneous water-borne poisonings
96. Harmful algal blooms
97. Acute ulceration response/environmental shock/delayed mortality syndrome
98. Traumatic lesions
99. Genetic anomalies

PROBLEM 89
Nutritional Deficiency

Prevalence Index

WF - 2, WM - 1, CF - 4, CM - 4

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Measurement of specific low nutrient levels in feed and/or fish
2. History and clinical signs

History

Outdated or improperly stored feed; feeding a monotonous diet (i.e., single food item); not feeding often enough or enough food at one time; poor growth; chronic mortalities; depressed or otherwise abnormal behavior; cannot find food (blind)

Physical Examination

Varies with specific deficiency, but most common clinical signs include the following: skeletal abnormalities; cataracts or other ophthalmic lesions; hematopathologies (e.g., anemia)

Treatment

1. Adjust diet to requirements of that fish species (evaluate current dietary formulation)
2. Provide varied diet if appropriate

COMMENTS

General Nutritional Requirements of Fish

Fish are efficient feed converters, with many food fish species producing 1 kilogram of fish for every 1.6 kilograms of feed. Nutritional requirements of fish are similar to those of mammals, but there are some important differences.

PROTEIN

Protein provides a major source of energy for fish, and subsequently fish require a higher percentage than warm-blooded animals (e.g., 30–36% for warm water fish vs. 16% to 22% for poultry). Protein requirements vary with fish species and fish size (greater in small fish). While most fish use some plant protein (e.g., soybean meal), most fish also require a certain amount of animal protein. Carnivorous fish, such as salmonids, need more high-quality protein than omnivorous/herbivorous fish, such as tilapia.

ENERGY

The primary energy sources for fish are fats and proteins. Fish can digest simple sugars efficiently, but as the sugar molecule becomes large and more complex, digestibility decreases rapidly. For example, glucose is much more digestible than starch. This is especially true for cold water species (e.g., trout).

Adverse effects of high-energy diets include the following:

1. Inadequate protein intake: since fish eat to satisfy an energy requirement, a diet high in energy (in relation to the amount of protein present) will prevent fish from consuming enough protein for a maximal growth rate, even if fish are fed ad libitum.
2. Excess fat deposition: reduces the dressing percentage (percent of live weight available after gutting), reduces the shelf life of frozen fish, and may cause pathological changes (fatty infiltration of liver), especially in salmonids.

Animal fats and highly saturated fats are poorly assimilated by fish. However, highly unsaturated fats, which are easily digested, are susceptible to auto-oxidation, resulting in feed spoilage. Thus, antioxidants are routinely added to fish diets. Vitamin E is the antioxidant of choice because of the often illegally high levels of synthetic antioxidants (e.g., butylated hydroxyanisol [BHA], ethoxyquin) that would be required and because it also prevents cellular auto-oxidation. Fish also have requirements for essential fatty acids (e.g., linolenic acid).

VITAMINS

Fifteen vitamins are essential for most fish, including vitamins A, D, E, K, thiamin, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid, B_{12}, biotin, choline, ascorbic acid, and inositol. However, not all species require all 15 vitamins in the diet. Most commercial diets are overly fortified with vitamins because of the high levels of oxidizable fats in the diets that can result in their inactivation. Early mortality syndrome in wild salmonines is caused by thiamin deficiency (McDonald et al. 1998; Brown and Honeyfield 2006).

MINERALS

Fish probably require the same minerals as warm-blooded animals for various physiological functions. In addition, fish use inorganic ions to maintain osmotic balance between themselves and the external environment. It is important to note that minerals in the water can make significant contributions to a fish's dietary requirements. The availability and biological activity of aqueous minerals are highly dependent on the composition and properties of the chemical soup in which the fish swim. The presence of certain minerals influences the activity of others, all of which are influenced by temperature, pH, etc. Fish can meet much of their calcium requirements by absorbing it through the gills, provided that adequate calcium levels are present in the water. Conversely, most natural waters are low in dissolved phosphorus and thus dietary phosphorus is essential. There is evidence that minimum iodine levels must be present in water to avoid hypothyroidism (goiter) in some marine fish (Crow et al. 1998).

Growth can also be influenced by changing dietary levels of magnesium (Mg), potassium (K), copper (Cu), iodine (I), selenium (Se), zinc (Zn), and iron (Fe). Goiter resulting from iodine deficiency has occurred in salmonids fed on all-meat diets. Fish feeds that are low in animal products may be deficient in trace minerals and thus may require supplementation.

Types of Feeds

While meal-type feeds can be used to feed some types of fish, feeds for most fish species must be in the form of large particles for them to be readily accepted by the fish. Thus feeds are processed to form pellets, extruded feeds, or flakes (Table II-89, Fig. II-89, B). Ninety days is the maximum storage time recommended for complete fish feed stored at ambient temperature; ascorbate is the most sensitive vitamin, although more heat-stable forms are now available.

Feeding Aquarium Fish

This group varies widely in their natural food habits and thus their nutritional requirements (i.e., herbivores, car-

Table II-89. Characteristics of various types of fish feeds.

Type of feed	Stability in water	Cost	Nutritional adequacy*
Flake	Excellent	Moderate	Moderate
Pellet	Poor to excellent*	Low	High
Freeze-dried	Good to excellent	Moderate to high	Moderate
Frozen	Poor	High	High
Live	Excellent	High	Highest

*Varies greatly with manufacturing process and commercial brand.

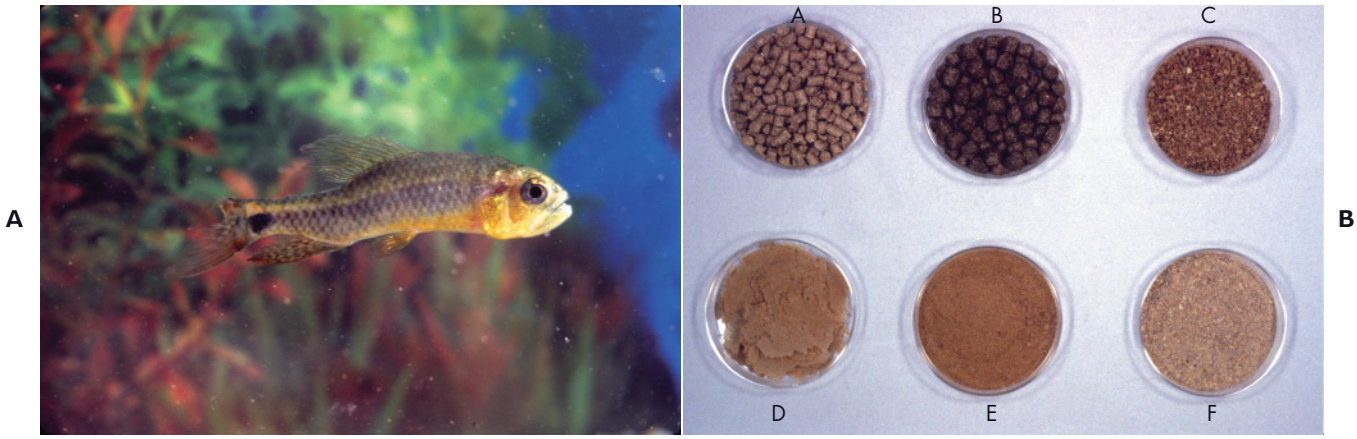


Fig. II-89. A. Chronic wasting in a killifish, as evidenced by the strongly concave abdomen. B. Major types of dry fish feeds: A = sinking pellet; B = floating (extruded) pellet; C = crumble; D = flake; E and F = mash. (*A* photograph courtesy of T. Wenzel.)

nivores, insectivores, omnivores). Diet formulations for aquarium species have been based mainly on the nutritional requirements of warm water food fish. Aquarium feeds also contain carotenoids and similar compounds to enhance pigmentation.

Fortunately, most freshwater aquarium fish can do well on a high-quality commercial diet. Flaked feeds are most commonly used, since diets with a hard texture or which sink rapidly may be poorly consumed (Lovell 1980), especially by small fish. However, water soluble vitamins are rapidly lost (within 30 seconds) from flake feeds upon contact with water (Pannevis and Earle 1994) and thus unless consumed immediately, the diet can be severely deficient. Dry diets should be used within 3 months of manufacture if they are stored at ambient temperature because vitamins may decay considerably by this time. Unfortunately, many companies that manufacture aquarium feeds do not place an expiration date or date of manufacture on their products. Flake or pellet feed should always be supplemented with other food items, such as various live or frozen products.

Live or frozen foods that are good supplements include larval ("baby") and adult brine shrimp (*Artemia salina* and *A. franciscana*), microworms (a nematode), water fleas (*Daphnia* spp.), krill, and earthworms. Tubificid worms are also a good nutritional source but are collected from organically polluted water and thus may harbor toxins. Tubificids and other aquatic oligochaete worms are also intermediate hosts for a number of fish parasites (e.g., see PROBLEM 63). Live fish are an excellent source of nutrients for carnivorous fish but may also transmit many diseases; parasites, mycobacteriosis, and other bacterial diseases are usually the most serious problems. Frozen whole fish are safer to feed but may not be as well accepted by the fish. Note that many bacterial pathogens (e.g., *Mycobacterium*) are not killed by freezing; some parasites and many viruses can survive for at least weeks in frozen fish.

The natural diets of marine reef fish are highly specialized and may not be satisfied by foods that are available in captivity. Owners should be aware of the natural food habits of potential pets, since this is a useful predictor of success in captivity. See Bower (1983) and Goldstein (1997) for marine species that do well in captivity. It is essential to provide a highly varied diet with emphasis on live or frozen preparations. Pannevis (1993) and McCartney (1996) provide details on nutritional needs of aquarium fish.

Feeding Larval Fish

In addition to the other important properties of practical diets already mentioned, diets for larval fish must also have the proper density to remain suspended by water currents to facilitate consumption by the fish. While fish with large yolk supplies, such as trout and channel catfish, can assimilate a wide range of nutrient sources, including artificial diets, immediately after absorption of the yolk sac, many other fish must begin feeding before their digestive system is well developed.

Many marine species begin feeding when they are a small size. There is at present no artificial feed that will completely replace live food for these individuals; this presents a number of problems:

1. There are trouble and expense involved in obtaining live food.
2. Some species require completely different types of food as they become older, requiring that a number of different live foods be available.
3. If the live food required cannot be cultured or for some other reason must be collected in the wild, there is the added danger of introducing pathogens along with the food, as well as introducing potential predators, such as aquatic insects (see PROBLEM 87); the live food most commonly used in raising larval fish is larvae of the brine shrimp (*Artemia salina*), but this is too large for some species that must be fed smaller food items such as rotifers.

There is evidence that inadequate hormone levels may lead to some developmental anomalies. For example, striped bass larvae often have a high incidence of failure to inflate the swim bladder. Treating prespawning female striped bass with thyroid hormone increased the incidence of normal swim bladder inflation and larval survival (Brown et al. 1988).

Young fish should be fed often. Some species need to have food constantly present to survive the early stages of life. The amount of food provided must also be constantly increased as the fish grow, requiring more total feed. But avoid overfeeding, which causes environmental problems.

Effect of Culture System on Nutritional Requirements

Fish in ponds are less at risk for nutritional problems if there is a sufficient amount of natural food in the pond. Thus, fish in farm ponds, which are relatively low density, rarely exhibit nutritional problems. However, commercial food fish ponds typically raise fish at high density, placing them at risk for nutritional disease. Many goldfish and koi ponds, especially those having filtration or aeration to allow greater fish densities, have little natural food available. Fish in raceways or cages have little access to natural food items and thus are totally reliant on the prepared diet for their nutritional needs (Hepher 1988). Typical hobbyist aquaria also have few natural food items, although some hobbyists often encourage the growth of algae and invertebrate feed items, such as in reef aquaria.

Feeding Food Fish

Feeding fish, as in other animal agriculture industries, constitutes a major expense to the farmer. Up to 70% of the fish farmer's total costs are for feed. Fish culturists also face two problems unique among farmers: first,

uneaten food quickly deteriorates in the water and makes relatively little contribution to fish production; second, uneaten food also contaminates the environment and may be detrimental to the fish's health (see PROBLEM 4). Feeding techniques are affected by a number of factors, including the following:

- **Physical factors:** Rate of water exchange, type of rearing facility (e.g., pond, raceway, cage), and size of fish will all influence feeding practices.
- **Temperature:** All fish species have a temperature range at which optimum feed conversion is obtained. This occurs around 30°C (86°F) for warm water fish; below about 12°C (54°F) feeding is erratic. Thus warm water fish, such as channel catfish, are fed daily only when the temperature is above 12°C. Tropical aquarium fish typically should be fed 1–2.5% of their body weight per day at 26°C (79°F), while goldfish kept at 20°C (68°F) only require 0.3% of body weight per day (Lewbart 1998).
- **Water quality:** Because of the intimate interrelationship between the aquatic environment and fish's metabolism, feeding practices must be used within the constraints that this relationship imposes. In warm water culture, dissolved oxygen (DO) levels in ponds that have heavy plant growth are related to photosynthetic activity, with the lowest DO levels in the early morning just before photosynthesis resumes. Thus feeding should be done after DO levels have risen. Feeding should not be done in late evening because DO begins to drop again and the nutrients in the feed simulate oxygen consumption (see PROBLEM 1).

DIETS FOR VARIOUS FOOD FISH SPECIES

Nutritional requirements for well-established food fish species, such as channel catfish, salmonids, carp, and Japanese eel (among others) are well defined, and thus, nutritional problems in these species usually result from improper feed handling and/or storage or occasionally from improper formulation at the feed mill.

For many other food fish species, nutritional requirements are less defined, which may be responsible for many problems encountered in propagating these species. Lovell (1989), National Research Council (1981, 1983, 1993), Steffens (1989), Tacon (1992), Wilson (1992), Webster and Lim (2002), and Halver and Hardy (2002), as well as specific research articles on the species of interest, should be consulted for specific nutritional requirements for various fish species.

Food-Borne Toxins

Many food-borne toxins have been experimentally induced in fish. These are summarized by Tacon (1992). A few have caused disease in clinical situations. Trout are extremely sensitive to aflatoxins, associated with moldy feeds, and develop hepatomas when levels as low as one part per billion are fed for several months (Lovell 1989). Unsaturated fatty acids, such as those found in fish oils, are readily oxidized, becoming rancid. Salmonids fed such rancid fats can develop lipoid liver disease, characterized by fatty infiltration of the liver and severe anemia (Tacon 1992).

Antinutrients, such as thiaminase, are present in many aquatic animal tissues and can cause vitamin deficiencies if fed raw to fish. Many other food products, especially plant products, have other types of antinutrients (Tacon 1992).

Some manufacturers reportedly add testosterone to their commercial aquarium feeds, since this enhances the color of many fish by stimulating breeding coloration. However, testosterone can have a major influence on sexual development. Exposure to high testosterone levels may cause sex reversal (from female to male) or sterility of some fish.

Taints (Off-Flavor)

Muddy or earthy tastes in fillets are a serious problem in pond-cultured fish, especially channel catfish in the United States (Tacon 1992); these taints are caused by soil bacteria (actinomycetes) or some cyanobacteria (see PROBLEM 96). Industrial wastes associated with taints include domestic sewage, phenols, or petroleum products (see PROBLEM 95).

Diagnosis of Nutritional Deficiency

Presumptive diagnosis of nutritional deficiency is based on compatible clinical signs, combined with evidence of an inadequate diet. Obviously, the diagnosis is much easier to make for species where the nutritional requirements are known. Unfortunately, deficiencies are most common in species with undetermined requirements. Definitive diagnosis requires identification of a specific nutritional deficiency in the diet.

Clinical signs of inadequate nutrition are most likely to be seen in young, rapidly growing fish that typically have the highest requirements for many nutrients. The most obvious sign of poor nutrition is starvation (Fig. II-89, A). A number of pathological changes have been induced in fish by specific nutrient deficiencies; the most common clinical signs are vertebral anomalies (scoliosis or lordosis), cataract, exophthalmos, fin erosion, fatty liver, and skin hemorrhage (Ghittino 1989; Roberts and Bullock 1989; Tacon 1992; Cahu et al. 2003). Pigment abnormalities have been linked to specific vitamin deficiencies in flatfish (Kanazawa 1993). Other common lesions are anemia and gill hyperplasia. All of these lesions are nonspecific.

Many of these lesions also occur with genetic defects (see PROBLEM 99) and have also been associated with a generally poor environment or husbandry.

Besides direct pathological changes, there is evidence that inadequate nutrition can increase susceptibility to disease, especially when fish are stressed (Blazer et al. 1989; Landolt 1989). Furthermore, stress may increase vitamin requirements (Tacon 1992). Vitamin E and C appear to play important roles.

Treatment of Nutritional Deficiency
Unless the case involves possible litigation (e.g., due to negligent feed preparation), a definitive diagnosis is almost never sought. Instead, the client is advised to obtain fresh feed or change the diet. Where nutritional requirements are uncertain (e.g., most aquarium fish), it is important to provide a varied diet.

PROBLEM 90
Hypercarbia

Prevalence Index
CF - 3
Method of Diagnosis
Rule-out of other problems combined with the following:
1. Measurement of aqueous CO_2 concentration >12 mg/l
2. History and clinical signs

History
Overcrowded system; use of liquid oxygen; poorly buffered ground water
Physical Examination
Dyspnea; chronic inflammation in kidneys and epaxial muscles
Treatment
1. Increase aeration
2. Decrease density
3. Run water through a packed column degasser
4. Add slaked lime (ponds only)

COMMENTS
Causes
Carbon dioxide (CO_2) is very soluble in water and levels can far exceed the atmospheric concentration. Hypercarbia can occur when using ground water, which may be low in pH and high in CO_2 (up to 100 mg/l may occur). Elevated CO_2 may also develop when using liquid oxygen, which allows a higher stocking density in raceways. The CO_2 concentration in ponds varies diurnally in parallel with pH, usually ranging from 0 mg/l in the late afternoon to 5–10 mg/l at daybreak (see Fig. II-1, D). Although CO_2 may exceed 10 mg/l in highly eutrophic ponds, diurnal hypercarbia peaks do not appear to be a problem. However, hypercarbia may exacerbate environmental hypoxia (see PROBLEM 1), and carbon dioxide is often much higher after a phytoplankton die-off.
Pathogenesis
Increased aqueous CO_2 inhibits diffusion of CO_2 out of the blood. High blood CO_2 reduces blood pH, which reduces hemoglobin's affinity for oxygen (Bohr effect). CO_2 also directly decreases the amount of oxygen that can be loaded by hemoglobin (Root effect). The net effect is reducing the amount of oxygen that can be transported to tissues. In salmonids, chronically elevated CO_2 has been associated with nephrocalcinosis and systemic granuloma, a multifocal deposition of chalky, white mineral in the stomach, kidney, and epaxial muscles (see PROBLEM 102).
Diagnosis
In a flow-through system, carbon dioxide is lowest at the inflow and highest at the outflow. In a pond, CO_2 is highest near the bottom of the pond. If samples are submitted to a reference laboratory for analysis, sample bottles must be filled completely to exclude air, kept below the temperature at which the water was collected (to prevent escape of CO_2), and analyzed within 2 hours of collection.
Treatment
Some fish can adapt to elevated CO_2 levels, but this adaptation must be gradual. There is also the risk of the fish developing nephrocalcinosis, at least in salmonids. Treating water with a buffer to increase the pH can also reduce dissolved CO_2. Above pH 8.34, free CO_2 is not present (see Fig. II-7).

Up to 10–12 mg/l of free CO_2 is usually tolerated if O_2 is high. Some fish can survive exposure to up to 60 mg/l (Hart 1944), which approaches narcotic levels (see "**Pharmacopoeia**"). If the CO_2 concentration in a pond exceeds 10–15 mg/l (e.g., after an algae die-off) it may be advisable to remove the excess CO_2 with slaked lime. Vigorous aeration also removes CO_2 from ponds (Ver and Chiu 1986). Aeration will reduce CO_2 levels in flow-through systems and is optimized by providing maximum surface area, such as through a packed column degasser (Aquatic Ecosystems) that is used to eliminate gas supersaturation.

PROBLEM 91
Hydrogen Sulfide Poisoning

Prevalence Index
WF - 4, WM - 3, CF - 4, CM - 3
Method of Diagnosis
Rule-out of other problems combined with the following:
1. Chemical measurement of hydrogen sulfide in water
2. History and clinical signs

History/Physical Examination
Acute to chronic stress response
Treatment
1. Aerate water
2. Raise pH
3. Lower temperature
4. Add potassium permanganate (freshwater only)

COMMENTS
Epidemiology/Pathogenesis
Hydrogen sulfide (H_2S) forms from the reduction of sulfate ion under anaerobic conditions. It is more of a problem in brackish water or marine systems, where there is a large amount of sulfate that can be reduced to sulfide. It can form on pond bottoms that become anaer-

obic because of high concentrations of organic matter combined with high metabolism (i.e., especially summer). Disturbing the bottom (e.g., seining) can release the toxic gas from the mud. It is also a problem in marine aquaria if anaerobic areas develop under rocks or if filter beds are not totally aerated. Some coastal aquifers also have high concentrations of H_2S. Paper mills and tanneries are also sources of H_2S. Hydrogen sulfide's main toxic action seems to be interference with respiration, causing hypoxia (Schwedler et al. 1985).

Diagnosis

Concentrations between 0.5 and 10 mg/l can cause acute mortality (Langdon 1988). Greater than 0.006 mg/l is toxic to some species; thus, any levels detectable with commercial test kits should be considered detrimental (Boyd 1990). Recommended maximum standards are <0.002 mg/l for fish and <0.012 mg/l for eggs (Piper et al. 1982). Presence of H_2S can often be detected from the characteristic smell of rotten eggs. Levels detectable by smell are not necessarily toxic. The threshold for odor concentration of H_2S in clean water is 0.025–0.25 µg/l (APHA 1992). It can also be tasted at relatively low concentrations. Acute poisoning is reportedly associated with the presence of purple-violet gills (Langdon 1988), but water testing is the recommended method of diagnosis.

Treatment

Vigorously aerating water or passing it over a packed column degasser (e.g., Aquatic Ecosystems) before use in flow-through systems will remove H_2S. In ponds, hydrogen sulfide formation can be prevented by maintaining aerobic conditions. It can be removed by oxidation with potassium permanganate, but permanganate must be used with caution in seawater (see "**Pharmacopoeia**"). Raising the pH (e.g., liming) and lowering the temperature also reduce H_2S toxicity. In brackish or marine aquaria, filter beds should be closely monitored to prevent the development of anaerobic zones. If a filter has stopped working, even for a few hours, extreme care must be taken when turning it on again, because, if the water has become anaerobic, H_2S may have formed. Thus, animals may need to be moved temporarily to avoid acute mortality when the filters are turned back on again.

PROBLEM 92
Chlorine / Chloramine Poisoning

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Chemical measurement of chlorine or chloramine in water
2. History and clinical signs

History

Acute to chronic stress response; fish added to tank within days of setting up tank; tap water used; recent water change in established tank; unrinsed chlorinated utensils; dyspnea

Physical Examination

See "**History**"

Treatment

1. Place immediately in chlorine-free, chloramine-free, highly oxygenated water
2. Aerate chlorinated make-up water for 24 hours (chlorine only)
3. Treat make-up water with chlorine or chloramine neutralizer

COMMENTS

Chlorine Poisoning

Chlorine is added to municipal (tap) water supplies to kill microorganisms (Boyd 1990). Like many toxins in water, chlorine is much more toxic to fish than to humans (Brooks and Bartos 1984). The amount of chlorine added varies considerably among different municipalities and can also vary considerably from time to time. Combined chlorine residual is the total amount of chlorine present in various forms (e.g., chloramine, hypochlorous acid). Municipal water systems generally require a minimum of 0.20 mg/l of combined chlorine residual at the tap; in actuality, there is usually 0.50–1.0 mg/l present. Water mains are routinely treated with high chlorine concentrations after being repaired, but this bolus of chlorine is rarely a problem because the chlorine is normally held out of the system and not allowed to reach a user's pipes. Inadequately rinsed, chlorine-disinfected utensils may contaminate water.

Chlorine toxicity can present as acute to subacute mortality associated with fish being added to a newly set-up tank or when fresh tap water is used for a water change. However, doing a partial water change with chlorinated tap water does not always cause toxicity because the chlorine may be quickly inactivated if a large amount of organic matter is present (e.g., in a long-established aquarium). Most aquarists are well aware of chlorine toxicity, making it uncommon. Chlorine is also used to treat industrial effluents (e.g., sewage, textiles, paper waste) before their discharge into waterways. Fish culture facilities should not be sited near chlorinated effluents.

Fish with acute chlorine poisoning will usually be dyspneic. Free chlorine reacts readily with organic matter, including gill tissue, causing acute necrosis and asphyxiation. Chronic exposure may also result in both extensive mucous secretion and hypertrophy of the gill epithelium (Leef et al. 2007).

Chloramine Poisoning

Many municipal water sources have high levels of natural organic matter, such as humic acids and fulvic acids.

Chlorine reacts with these organics, producing haloacetic acids (e.g., trichloroacetic acid) and trihalomethanes (e.g., chloroform). Trihalomethanes and possibly haloacetic acids are carcinogenic (USEPA 1989) and thus, potentially dangerous to humans (Christman et al. 1991). To eliminate trihalomethanes from drinking water, many municipalities add ammonia to the chlorine during disinfection. Reaction of ammonia with chlorine produces a more chemically stable disinfectant: chloramine. Chloramines are created by adding an excess of chlorine, resulting in monochloramine (other chloramines impart a taste to the water, and so the chemicals' ratio is designed to avoid their production). Chloramine, like chlorine, is highly toxic to fish (Tompkins and Tsai 1976). Acute exposure induces both respiratory and acid-base disturbances that are suggested to be directly related to increased mucus production from gill irritation (Leef. et al. 2007). Note that some chloramine preparations (e.g., chloramine-T) are used to treat skin and gill pathogens (see "**Pharmacopoeia**" for more details on toxicity of these compounds).

Diagnosis

Commercial test kits for chlorine and chloramine are available (Chemetrics, Inc., Hach Company), but presumptive diagnosis can often be made from the history. It is important to determine if fish may have been exposed to chlorine or chloramine-treated water. The disinfectant used in a particular municipality can be determined by contacting the public works department (Kowalski 1984). The threshold for smelling chlorine is 0.20–0.40 mg/l (Anonymous 1989).

Chlorine levels of 0.10 mg/l are common in tap water and can be acutely fatal in aquaria with low organic matter (e.g., newly established aquaria). Any detectable amount of chlorine is undesirable, with 0.003 mg/l considered to be a maximum tolerable limit for continuous exposure (USEPA 1973; 1979–1980). Sublethal exposure can cause hemolytic anemia and Heinz body formation in erythrocytes (Buckley 1976). Chloramines should be undetectable by commercial kits before water is used for fish.

Chlorine or chloramine poisoning must be differentiated from other poisons (see PROBLEMS 91, 93, and 95) and from environmental shock (see PROBLEM 97).

Prophylaxis

Chlorine is easily removed from water by vigorous aeration for 24 hours or by adding commercial dechlorinating agents. Chloramines are not easily removed by aeration. The water must be filtered through activated carbon or treated with a chemical neutralizer, such as sodium thiosulfate, to break the chlorine-ammonia bond. Because the chemical neutralization releases ammonia, this must also be removed (see PROBLEM 4), although much of the residual ammonia can be removed naturally in an aquarium having active biological filtration; its effect is also much less important at lower pH. Heating the water to near boiling will also drive off chloramines. Most commercial chloramine removers have additives for reducing ammonia toxicity. Some commercial chloramine neutralizers do not remove ammonia but simply cause the ammonia test to read negative. Some commercial products, such as Ammo-Lock2 (Aquarium Pharmaceuticals) and AmQuel (Kordon), react with the ammonia to form nontoxic, inert, moderately stable substances. With these products, the ammonia is bound but not actually removed.

Treatment

Fish exposed to acute chlorine poisoning appear to have improved survival if the water is supersaturated with oxygen for several days. Lowering the temperature may also help (G. Lewbart, personal communication).

PROBLEM 93
Metal Poisoning

Prevalence Index

WF - 3, WM - 3, CF - 3, CM - 3

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Chemical measurement of metal in water
2. History and clinical signs

History

Metal plumbing used to carry water source; metal in contact with water (e.g., rocks, ornaments); metal in the water supply; copper-containing medications

Physical Examination

Varies with toxicosis

Treatment

1. Remove fish to another system
2. Water change
3. Add EDTA
4. Add ion exchange filter

COMMENTS

Epidemiology/Pathogenesis

Fish are much more sensitive than humans to aqueous metals (Table II-93), which is one reason why water that is safe for human consumption may be highly toxic to fish. Metals are most toxic in low-alkalinity water, which allows a high concentration of metal to remain dissolved (and thus toxic).

Lead, copper, or galvanized (zinc-coated) iron plumbing may leach metals. Since more and more metal will dissolve into the water over time, the longer that water sits in a pipe, the higher the metal concentration. Thus, water that first comes out of a pipe has the highest metal concentration. Ground water, especially soft, acid water, may have toxic concentrations of metals. Rainwater

Table. II-93. Metal concentrations associated with toxicity in freshwater fish (mg/L unless stated otherwise). [1]

Metal/metal salt	Levels in water associated with fish kills	Acceptable continuous exposure levels in water for fish culture (2)	Sources	Diagnostic clinical features (3)	References
Aluminum	>0.1–5 (low pH); also toxic at pH > 8 (alumate form)		Tank fittings in low pH or saltwater; acid rain		Brown et al. (1983)
Antimony (potassium tartrate salt)	>12–20				
Arsenic	>1–2	<0.7			
Arsenite	>14				
Cadmium	>1.0–3.7 >5.2	<0.0005 (soft water) <0.003 (hard water)	Electroplating; superphosphate; galvanized pipe (4)		
Cadmium salts	>0.1				
Chromates	>3.3–133		Corrosion inhibitor in cooling towers; metal plating/anodizing; leather tanning; hexavalent chromium most commonly used		
Cobalt	>30				
Copper	>0.03–0.7 (soft water) >0.6–6.4 (hard water)	<0.006	Mining waste; low alkalinity ground water; plumbing pipes; bronze, brass fittings; antifouling paints for sea cages (may accumulate in sediments); see also "Pharmacopoeia."		Jeffree and Williams (1975)
Copper nitrate	>0.02				
Copper sulfate	>0.14	<0.002 maximum <0.00005 average			
Iron	>0.5	<0.1	Well or spring water; anoxic reservoir water; rising pH, O_2; acid drainage; industrial effluents; corroding iron pipes	Precipitating iron (ferric hydroxide) on gills impairs respiration; stains laundry, porcelain, and concrete; some persons can detect a bittersweet, astringent taste at >1 mg/L.	Wedemeyer et al. (1976) Langdon (1987a)
Lead	>1.0–31.5	<0.02	Lead or galvanized plumbing pipes (4); red paint; lead solder joints; industrial, mine, or smelter discharge; weights used to hold aquarium plants	Sigmoid spinal curvature; caudal cutaneous melanosis; erythrocytic stippling (chronic)	Hine (1982) Bengtsson (1975) Untergasser (1991)
Lead salts	>0.5	—			
Manganese	>75	<0.01	Well or spring water; anoxic reservoir water; batteries; steel or aluminum alloys	Manganese oxide precipitates on gills, impairs respiration; stains laundry and porcelain at >1 mg Mn/L; permanganates are most toxic species	
Manganese chloride	>0.5	—			
Mercury	>0.17	<0.0002	Mining waste		
Mercuric chloride	>0.0008				
Methyl mercury	>0.07				
Nickel	>4.5–9.8	<0.01	Metal plating baths; corrosion product of stainless steel and nickel alloys		
Nickel salts	>0.1	—			
Selenium	>8–72	<0.05	Coal power stations (coal ash, fly ash); drainage from seleniferous soils in semi-arid areas		Gillespie and Bauman (1986) APHA (1992, 2005)
Silver	>0.006–0.07	<0.17 µg/L	Surface-finishing; photographic film manufacturers and processors		
Silver sulfide/thiosulfate complex	>280–360	—			
Tin	>55	—		Poorly soluble in natural waters (<100 µg/L)	APHA (1992, 2005)

Continued.

Table. II-93 Metal concentrations associated with toxicity in freshwater fish (mg/L unless stated otherwise). cont'd.

Metal/metal salt	Levels in water associated with fish kills	Acceptable continuous exposure levels in water for fish culture (2)	Sources	Diagnostic clinical features (3)	References
Tri-n-butyl tin (TBT)	>0.0015–0.02 mg/l	<0.02 µg/l	Antifouling paints for boats, nets, etc.		Short and Thrower (1987)
Uranium	>3–135	—			
Zinc	>0.4–1.76	<0.005	Galvanized tanks; de-zincification of brass; white paint; mining waste; low alkalinity ground water; antifouling paints	Over 5 mg/L causes bitter, astringent taste and opalescence in alkaline water; mean concentration in U.S. drinking waters = 1.33 mg/L	APHA (1992, 2005)

(1) Modified from Langdon (1988), with data provided in the listed references, as well as from USEPA (1973, 1979–1980), Wedemeyer et al. (1976), Bengtsson (1975), Chen et al. (1985), Sorensen (1991), APHA (1992, 2005).
(2) "Safe" levels are generally concentrations that are 10–100 times lower than the lowest concentrations reported to kill fish. Thus, these are usually conservative estimates.
(3) Most signs of metal poisoning are nonspecific.
(4) Lead and cadmium can enter water with deteriorating galvanized pipe because the zinc used for galvanizing is contaminated with these metals.

runoff may also be a source of metal poisoning in poorly buffered soils that may leach aluminum or other metals from soils or mine waste.

Metals may be introduced into aquaria from metal aquarium hoods or from objects placed into the tank; this may include not only metal objects, but also ceramic ware that has lead glaze and certain rocks. Only items known to be safe for aquarium use should ever be placed into a tank. Overdosing with copper that is used as an algaecide or to treat ectoparasites may lead to poisoning. Over-the-counter aquarium remedies for freshwater fish that include copper may be toxic, even when the recommended dosage is used, because copper toxicity varies greatly depending upon water conditions (see "**Pharmacopoeia**").

Water from the hypolimnion (see PROBLEM 3) of lakes or reservoirs used in fish hatcheries may be high in copper, zinc, iron, and manganese because of mobilization of the metals from anaerobic conditions (Grizzle 1981). Oxidized manganese may be toxic (see "**Potassium Permanganate**" in "**Pharmacopoeia**").

Clinical Signs

Clinical signs of metal poisoning vary with the element and somewhat with the fish species. As with most toxins, signs are mostly nonspecific. The most common cause of metal poisoning is copper. Like most heavy metals, copper toxicosis primarily affects the gills, resulting in osmoregulatory dysfunction. Kidney and liver may also be affected (Cardeilhac and Whitaker 1988). Copper is also immunosuppressive and thus may potentiate infectious disease epidemics (Knittel 1981). See the "**Pharmacopoeia**" for more information on copper. Sorensen (1991) discusses metal poisoning in detail.

Diagnosis

Definitive diagnosis of metal poisoning requires the measurement of toxic metal levels in water. However, determining whether a metal concentration is toxic is often more complicated than simply measuring the total amount of metal in the water, because the toxicity of a metal is primarily due to its dissolved ionic form rather than the total concentration. Some metals form oxides, hydroxides, and carbonates in water. Clay and organic material adsorb and/or chelate metals, inactivating (i.e., detoxifying) them. Calcium and magnesium also reduce heavy metal toxicity by competing with heavy metal binding sites on the gill (Pagenkopf 1983). Thus, it is hard to assess the probable effect of a metal when the above complications are present (e.g., water from a high hardness, high alkalinity, pond with considerable suspended clay and organic matter).

If metal toxicity is suspected and if it is economically justifiable to confirm the cause, it is best to send samples to a specialized laboratory. However, the clinician should be aware of the limitations of analysis. Atomic absorption spectroscopy is most commonly used for highly accurate metal analysis. This method determines the total amount of metal in a sample. However, more gentle extraction methods are also used (Riggs et al. 1989) because of the aforementioned considerations.

It can also be advisable to submit affected fish for determination of metal concentration in target tissues (usually gill, liver, and kidney). Extreme care must be taken to avoid contamination of tissue samples during preparation, so it is usually advisable to submit live fish or freshly iced, live fish and have the analytical laboratory prepare specific tissues.

Commercial test kits are available from aquarium suppliers and other sources (Aquarium Systems, Inc., Chemetrics, Inc., Hach Company, LaMotte Company) for measuring total copper, iron, and other metals. Such kits are relatively reliable for determining metal levels in waters low in organics and suspended sediment (e.g., typical aquarium water, tap water, or ground water).

IRON TOXICITY

Iron toxicity is not due to direct toxicity of the metal, but rather to the precipitation of iron oxides on the gills when anaerobic water (e.g., from a well) that has soluble, reduced iron is exposed to air (Wedemeyer et al. 1976; Langdon 1988). Diagnosis can be presumptively based on typical clinical signs; however, measuring iron levels in the water is also advisable. Waters with high iron content often stain concrete and other structures brown. Manganese toxicity acts similarly.

Treatment

Avoiding exposure to contaminated water is the best approach. When necessary, water can be treated to remove toxic metals. Ion exchange filters (e.g. Cole-Parmer) adsorb copper, zinc, lead, and other heavy metals. Pumps delivering a measured amount of EDTA will chelate heavy metals (J. Hinshaw, personal communication). Ion exchange filters and metal chelators are less effective in high-hardness water. They also remove essential heavy metals (Ca^{++}, Mg^{++}) which may need to be re-added to the water for some fish. They are also expensive, being feasible only for hatcheries, research facilities, or recirculating systems.

Iron toxicity can be avoided by allowing the iron to settle out in a pond (the water in the pond should have a 1- to 2-day transit time). A quicker method is to vigorously aerate the water in a tower and then run it through a sand filter to remove the iron precipitate. It can then be used immediately (Boyd 1990).

PROBLEM 94
Cyanide Poisoning

Prevalence Index

WM - 3

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Measurement of cyanide in tissues
2. History and clinical signs

History

Fish determined to be collected using cyanide

Physical Examination

Peracute exposure:

- Laterally recumbent with weak to strong opercular movement and some fin movement
- Dark red liver, sometimes with red clots

Treatment

Supportive therapy

COMMENTS

Epidemiology/Pathogenesis

Wild-caught marine aquarium fish often display very high mortalities during shipment and after being held in retailers' shops. In many cases, fish can appear normal but then die suddenly without any apparent clinical signs. This delayed mortality syndrome (DMS; also see PROBLEM 97) can often cause losses of 80% or more in fish shipped from reefs to aquarium retailers (Rubec et al. 2001).

Cyanide intoxication has long been suspected to play a role in this serious form of delayed mortality syndrome. Since the discovery in the 1960s that cyanide could be used to temporarily stun fish, this poison has been widely used in waters of the tropical Pacific for the collection of reef fish. In the aquarium fish trade, it has been used extensively in the Philippines (where many marine aquarium fish are collected) and elsewhere. Prepared from sodium cyanide tablets dissolved in water, the solution is squirted into crevices in the reef to temporarily stun the fish, allowing their rapid capture (Rubec et al. 2001). This method of collection is much faster than collecting unanesthetized fish with nets, which is why it has been used on coral reefs, where fish can easily hide.

Cyanide has also been used in the substantial live trade in food fish in Asia and the Western Pacific that are sold in high-end restaurants for diners who can then choose a live fish for their dinner. It was estimated that 20,000–25,000 tons of live reef fish were collected for this trade in 1995. This does not include domestic consumption, the fish that do not recover from the cyanide, or fish that do not reach the market alive. The major player in the live food fish trade is Hong Kong (Morton 1996).

While cyanide has been suspected to be a serious threat to coral reefs and to be a contributor to the major decline in large reef fish in the Indo-Pacific region, the true impact of this toxin on coral reef health and fishery abundance is uncertain, especially in relation to other destructive fish collection methods (e.g., blasting of reefs with explosives) (Mous et al. 2000). Its use for collecting aquarium fish appears to have declined in some areas (e.g., Philippines), but it still appears to be used widely in other areas (e.g., Indonesia). Hard data on the actual prevalence of its use in collecting fish are difficult to determine. Nevertheless, since some estimate that 50% of cyanide-collected fish may be overdosed and die immediately (Rubec 1986), this is a very wasteful collection method.

Cyanide is a respiratory poison that is a powerful inhibitor of cytochrome oxidase, a hemoprotein. Cyanide preferentially binds to iron porphyrins in cytochrome oxidase, stopping electron flow in the respiratory chain

in mitochondria, and thus preventing the normal use of oxygen by the tissues. It can damage the liver, spleen, heart and brain of fish. At sublethal doses, it causes unconsciousness. If the fish does not die, it will rapidly recover consciousness and apparently normal behavior, typically within 60 minutes (Hanawa et al. 1998). The lethal dose is very close to the "anesthetic" dose: For humbug damselfish, 50 mg/l for 60 seconds causes unconsciousness with no mortality, but 50 mg/l for 120 seconds causes 100% mortality (Hanawa et al. 1998).

Clinical Signs/Pathology

Peracute cyanide intoxication induces rapid immobilization, sometimes mistakenly referred to as "anesthesia". During recovery from peracute exposure, fish may be laterally recumbent with weak to strong opercular movement and some fin movement. The liver of exposed fish may be dark red and/or have red clots (Hanawa et al. 1998). However, none of these signs are associated with DMS. An unsubstantiated sign associated with cyanide-associated DMS is unusual feeding behavior: either anorexia or hyperphagia, causing starvation due to inability to assimilate food (Herwig 1977).

Note that cyanide is not a true anesthetic (see "**Anesthetics**" in "**Pharmacopoeia**"), since it does not relieve pain or reduce sensitivity to sensory input; it simply causes unconsciousness due to respiratory failure. It is an inhumane method of collecting fish.

Diagnosis

Definitive diagnosis of cyanide poisoning requires the identification of cyanide in target tissues. Cyanide is rapidly detoxified, usually making it undetectable in tissues within several days of peracute exposure (Mak et al. 2005). While some reference laboratories can measure tissue cyanide levels, there is no simple field test at present, although such a test might be available in the future (Mak et al. 2005). Rubec et al (2003) provides data on which fish species collected in the Philippines have the highest prevalence of cyanide exposure.

While cyanide has been suspected of causing DMS, its role is uncertain because there are no scientific studies that prove that cyanide exposure can cause DMS. While fish may die more readily if they are acutely stressed (e.g., placed in an aquarium bag) *immediately* after cyanide exposure (Hanawa et al. 1998), there are no data showing that fish that have been exposed to cyanide are still more susceptible to stress (i.e., DMS) *several days or weeks* later. In addition, we do not know how other stressors, as well as infectious agents, may contribute to morbidity and mortality of DMS in marine ornamentals (Fenner 1998).

Treatment

Since the chronic effects of cyanide poisoning as a part of DMS are unclear, treatment methods are not well defined. However, reducing stress and quickly treating opportunistic infections will greatly reduce morbidity and mortality regardless of whether cyanide plays a role in this phenomenon. Starvation plus stress might play an important role in DMS in marine ornamentals (Hall and Bellwood 1995). The Marine Aquarium Council (www.aquariumcouncil.org), in cooperation with the International Marinelife Alliance, certifies wholesalers and retailers that provide fish that are certified to be collected without the use of cyanide. Because of the reluctance of many collectors to switch to net collecting, there is interest in using possibly less damaging agents, such as clove oil, to capture fish (Helfman 2007).

PROBLEM 95
Miscellaneous Water-Borne Poisonings

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 4

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Toxicological exam
2. History and clinical signs

History

Acute to chronic mortality with evidence of exposure of fish to toxin(s)

Physical Examination

Varies with toxin

Treatment

1. Place fish in unpoisoned system, or add activated carbon, or change water
2. Eliminate exposure to toxin

COMMENTS

Epidemiology/Pathogenesis

A wide range of toxins can affect fish (Di Giulio and Hinton 2008). Many are more toxic to aquatic organisms than they are to terrestrial animals. Thus, insecticides, herbicides, nicotine (cigarette smoke), and household cleaners can be lethal, even if they only reach the water as an aerosol. Objects that are not tested safe for aquarium use can be toxic. For example, various soft plastics can leach plasticizers (softening agents) or may be treated with insecticides or fungicides (e.g., foam padding used for furniture manufacture) (Untergasser 1991). Clinical signs will obviously depend on the type of poisonous exposure.

In ponds or systems that use surface water (e.g., trout raceways), poisoning may occur after rainfall, which may wash acids or agricultural chemicals into the water. However, most fish kills caused by agricultural chemicals result from aerial spraying of crops. Because crop spraying pilots are well aware of the toxicity of agricultural chemicals to fish, poisoning caused by agricultural spraying is now rare. When it occurs, it is usually caused by either the use of new chemicals not before applied in an

area, the use of emergency-use pesticides for unusually heavy outbreaks of some pests, or the use of pesticides contrary to label recommendations (Mitchell 1995).

Susceptibility varies greatly among species, and not all react similarly. Invertebrates often exhibit quite different susceptibility to toxins than fish (may be more or less susceptible). Of the pesticides, chlorinated hydrocarbon insecticides have the greatest potential for harming fish. In general, poisons are more toxic at higher temperatures and may be affected by pH, hardness, alkalinity, and DO. Young fish are usually more susceptible than older fish (Cope 1971).

Diagnosis

When a fish kill occurs, some type of poisoning, especially pesticide-related, is one of the first thoughts that come to the mind of a fish culturist. However, poisonings are rarely a cause of kills in fish culture, and the clinician should rule out other more common causes of kills, such as hypoxia (see PROBLEM 1), or an infectious disease epidemic. The history is critical in determining the cause of miscellaneous water-borne poisonings. Specifically, it is necessary to have some idea about the type of toxin that the fish are or were exposed to, since it is impossible to analyze for all possible toxins. If more than one fish species is present, all species will likely die. The death of other animals, such as frogs, turtles, snakes, and birds, is also strongly suggestive of poisoning. Lack of algae (killed by herbicides) or zooplankton (killed by insecticides) in the water may indicate a pesticide kill.

Acute vs. Chronic Poisonings

Both acute and chronic poisonings are often difficult to diagnose (Beyer et al. 1996). Chronic, sublethal toxicity is often insidious, taking a long time to develop. Furthermore, whether certain low levels of poison are toxic can be hard to decide. Most poisons can cause chronic toxicity at 10- to 1,000-fold or lower concentrations than the acute concentrations shown in Table II-95. Note that toxicity can vary greatly among fish species. For example, the fungicide benlate is much more toxic to channel catfish than to bluegills. However, channel catfish are more resistant to certain other toxins.

Acute toxicity (i.e., fish kill caused by poisoning) can also be difficult to definitively diagnose because many poisonings are onetime events where the poison quickly dissipates after the kill. For example, although short-acting pesticides are fortunately replacing long-acting toxins, they present a greater diagnostic challenge. A large die-off within only a few hours is suggestive of poisoning. Usually, all sizes of fish will be affected, but sometimes the smaller fish will be first to appear distressed. Prompt response is often crucial to diagnosis.

Sample Collection/Submission

Collect samples quickly and preserve them in a fashion that will allow accurate analysis, if a definitive diagnosis is required. Chain of custody should not involve the fish owner, if possible, to avoid any challenge to the validity of samples if litigation is involved. If the owner must collect the samples, a witness (e.g., law enforcement officer) should be present when samples are collected. The methods for collection depend on the type of toxin that is suspected. Most miscellaneous poisonings probably go undiagnosed because it is difficult to obtain this information. It can also be advisable to submit affected fish to determine toxin concentration in target tissues (gill, liver, and kidney are common targets). Extreme care must be taken to avoid contamination of tissue samples during preparation, so it is best to submit live fish or freshly iced, live fish and have the analytical laboratory prepare tissues. Specific recommendations should also be sought regarding sample preparation, depending on which toxin will be sought. Such analyses can be expensive and may not be viable options for the owner. Analysis costs may range from $100 if a specific toxin is suspected, to several thousand dollars or more if it is not known what the toxin might be.

Samples can also be preserved for histopathology. Most lesions induced by toxicants are suggestive of a toxic insult but are nonspecific (e.g., degeneration, necrosis, hyperplasia) (Meyers and Hendricks 1982; Schlenk and Benson 2001). Only a few toxicants cause lesions in aquatic animals that may be useful for diagnosis, although virtually none are pathognomonic (Table II-95).

Treatment

Separation of the fish from the toxin is essential and may be accomplished either by placing fish in a clean system or by diluting and/or removing the toxin by adding clean water or activated carbon, where this is feasible.

Avoidance is the best method of control. The use of pesticides in aquatic areas should be discouraged. Aquarium owners should be made aware of the exquisite sensitivity of fish to even airborne toxicants. For example, fish are highly susceptible to even small amounts of airborne nicotine in smoke. Pesticides sprayed over fields can also drift a considerable distance, reaching ponds. Advise owners to plant high vegetation to intercept airborne drift of pesticides and construct barriers (e.g., ditches) to divert runoff from treated fields. Advocate proper methods of pesticide application and dispense them in a proper manner (i.e., don't contaminate waterways).

Table II-95. Water-quality standards and levels associated with fish kills in freshwater (mg/L unless specified otherwise). [1]

Parameter	Levels in water associated with fish kills	Acceptable continuous exposure levels in water for fish culture	Sources	Diagnostic clinical features (2)	References
TOTAL HARDNESS AS $CaCo_3$	>200 (chronic CO_2 excess) >800 (all causes)	20–200			
TOTAL SUSPENDED SOLIDS	<5,000–100,000	<80 (most fish) Secchi disk reading <25 cm (see PROBLEM 1); For salmonids, <5 best, but not >50	Clay; silt; algae; floods; earthmoving; sawdust and other suspended matter; inadequate solids removal in intensive closed systems	If caused by algae: low DO (see PROBLEM 1); if caused by inanimate material: 1) Low fish production in ponds (inhibits algae growth) 2) "Coughing" to clear gills; gill epithelial hyperplasia 3) Settles on eggs, causing suffocation and secondary infection 4) Add calcium or alum to reduce turbidity in ponds	
TOTAL DISSOLVED SOLIDS	>5,000–20,000	<400			
NITRATE					See PROBLEM 6
MISCELLANEOUS AGRICULTURAL CHEMICALS					
Potassium salts	>1,500		Fertilizer		
Ammonium salts	>50		Fertilizer		
Phosphorus (elemental)	>0.02–4.0 (acute) >0.0001–0.002 (chronic)		Industry		Fletcher et al. (1970)
Lime (Calcium oxide, calcium hydroxide)	causing pH > 9–10				See "**Pharmacopoeia**"
MISCELLANEOUS POISONS					
Chlorine	>0.10–4.0	<0.003			See PROBLEM 92
Fluoride salts	>5.0				
Sodium arsenite, arsenic trioxide	>2.0–20				
Cyanides	>0.03–0.23	<0.005	Iatrogenic overdose from fish collecting; mining waste; gas works; steel mills; ferrocyanide (see "**Salt**" in "**Pharmacopoeia**")		Also see PROBLEM 94
Hydrogen sulfide	>0.5–10	<0.002	Anaerobic organic decay; paper mills; tanneries	Purple-violet gills (acute)	See PROBLEM 91
Methane (marsh gas)		>65 apparently not harmful	Anaerobic organic decay	Bubbles trapped in a glass jar are easily ignited with a match	McKee and Wolf (1963) Boyd (1990)

Continued.

Table. II-95 Water-quality standards and levels associated with fish kills in freshwater (mg/L unless specified otherwise), cont'd.

Parameter	Levels in water associated with fish kills	Acceptable continuous exposure levels in water for fish culture	Sources	Diagnostic clinical features (2)	References
Nicotine	>1				
Holothurin			Biotoxin released by holothuroids (sea apples) in reef aquaria	Acute deaths; highly toxic to fish, less toxic to invertebrates	Knop (2004b)
Algal toxins					See PROBLEM 96
ORGANOCHLORINE (CHLORINATED HYDROCARBON) PESTICIDES					
Endrin	>0.0003–0.002	<0.003 µg/L (ppb)	Agricultural discharges	Paralysis (acute); spinal deformities and vertebral fractures (chronic); decreased egg viability; lipophilic toxins that may be mobilized during fasting (e.g., winter); very persistent pesticides in environment	Gilbertson (1985) Westin et al. (1985)
Chlordecone (Kepone)	>0.004–0.07	<0.001 µg/L			
Endosulfan	>0.01	<0.01 µg/L			
Pentachloraphenate	>0.1	<0.1 µg/L			
Aldrin	>0.013–0.05	<0.01 µg/L			
Heptachlor	>0.019–0.25				
Dieldrin	>0.008–0.05	<0.005 µg/L			
Chlordane	>0.02–0.08	<0.004 µg/L			
Lindane (BHC)	>0.23–0.8	<0.02 µg/L			
Toxaphene (camphenes)	>0.003–0.018	<0.01 µg/L			
DDT	>0.008–0.027	<0.003 µg/L			
CARBAMATE PESTICIDES					
Carbaryl (Sevin®)	>0.5–10	<0.02 µg/L		Depressed brain acetylcholinesterase; vertebral deformities; muscular/neural lesions; moderately persistent pesticide in environment	Post (1987)
Zectran	>2.5–17				
ORGANOPHOSPHATE PESTICIDES					
Diazinon	>0.2–5.2	<0.002 µg/L	Agricultural discharges; livestock discharges	Depressed brain acetylcholinesterase; weakness; vertebral fractures; perivertebral hemorrhage (acute and chronic); relatively nonpersistent pesticide in environment	Schneider (1979) Also see "Organophosphate" in "**Pharmacopoeia**"
Malathion	>0.1–30	<0.008 µg/L			
Parathion	>0.3–1.6	<0.001 µg/L			
Trichlorphon	>0.8–100.0	<0.001 µg/L?			
Fenthion (Spotton®)	>0.9–2.5				
Chlorpyriphos (Dursban®)	>0.01				
Azinphos-methyl (Guthion®)	>0.005–0.09				
Coumaphos (Co-Ral®)	>0.3–1.1				
PYRETHRIN INSECTICIDES	>0.0005–0.001	<0.001 µg/L		See also "Pyrethroid" in "Pharmacopoeia."	
Pyrethrum (Pyrethrum®)					
Permethrin (Ambush®)					
Resmithrin (Synthrin®)					
INSECT GROWTH REGULATOR INSECTICIDES					
Fenvalerate (Ectrin®)					

IVERMECTIN ANTHELMINTICS	<0.1 mg/L?	<0.001 µg/L	Livestock discharges		Palmer et al. (1987) Also see "**Pharmacopoeia**"
PISCICIDES					
Rotenone (derris root, cube root)	>0.006–4			Rotenone: Gills bright red even though clinically hypoxic; natural decay takes days (high temperature) to weeks (low temperature)	Cailteux et al. (2001)
Antimycin	>0.05–20 µg/L 1–200 µg/L			Antimycin: Most persistent and toxic at low pH (persists for 1 day to over 1 week, depending upon pH); temperature less important	
HERBICIDES/ALGAECIDES			Agricultural discharges; treatment of plants in waterways	Some persist for months in sediment (e.g., diquat, paraquat), especially granular forms	See "copper" and "diquat" in "**Pharmacopoeia**"
Copper sulfate	>0.14–0.5				
Simazine	>10.0	<.01			Reid and Anderson (1982)
Acrolien	>0.14				
Glyphosphate (Roundup®)	>12–130				
Chlorthalonil	>10–20				Davies and White (1985)
2,4-D (Weedone®)	>2.0–96.5	<0.004 µg/L			
Paraquat	>840				
Diuron	>4–152				
Diquat	>8–350				
Silvex	>1				
Endothall (Aquathol®)	>0.3–450				
FUNGICIDES					
Trifluralin (Treflan®)	>0.04–2.2			Muscular/neural lesions; vertebral deformities	
Benomyl (Benlate®)	>0.016–2.2				
Captan	>0.017–0.20				
Triphenyl tin (Du-Ter®)	>0.020–0.100				
Antimildew agent			Silicone sealant not approved for aquaculture use (bathroom caulk)		
FOREST FIRE RETARDANTS					
Fire-Trol 831	>1,000				
MOTHPROOFING AGENTS					
Eulan WA New (chlorophenylid)	>0.5–5.4				
Mitin N/Mitin FF (fenurons)	>0.07–11.2				
DETERGENTS			Household and industrial laundering; other cleaning operations	Hemorrhage; excess mucus; gill subepithelial edema; epithelial disruption	Wedemeyer et al. (1976)
Sodium dodecyl sulphate	>28–32	<0.1			
Dodecyl benzosulphonate	>5	<0.1			
Sulphonates	>4	<0.1			

Continued.

Table. II-95 Water-quality standards and levels associated with fish kills in freshwater (mg/L unless specified otherwise), cont'd.

Parameter	Levels in water associated with fish kills	Acceptable continuous exposure levels in water for fish culture	Sources	Diagnostic clinical features (2)	References
PHENOLS					USEPA (1973)
Phenol	>7.5–56	<0.10	Industrial effluents; landfills; coal, petroleum processing; wood distillation; municipal, animal wastes	Low taste threshold by humans	
o-Cresol	>2.3–29.5	<0.10			
m-Cresol	>6.4–24.5	<0.10			
Resorcinol	>14	<0.10			
Hydroquinone	>0.30	<0.10			
POLYNUCLEAR AROMATIC HYDROCARBONS (PAHS)					
Naphthalene	>165	<1.5?	By-products of petroleum processing or combustion	Very insoluble in water, but many are very carcinogenic	
Anthracene					
Benzo(a)pyrene					
Phenanthrene	>1–2				
MISCELLANEOUS PETROCHEMICALS					
Diesel oils, car oils			**Surface waters**: Usually oil spills **Ground waters**: Leaking underground fuel storage tanks; industrial wastes; landfills; underground waste dumps	Fish exposed to petroleum develop hemosiderosis (excess deposition of hemosiderin, a yellow-brown, Perl's Prussian blue–positive pigment)	Malins et al. (1984) Poirier et al. (1986) Khan & Nag (1993) Smith (1968)
Diesel fuel	>50–1,000				
Crude oil	>167				
Toluene					
Benzene	>10–260				
Hexachlorobenzene	>toxic than benzene				
Aniline, toluidine	>100				
PHTHALATE ESTERS	731–1,300	<0.3 µg/L	Plasticizers (softening agents), especially for PVC (polyvinyl chloride) plastics		
PHOSPHATE ESTERS			Lubricants; oil additives; plasticizers	Depressed brain acetylcholinesterase activity	Nevins and Johnson (1978)
Pydraul 115E	>45–100				
Pydraul 50E	>0.72–3				
Houghotosafe 1120	>1.7–43				
POLYCHLORINATED BIPHENYLS (PCBs, Arochlor®)	>0.015–61 (acute) >0.003 (chronic)	<0.002 µg/L	Transformer lubricants; heat exchangers; hydraulic fluids; plasticizers; many sources	Very stable in environment; chronic problems most serious, especially reproductive impairment, egg mortality; transformer oils with PCB less toxic than PCB alone	Mayer and Mayer (1985) Murty (1986b)

[1] Modified from Langdon (1988), with additional data provided in the listed references, as well as from USEPA (1973, 1979–1980), Wedemeyer et al. (1976), Alabaster and Lloyd (1982), Hine (1982), Piper et al. (1982), Hellawell (1986), Murty (1986a, 1986b), Meyers and Hendricks (1982), Bengtsson (1975), Wellborn et al. (1984), Johnson and Finley (1980), APHA (1992, 2005), and Mitchell (1995). Also see the "**Pharmacopoeia**" for details on various drugs.

(2) Toxin-induced lesions are typically nonspecific; similar lesions are often induced by many different types of agents (Mallatt 1985; Meyers and Hendricks 1982).

PROBLEM 96
Harmful Algal Blooms (HAB)

Prevalence Index

WF - 4, WM - 4, CF - 4, CM - 2

Method of Diagnosis

Rule-out of other problems combined with identification of specific harmful alga in concentration sufficient to be pathogenic

History

Acute to chronic mortality consistent with exposure of fish to harmful alga: most commonly, behavioral abnormalities and/or dyspnea; floating algae ("scum") on water; red, brown, or green discoloration of water

Physical Examination

Varies with alga, but often neurological signs; dyspnea; algae lodged in gills

Treatment

MARINE CAGES

1. Reduce or stop feeding and handling
2. Eliminate exposure to alga

PONDS AND AQUARIA

1. Reduce or stop feeding and handling
2. Place fish in unpoisoned system or add activated carbon or change water

COMMENTS

Epidemiology/Pathogenesis

Noxious phytoplankton causing harmful algal blooms (HAB) are becoming an increasingly serious threat to fish culture (Fig. II-96, A, B, C, D), with the great majority of problems occurring in near-coastal marine systems (e.g., cage culture or aquaculture operations that use coastal or estuarine water). Harmful algal blooms have prevented the development of aquaculture in some areas (e.g., Sunshine Coast of the Strait of Georgia, British Columbia). There is evidence that eutrophication (e.g., from agricultural, urban and industrial sources) is responsible for many of the blooms (Smayda 1990). However, there is little evidence that properly sited farms play any role in causing blooms. A proper site usually is one that is in moderate to deep water, having moderate or greater vertical water mixing, and having a strong tidal velocity to flush away nutrients. Flushing may also be important in preventing the accumulation of noxious algae cysts (resting stages) in the sediment underlying the cages. Nonetheless, farms in shallow, poorly flushed sites that are sensitive to nutrient additions may contribute to bloom production. Fish reared in aquaculture are typically at significantly greater risk than wild fish because they are unable to escape when a bloom approaches. However, fish kills and disease in wild populations have also been associated with certain harmful algae (Bruslé 1995; Noga et al. 1996; Rensel and Whyte 2003).

Many types of harmful algae have been implicated in fish morbidity and mortality (Table II-96). Noxious algae can harm fish in one of several ways. Some cause hypoxia by mechanically obstructing respiration or physically/chemically damaging the gills (Kent 1992). Some algae produce potent toxins; neurotoxins are especially common. Clinical signs of algal neurotoxicity include disorientation, loss of equilibrium, and sporadic hyperactivity. Other algal toxins damage skin, liver, blood or other organs. Sublethal exposure to some harmful algae increase susceptibility to infectious disease (Albright et al. 1993; Noga et al. 1996). Algal blooms can also stress or kill fish due to removal of oxygen or excess production of oxygen (see PROBLEMS 1 and 11, respectively, for details).

DINOFLAGELLATES

Many dinoflagellates have been implicated or suspected in fish kills. The red tide dinoflagellate (*Karenia brevis* = *Gymnodinium breve* = *Ptychodiscus brevis*) causes mass mortalities of fish and invertebrates in states bordering the Gulf of Mexico, United States (Steidinger and Baden 1984; Steidinger et al. 1998). Another related dinoflagellate species, *Karlodinium veneficum* (formally *K. micrum*) kills fish along the east coast of the United States (Deeds et al. 2002; Place et al. 2008). Both species produce potent, well characterized, polyketide toxins which have been measured at fish kill sites at concentrations which cause mortality in the laboratory (Deeds et al. 2002; Place et al. 2008). *Karlodinium veneficum* has also caused mortalities at several aquaculture operations (Deeds et al. 2002).

Pfiesteria piscicida and some related algae (*Pseudopfiesteria shumwayae* and *Cryptoperidioiopsis* species) have been identified in major estuaries along the western Atlantic coast of the United States, including the Chesapeake Bay and Albemarle-Pamlico Estuary (Litaker et al. 2002). There is evidence that some of these "Pfiesteria" algae can cause acute mortality in cultured marine and estuarine fish, especially in intensive aquarium systems (Smith et al. 1988; Burkholder et al. 1992; Vogelbein et al. 2002). Sublethal exposure may also cause massive skin damage in surviving fish (Noga et al. 1996; Vogelbein et al. 2002). Interestingly, the algae can directly feed on skin, causing major skin damage and eventually death (Vogelbein et al. 2002). While a toxin has been suspected in some cases (Smith et al. 1988), this has yet to be proven by identification of the toxin. The ability of Pfiesteria algae to bloom in aquaria means that they can be easily introduced into a culture system with contaminated water or fish (Smith et al. 1988). Pfiesteria species were also linked to numerous fish kills and disease epidemics in wild fish populations (Noga et al. 1996; Burkholder et al. 1999). However, more recent evidence indicates that their very low cell densities in the wild make them highly unlikely to play a role in these

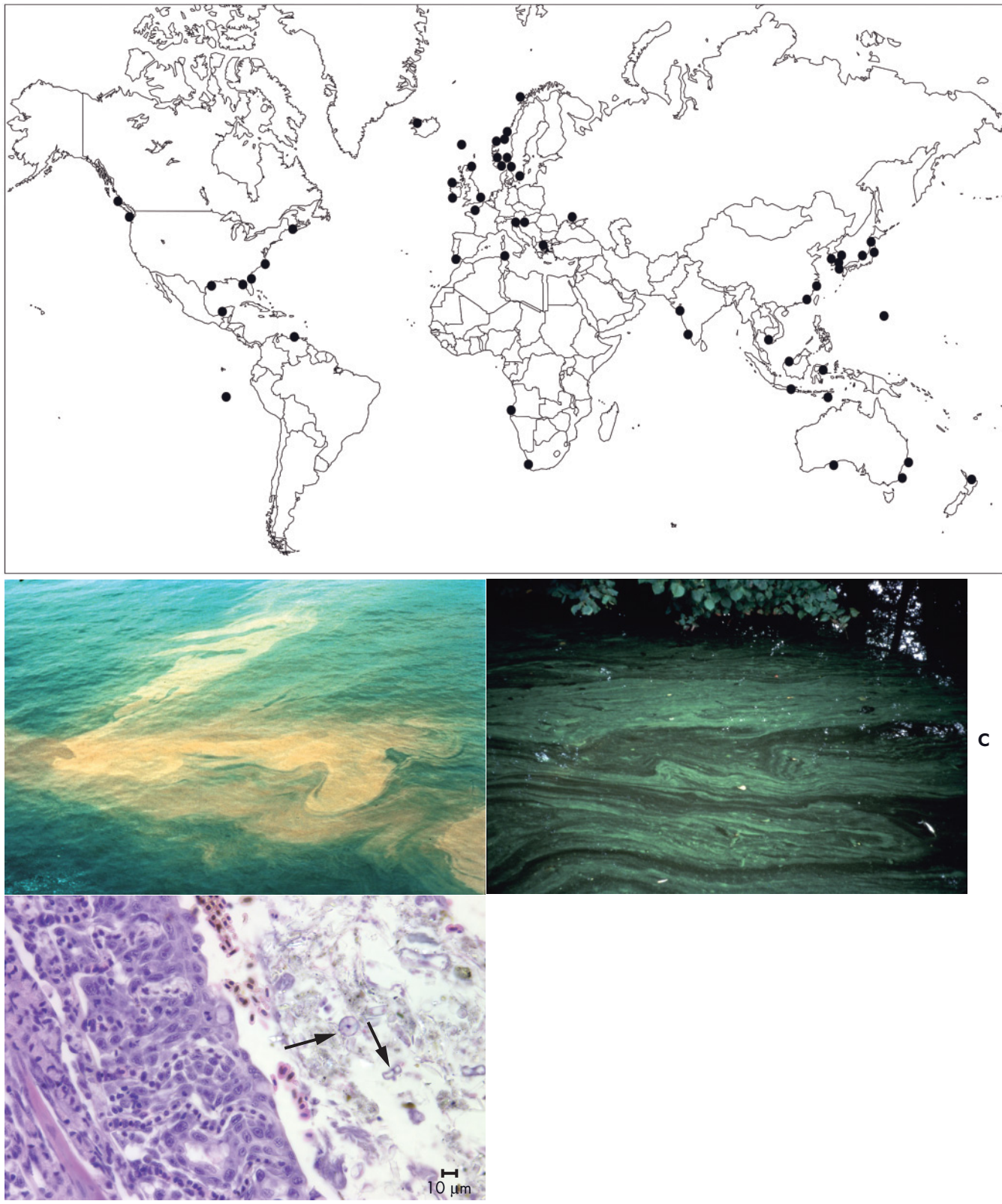


Fig. II-96. A. Global distribution of kills in wild and cultured fish associated with noxious phytoplankton. For more details on distribution of harmful algal blooms, see www.issha.org. B. Red tide caused by *Noctiluca miliaris* bloom. C. Freshwater algal bloom caused by cyanobacteria (blue green algae). D. Histological section of Atlantic salmon gill with *Chaetoceros* diatoms lodged between the primary lamellae. Diatoms (*arrows*) are refractile structures, some retaining green-brown cytoplasm. Note hemorrhage, severe epithelial hyperplasia, and chronic inflammation from physical trauma. Hematoxylin and eosin. Bar = 10 µm. (*A* modified from Sundstrom et al. 1990; *B* photograph courtesy of H. Möller; *C* photograph courtesy of H. Paerl; *D* photograph courtesy of T. Peterson and M. Kent.)

Table II-96. Taxonomic groups of noxious algae associated with toxicoses to fish. Many of the listed algae have only been associated with fish kills and have not proven to be the cause of mortalities. The pathogenesis of most noxious algae in causing fish morbidity/mortality is uncertain (data from Steidinger and Baden [1984], Davin et al. [1988], Kent [1992], Kent and Poppe [1998], Chang et al. [1990], Rensel and Whyte [2003], and K. Steidinger [personal communication]). Note that "algae" is a generic term and includes members of the botanical kingdoms Plantae and Chromista, as well as certain bacteria. Note also that the dinoflagellates *Pfiesteria* and *Pseudopfiesteria* are heterotrophs (do not produce chlorophyll or other photopigments).

Dinoflagellates (class Dinophyceae)
- *Alexandrium angustitabulatum*†
- *Alexandrium catenella*
- *Alexandrium excavatum*
- *Alexandrium fundyense*
- *Alexandrium monilatum*
- *Alexandrium tamarene*
- *Amphidinium carterae*
- *Amphidinium klebsii*
- *Amphidinium rhynocephalum*
- *Cochliodinium polykrikoides*
- *Cryptoperidiniopsis brodyi*
- *Gambierdiscus toxicus*
- *Gonyaulax spinifera*
- *Gymnodinium galatheanum*
- *Gymnodinium pulchellum*
- *Gymnodinium sanguineum*
- *Gymnodinium venificum*
- *Gyrodinium cf. aureolum*
- *Karenia (Gymnodinium) mikimotoi*
- *Karenia brevis (Gymnodinium breve)*
- *Karlodinium micrum*
- *Karlodinium veneficum (Gymnodinium veneficum)*
- *Lingulodinium polyedra*
- *Noctiluca miliaris*
- *Peridinium polonicum (F)*
- *Pfiesteria piscicida*
- *Prorocentrum balticum*
- *Prorocentrum concavum*
- *Prorocentrum minimum*
- *Pseudopfiesteria shumwayi*
- *Pyrodinium bahamense var. compressa*

Green algae (class Chlorophyceae)
- *Chaetomorpha minima*

Cyanobacteria (blue-green algae)
- *Anabaena flos-aquae (F)*
- *Anacystis marina (F)*
- *Aphanizomenon flos-aquae (F)*
- *Microcystis aeruginosa (F)*
- *Nodularia spumigena*
- *Oscillatoria agardhii (F)*
- *Oscillatoria rubescens (F)*
- *Schizothrix calcicola*

Yellow-green algae (class Chrysophyceae)
- *Ochromonas danicum (F)*
- *Ochromonas malhamensis (F)*
- *Ochromonas minuta (F)*

Prymnesiophytes (class Prymnesiophyceae)
- *Chrysochromulina polylepis*
- *Chrysochromulina leadbeateri*
- *Prymnesium calathiferum*
- *Prymnesium parvum*
- *Prymnesium patelliferum*

Rhaphidophyceans (class Rhaphidophyceae)
- *Chattonella marina*
- *Heterosigma akashiwo*
- *Chattonella antiqua*

Diatoms (class Bacillariophyceae)
- *Chaetoceros concavicorinus*
- *Chaetoceros convolutus*
- *Corethron sp.*

Silicoflagellates (Phylum Heterokontophyta)
- *Dictyocha speculum*

†*Alexandrium* species were previously classified as *Gonyaulax*, *Protogonyaulax*, or *Gessnerium*.
F = freshwater algae. All others are marine or estuarine.

events (Vogelbein et al. 2008); instead, these mortalities appear to be due to *Karlodinium veneficum* which is difficult to morphologically distinguish from Pfiesteria algae (Vogelbein et al. 2008, Place et al. 2008).

Some dinoflagellate toxins are transferred up the food chain (e.g., *Alexandrium*) and have caused mortalities in wild fish that consume tainted zooplankton along the Northwest Atlantic coast (White 1981a, 1981b; Botana 2008).

PRYMNESIOPHYTES

Prymnesium parvum causes mortality in brackish and marine pond fish in Europe and in the Middle East (Shilo 1981; Gordon and Colorni 2008). It produces a hemolytic toxin. *Chrysochromulina polylepis* causes hemolysis. While not toxic, *Phaeocystis pouchetii* blooms can produce a thick, mucilaginous matrix, suffocating marine life.

RHAPHIDOPHYSEANS

Chattonella antiqua and *Heterosigma akashiwo* presumably kill fish by generation of superoxide anion radicals (O_2^-) that cause severe gill damage (Nakamura et al. 1998).

DIATOMS

Chaetoceros spp. (*C. concavicornis* and *C. convolutus*) and occasionally *Skeletonema* or *Thalassiosira* spp. have been associated with mortality in seawater-cultured salmonids. The spines of the alga apparently cause it to become lodged on or in the gill tissue, inducing a foreign body reaction and traumatizing the gill tissue (Fig. II-96, D). Epithelial hyperplasia causes hypoxia. In some cases, hyperactive mucus production appears to be primarily responsible for the hypoxia (Rensel 1993).

SILICOFLAGELLATES

Dictyocha speulum causes a gill pathology that is similar to that of diatoms.

CYANOBACTERIA

Die-offs of cyanobacteria are well known to cause environmental hypoxia (see PROBLEM 1). A die-off appears as a change in the water's color from green or green-brown to light brown. The water often smells because of cyanobacterial decomposition. Cyanobacteria are also the major cause of off-flavor, which results from the uptake by the fish of chemicals (primarily geosmin and 2-methylisoborneol) produced by cyanobacteria (e.g., *Oscillatoria*, *Anabaena*) (Smith et al. 2008). The chemicals impart an undesirable flavor to the fillet, preventing fish from being harvested until the compounds leave the fish. The problem is most common in ponds receiving large additions of feed or fertilizer (Paerl and Tucker 1995).

Cyanobacteria are well known to be toxic to mammals. While they have been suspected in a number of freshwater fish kills, there are few documented cases. *Aphanizomenon flos-aquae* (English et al. 1993) and *Anacystis marina* (A. Goodwin, personal communication) have been associated with mortalities of channel catfish in ponds.

Diagnosis

Any of the previously described clinical signs without evidence of another etiology suggests a possible noxious algae problem. The presence of an obvious bloom (Fig. II-96, B) is also supportive. Important differentials are other toxins (see PROBLEMS 93 and 95). Almost all documented noxious algae blooms have occurred in brackish or marine waters.

Many algae are suspected of causing fish morbidity/mortality but have not yet been proven as a cause. The number, as well as the type of algae present, are important in making a diagnosis. A certain minimum concentration (bloom concentration that varies with algal species) is required to implicate an algal organism as a cause of morbidity. Definitive identification of an unidentified noxious algae requires examination of the sample by a trained expert, since to the untrained observer, noxious algae can look similar to closely related, nontoxic algae.

Samples must be collected as soon as possible after a bloom is suspected since blooms can rapidly dissipate. Fresh samples are best for identification. Fresh samples should be kept at ambient water temperature or slightly lower but should not be iced. One of the best ways to do this is to wrap the container in wet newspaper and let evaporation cool the sample for transport (K. Steidinger, personal communication). If fresh samples cannot be examined within 24 hours of collection, they should be preserved in Lugol's iodine solution and kept refrigerated and in the dark until examination. Alternatively, samples can be preserved in 1–2% neutral buffered formalin. For delicate species (i.e., thinly armored or unarmored dinoflagellates), 2% gluteraldehyde in borate buffer is better. Some algae, especially unarmored forms, are considerably altered by fixation. The reference laboratory should be consulted for proper sample submission.

Because of the lack of specific pathology for most HAB, histology is not very useful, except for helping to initially rule out other problems (e.g., infections, etc.). However, taking fresh wet mounts of gill can allow rapid identification of certain gill-damaging algae (e.g., *Chaetoceros*); these algae are often washed off the gill during histological processing.

Management and Treatment

MARINE CAGE CULTURE

The speed with which many noxious algal blooms can affect cage-cultured marine fish often makes it difficult to successfully respond the first time that a bloom event occurs on a farm. However, if blooms continue to recur, there are some strategies that have been used with varying success to reduce the impact of subsequent blooms on the farm at risk. First, the farm should have a routine monitoring program that surveys water samples for the presence and abundance of the noxious alga. Once identified by an expert, many noxious algae can be easily recognized by a fish farm technician using a compound microscope. Also, molecular probes are being developed for many noxious algae and their toxins and some are commercially available (Abraxis, Jellett Biotek, Mercury Science).

Monitoring intensity will vary, but should be greatest during times of greatest risk of bloom occurrence and when cell numbers are near toxic levels. Aerial surveys might also assist in detecting approaching or developing blooms. It is sometimes possible to detect the onset of a bloom by observing changes in behavior (anorexia, lethargy, disorientation, etc.), some of which might be peculiar to a specific noxious alga. It can be useful to have some small, portable cages always set up so that fish can be observed and sampled more easily.

When the action level has been reached, bloom impact might be reduced in one of several ways (Rensel and Whyte 2003). For all types of blooms:

1. Reduce feeding and handling: Not handing the fish will reduce stress. Immediately withholding feed during a bloom event will reduce oxygen demand of the fish. However, prolonged fasting (several weeks) is not recommended and thus this is not effective during long-lasting HAB.
2. Physically move the cage away from the bloom: This is often the preferred method but is risky (possible loss or damage of cage or fish) and expensive. It also may require government approval.
3. Submerse cage to a lower water depth: This requires the use of a cage specifically designed to withstand this procedure. Also, salmonids, being physostomous (their swim bladder opens to the digestive tract), must occasionally gulp air to fill their swim bladder. Thus, they cannot remain below the water indefinitely.

For certain types of HAB, it might also be useful to:

4. Inject air using large pipes or air diffusers to move deeper (clean) water to the surface of the cage: This can disperse blooms that are concentrated near the water's surface. The pipe for air injection must not be too deep or it might induce gas bubble disease (PROBLEM 11).
5. Increase oxygenation by supersaturating the water with pure oxygen: This can reduce mortalities caused by clinical hypoxia from gill-damaging algae (e.g., *Chaetoceros*). Supersaturation must be below levels that can cause gas bubble disease (typically <300% or 400 mm Hg).
6. Treat water with clay to flocculate algal cells: Cell concentrations of a number of HAB can be significantly reduced by adding clay, but a number of environmental concerns, especially the effect of clay accumulation on benthos, remain to be resolved.

The latter three methods mentioned above are not yet commonly used.

When a bloom is approaching a farm, it is common to harvest fish just prior to its arrival if possible. The sale of

fish slaughtered during a bloom is not advised; but fortunately, many, if not most, toxins from HAB do not appear to accumulate in edible fish tissues to levels that are toxic to humans. This is in sharp contrast to the serious problems that HAB cause for human consumption of edible shellfish.

CLOSED AQUACULTURE SYSTEMS

In ponds, algicides (copper, simazine, endothall) can inhibit many harmful algae, but extreme care must be taken to avoid oxygen depletion (see PROBLEM 1). Treatment with copper can also cause release of toxin from dying algae, exacerbating the losses (Deeds et al. 2002). Treating with potassium permanganate instead of copper has been found to result in less mortality in some cases (Deeds et al. 2002). With any algicidal treatment, bloom recurrence may occur. Cyanobacteria are significantly inhibited at 5–10 ppt salinity (Paerl and Tucker 1995). Relatively small volumes of water (e.g., for aquaria) can be disinfected and detoxified using ozonation and activated carbon filtration before adding or use (K. Steidinger, personal communication).

PROBLEM 97

Acute Ulceration Response (AUR)/Environmental Shock/Delayed Mortality Syndrome (DMS)

Prevalence Index

WF - 2, WM - 2, CF - 2, CM - 2

Method of Diagnosis

Rule-out of other problems combined with the following:

1. Measurement of water quality under old and new conditions
2. History
3. Identification of uninfected skin ulcers (early stage only)

History

Acute mortality or disease outbreak after large (50% or greater) water change; acute mortality or disease outbreak after transferring fish to a new culture system or imposing some other type of acute stress

Physical Examination

Acute stress response

Treatment

1. Reduce stress during transport or other manipulations
2. More frequent and smaller water changes in culture systems
3. Prophylactic antibiotics to reduce opportunistic infections

COMMENTS

Causes of Environmental Shock

Rapid changes in culture conditions can be dangerous to fish, even if these changes are within the normal physiological range for that species. Thus, if fish are moved to a new aquarium where the pH, hardness, or temperature is quite different from their previous environment, it can cause severe stress. Stress is also imposed by the manipulations involved in the transfer, such as netting the fish; confining them in a transport container; transporting them, with consequent build-up of toxins (e.g., ammonia, CO_2) and changes in pH and temperature; and then exposure to a novel environment.

Pathogenesis

Fish may adversely respond to such stress in several ways. The most severe reaction is peracute mortality. Some highly stress-prone species may die within minutes to hours of capture. If the fish survive the transport and/or transfer to a new environment, they may become sick within several hours to several days after being placed into the new culture system. For example, striped bass are highly stressed by simply moving them from a relatively large volume of water (e.g., large aquarium or pond) to a small volume of water (small aquarium).If the confinement (stress) is severe enough, they will begin to slough their skin as soon as 15 minutes after the initiation of the stress (Udomkusonsri et al. 2004) and this can lead to loss of large areas of skin on the entire body within 1 hour (Udomkusonsri and Noga 2005). This can then result in severe secondary infections (e.g., due to water molds) and acute mortality, within 24 hours of the acute stress (Noga et al. 1994). This acute ulceration response (AUR) also occurs in many other fish, including channel catfish, freshwater angelfish and guppies (Udomkusonsri and Noga 2005). This pathological skin response is part of a broader physiological response that has often been referred to as delayed mortality syndrome or delayed capture mortality syndrome. DMS is accompanied by a number of adverse physiological changes. Like AUR, DMS is also associated with opportunistic infections, such as water molds (see PROBLEM 34), many bacteria, or ectoparasitic protozoa, which take advantage of the stressed host.

While infections that are caused by AUR/DMS are usually evident within 2–5 days of the stressful event, they may not appear until over 1 week later. A critical period of about 2–3 weeks after the acute stress is the most likely time that fish will become sick because of AUR/DMS; thus, close observation is warranted.

The mechanisms responsible for AUR/DMS are unknown, although some attendant physiological changes resemble the shock response in mammals. For example, the stress hormone epinephrine can at least partly reproduce AUR lesions (Noga et al. 1998). Severe exercise can kill fish, but the pathogenesis does not appear similar to capture myopathy of feral mammalian hoofstock. Wild-caught fish are more susceptible to AUR/DMS than captive-bred fish. This may explain why disease outbreaks are especially common after shipping tropical marine fish. In general, the hardy fish species appear to be most resistant to DMS (Table II-97),

Table II-97. Some tropical freshwater aquarium fish that are relatively resistant to environmental fluctuations. This information is intended as a general guideline. Some species or strains within this group may not be as resistant.

Goldfish
Koi
Guppies and mollies (*Poecilia* spp.)
Platies and swordtails (*Xiphophorus* spp.)
Zebrafish
Danios (*Danio* spp.)
Kissing gouramies
Siamese fighting fish
Oscar
Jewel cichlid
Firemouth cichlid and related cichlids (*Cichlasoma* spp.)
Pacu
Ctenopoma

although not entirely immune. The same is probably true for AUR.

Diagnosis

Diagnosis of AUR/DMS is usually based on the history and clinical signs (i.e., acute mortality with no detectable cause; delayed mortality caused by opportunistic infections). This is obviously a rule-out diagnosis because many other problems have a similar presentation. For example, an acute stress response can occur because of many water-quality problems. AUR/DMS is differentiated from other water-quality problems in that water-quality conditions are within the normal range for that species. Thus, the stress is caused by an inability to acclimate to conditions within the normal range and is typically potentiated by other stressors (e.g., confinement; see "**Acclimation**," **p. 65**). If the fish are examined early enough after the stress has occurred, a definitive diagnosis of AUR can be made by identifying significant skin ulceration using the fluorescein test (Fig. II-97) in the absence of any microbial infection. However, the ulcers induced by AUR are quickly colonized by opportunistic microbes, making it difficult to diagnose a spontaneous case before an infection is established. However, the fluorescein procedure can be used to determine whether or not AUR is present in fish that have just been stressed; if AUR is detected, then appropriate prophylactic measures can be instituted to prevent secondary microbial infection. Some aquarium fish are generally considered more resistant to environmental stress (i.e., "hardy" species, Table II-97).

Treatment

If environmental shock is suspected, immediately returning the fish to the previous environmental conditions may be helpful but is usually not feasible. Symptomatic treatment should be used to control morbidity and mortality. Symptomatic treatment includes using appropriate medications to control opportunistic infections and can also include the use of salt, which acts as an osmoregulatory enhancer, since osmoregulatory dysfunction appears to be a major sequela. Adding calcium also helps (Grizzle et al. 1990).

Prophylaxis

Prevention is especially desirable for AUR/DMS because of its potentially devastating consequences, as well as lack of specific treatments; this involves the proper handling of fish during manipulations, such as transport. Acute ulceration response/delayed mortality syndrome is probably one of the major reasons why mortalities are frequently high immediately after shipping fish.

While frequent water changes are useful for maintaining good water quality in aquaria, avoid major changes in environmental conditions. It is usually best to replace 25% or less of the water at any one time to prevent environmental shock, although larger water changes are tolerated if the fish are acclimated to them.

Before transport, feed should be withheld for up to 48–72 hours because fish are adapted to intermittent feeding and thus it requires a long time to achieve a noticeable physiological effect that will reduce oxygen consumption and production of carbon dioxide, ammonia and feces (Wedemeyer 1992).

PROBLEM 98
Traumatic Lesions

Prevalence Index

WF - 1, WM - 1, CF - 2, CM - 2

Method of Diagnosis

Usually rule-out of other problems combined with history and clinical signs

History

One or several (but not all) fish hiding in corners or elsewhere in aquarium; obvious attack of one or more individuals by tankmates; new fish recently introduced into established aquarium; shy or peaceful fish or smallest fish most affected; normal or more intense coloration in aggressors with faded or otherwise changed color pattern in affected fish; decrease in fish numbers in a rapidly growing population (e.g., fingerlings); large size variation among individuals; fish-eating birds near pond; overcrowding

Physical Examination

Fins ragged or scales missing in submissive individuals; in salmonids, dorsal fin especially damaged or missing, with traumatic wound on dorsum (soreback); opportunistic infections; corneal edema or ulceration, especially in large individuals

Treatment

AQUARIUM FISH (AGGRESSION PROBLEMS)

1. Hospitalize attacked individual(s) and treat with antibiotics (topical treatment if small, focal lesion)
2. Remove aggressor(s) from aquarium

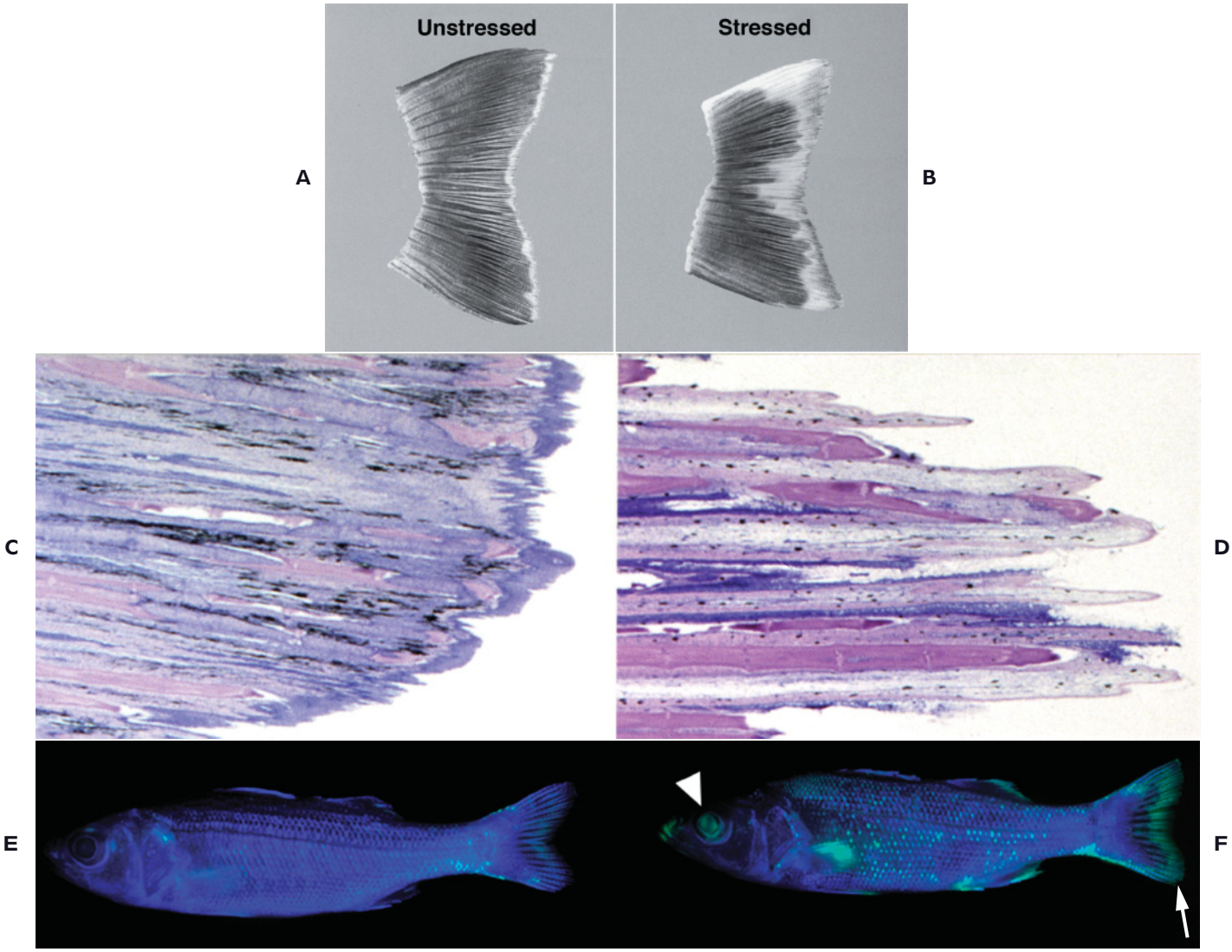


Fig. II-97. Severe ulceration in hybrid striped bass due to acute confinement. A. Caudal fin of unstressed fish. B. Caudal fin of fish stressed by placing in a confined space (small aquarium) for 45 minutes. C. Histological section through the caudal fin of the unstressed fish. D. Histological section through the caudal fin of the stressed fish. Note the entire loss of the basophilic epithelium. Hematoxylin and eosin. E. Unstressed fish treated with fluorescein. F. Fish stressed by confining in a small aquarium for 45 minutes and then treated with fluorescein. Note the bright yellow-green coloration on the fins (*arrow*), indicating that the skin is entirely sloughed (i.e., ulcerated). The cornea of the eye is also ulcerated (*arrowhead*). (*E* and *F* photographs by P. Udomkusonsri and E. Noga.)

3. Change position of rocks and other objects in aquarium
4. Reduce (or increase) density
5. Feed lightly while introducing new fish
6. Place new fish in a clear plastic box for up to several days

OTHER PROBLEMS (DEPENDING UPON ETIOLOGY)

1. Decrease stocking density
2. Increase feeding rate
3. Treat infected wounds if severe
4. Reduce exposure to sunlight
5. Prevent access of fish-eating wildlife

COMMENTS

Causes of Traumatic Lesions

AGGRESSION

Aggression is a common cause of trauma and even mortality in aquarium fish. Some fish, such as large cichlids, are highly prone to being aggressive. Aggression develops out of the instinctual behavior of many fish to form

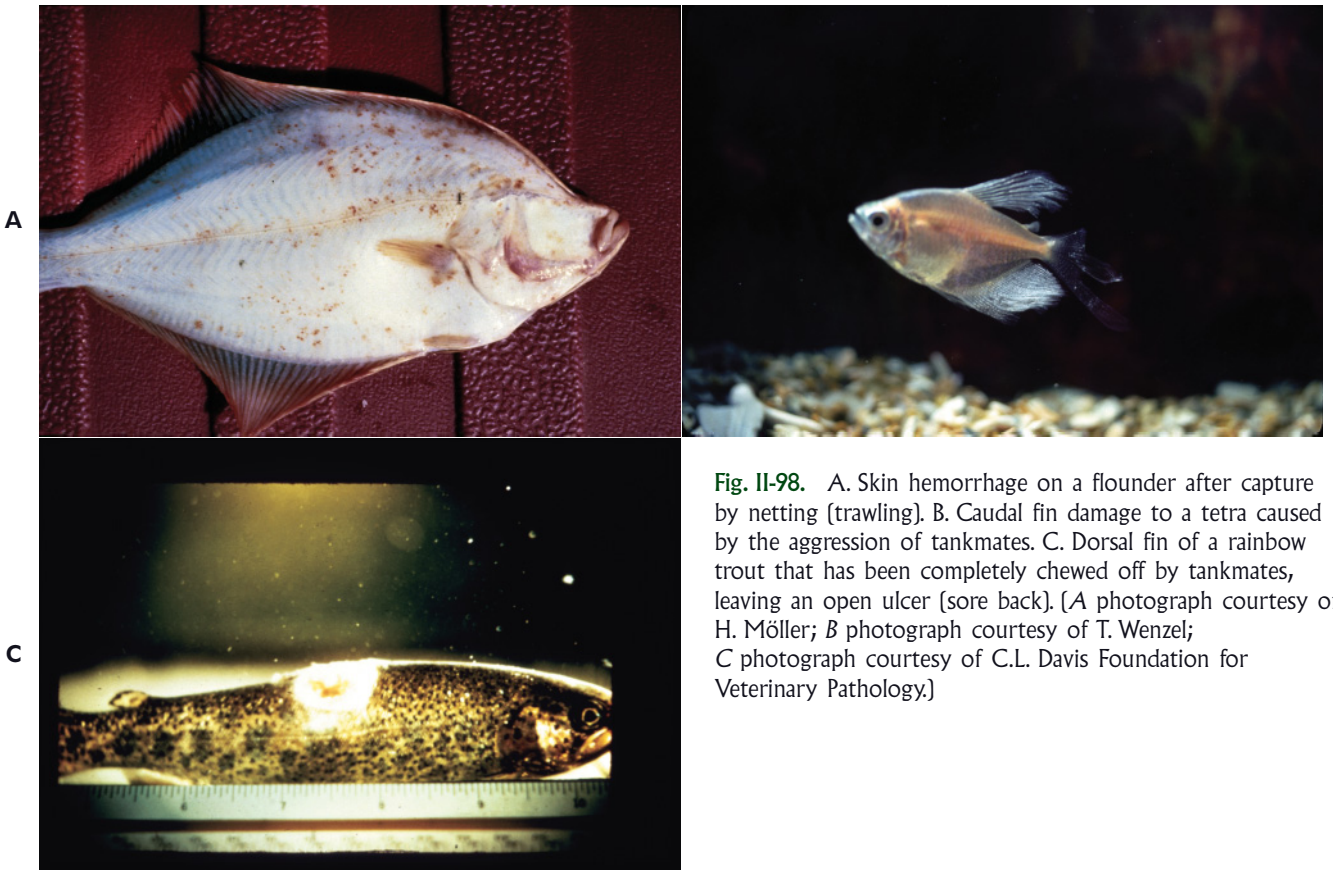


Fig. II-98. A. Skin hemorrhage on a flounder after capture by netting (trawling). B. Caudal fin damage to a tetra caused by the aggression of tankmates. C. Dorsal fin of a rainbow trout that has been completely chewed off by tankmates, leaving an open ulcer (sore back). (*A* photograph courtesy of H. Möller; *B* photograph courtesy of T. Wenzel; *C* photograph courtesy of C.L. Davis Foundation for Veterinary Pathology.)

territories. Thus, this is usually not a problem in species that are not territorial (e.g., neon tetras). However, some species, such as some barbs, tend to nip fins of slower moving species (Fig. II-98, B). Aggression-induced lesions can also result from courtship rituals.

Most territorial aquarium species will almost always form dominance hierarchies, unless the fish are crowded to the extent that territories cannot be successfully maintained by any one fish. In the latter case, aggression will not be a problem. However, in most tanks, fish will be in a low enough density to allow dominance hierarchies to form.

Salmonids are territorial and when overcrowded or underfed, will nip fins, especially the dorsal fin. This aggression may result in the entire loss of the fin, with formation of a large open wound on the back (sore back; Fig. II-98, C). Channel catfish are relatively nonaggressive, except when sexually mature.

PREDATION

Piscivorous birds, including anhingas, diving ducks, grackles, ospreys, eagles, cormorants, pelicans, herons, and egrets, can consume extremely large amounts of fish (up to 450 grams [1 lb] of fish per day). Anhingas and cormorants are serious problems in catfish and baitfish ponds. Herons can also damage fish when attempting to capture them. Piscivorous birds not only feed on fish but some also will consume fish feed; some also carry damaging parasites (e.g., see PROBLEM 58).

Many piscivorous insects will eat fish eggs or larvae. Important predators include the hemipterans (true bugs; e.g., water scorpion [*Ranatra* sp.], water bugs [*Belostoma* spp. and *Lethocerus* spp.], and backswimmer [*Notonecta indica*]), the coleopterans (predaceous diving beetle [*Cybister* sp.]), and the odonates (dragonflies). The true bugs and beetles must breathe air, while the nymph stages of dragonflies have gills. Adult dragonflies feed on larger fish.

Tadpoles are not piscivorous, but rather compete with fish for food. Frogs are highly carnivorous. *Hydra*, a small coelenterate, can kill small fry in aquaria. It is one of the most common problems when raising (freshwater) larval fish, especially when feeding brine shrimp, which acts as a food source for the hydra (R. Goldstein, personal communication). Certain copepods can prey on very small fry immediately after hatching (Valderrama 1999), and polychaete worms (bristleworms) may prey on small fish in marine aquaria.

CANNIBALISM

Many fish are cannibalistic and will eat their tankmates if given a chance. This can be a serious problem in young, rapidly growing fish (i.e., fry, fingerlings), where there is often a large difference in individual growth rates. The

two principal causes of sibling cannibalism are genetic and behavioral (Hecht and Pienaar 1993). Genetic forms are caused by size variation in a cohort; genotypic differences cause variation in individual growth rates. Behavioral forms are induced by environmental limiting factors. Important food fish aquaculture species displaying cannibalism include walking catfish, snook, barramundi, walleye, striped bass, European sea bass, nebulosus sea trout, chub mackerel, Atlantic cod, European eel, common carp, Northern pike, yellowtail, and dolphin (Hecht and Pienaar 1993).

CONFINEMENT

Trauma may occur when fish are gathered in a net (Fig. II-98, A) or as a sequela of transport. Close confinement may lead to abrasions from sharp fin spines and puncture wounds. Idiopathic corneal edema, which may lead to ulceration, occurs when some fish are transported (Brandt et al. 1986). Corneal abrasions and ulcerations are common sequelae of trauma, especially in large individuals; this can result not only from fighting, but also from bumping into sharp objects, such as rocks or coral, in an aquarium. Ophthalmic trauma, which can eventually lead to phthisis bulbi, is common in large aquarium fish.

LIGHT

Excessive ultraviolet (UV) radiation causes sunburn in salmonids (Bullock and Roberts 1979). Common in midsummer in northern latitudes, it is usually seen in small 2- to 3-inch fingerlings that are moved from indoors to outdoors, clear water rearing units. There is low mortality, but there may be high morbidity. Damaging UV radiation can penetrate up to 1 meter in clear water (Bullock and Roberts 1979). Sunburn has also been observed in ornamental carp (Cecil 2001). Excessive light may also cause eye damage (Piper et al. 1982; Whitaker 2001).

Fish exposed rapidly to bright light may be startled. Salmon smolts reared in subdued light may burrow into the base of nets when transferred to net-pens, damaging their heads (e.g., skin loss) (Grant 1993).

ELECTRICITY

Electrical shock can damage fish from electrocution (Langdon 1988), such as a lightning strike. Electroshock from electrofishing gear can cause serious damage to both the spinal column (fractures, compressions) and muscles (hemorrhage) of several fish species. There appears to be a relationship between the number of electrical pulses per unit time and the number of injured fish (Snyder 1995). Electrofishing over spawning or nursery grounds can damage developing embryos and fry (Dwyer et al. 1993).

Clinical Signs/Diagnosis

Traumatic lesions can grossly mimic lesions with an infectious etiology. The history is extremely important in making a diagnosis of trauma. There should be no pathogens in fresh traumatic lesions, but older lesions are often secondarily infected. Uninfected, traumatic lesions that are caused by aggression often have little hemorrhage (Fig. II-98, B), but there are exceptions. In salmonids, dorsal fin damage caused by aggression (Fig. II-98, C) can appear grossly identical to sunburn lesions.

When cannibalism is occurring, the actual observation of fish feeding on tankmates may be missed. But, cannibalism is strongly suggested by an unexplained decrease in numbers of fish in a healthy population, along with a size variation in the population that is large enough to allow the larger fish to consume the smaller fish. The larger fish often are much larger than the average fish in the population and often will have a full stomach because of constant feeding. Presence of piscivorous birds on or near pond banks and live or dead fish with puncture wounds is strongly suggestive of bird feeding activity.

Treatment

MEDICAL

In aquarium fish, if lesions are small, medical treatment is often unnecessary, so long as the affected fish is isolated and watched closely for secondary wound infections. However, ophthalmic lesions should be treated aggressively to avert blindness. Ophthalmic and other focal lesions can be treated with topical antiseptics or antibiotics (see "**Pharmacopoeia**"). More serious or extensive lesions should be treated with systemic antibiotics. Other medications may also be needed, depending on the type of secondary infections present. In salmonids, infected wounds should be treated for the specific infection.

Environmental

AGGRESSION

In freshwater aquarium fish, aggression can be reduced by choosing compatible tankmates (community-tank fish) (see p. 5), stocking fish of about the same size (so that one large fish does not bully the others), keeping at least five individuals of one species (so that aggression is not directed against only one submissive individual), and providing adequate hiding places (e.g., rocks, flower pots) for submissive individuals. Also, it may help to feed lightly when introducing a new fish and to place the new fish in a clear plastic box for up to several days, which may accustom the tankmates to the new fish's presence.

The great majority of marine aquarium fish are territorial and thus considerable care should be taken in choosing compatible tankmates. According to Bower (1983), "a (marine) aquarium should contain only one individual of a particular species, of a particular body shape and of a particular color or pattern of colors," to reduce recognition, and thus attack, of tankmates. Aggression in salmonids is remedied by reducing stocking density and/or by increasing feeding rate.

CANNIBALISM

Cannibalism can be reduced by providing more frequent feedings to growing fish, so that individual growth rates

are more uniform. Frequent grading should also be done to remove large individuals from the population. Reducing density may also reduce cannibalism.

PREDATION

Covering culture systems with bird netting or other physical barriers (available from aquaculture supply companies) will eliminate bird problems but is often economically unfeasible. Perimeter fencing around ponds can provide protection from wading birds, such as herons. Scare tactics (e.g., dummies, devices that produce loud noises; available from aquaculture supply companies) often work for a while, but then the birds become accustomed. Scare tactics cannot be legally used against threatened or endangered species (e.g., bald eagle). Most piscivorous birds are migratory species, and in the United States and elsewhere are protected by law from being killed. However, farmers in the United States can obtain a depredation permit from the U.S. Fish and Wildlife Service, which allows a limited number of defined species to be killed. This is done to reinforce the effectiveness of the scare tactic that is used as the primary deterrent to bird feeding. Once the permit is issued, the USDA Office of Animal Damage Control provides assistance with the control effort. Littauer (1990), Stickley (1990), Avault (1995), and Cowx (2003) provide more details on controlling birds.

Predaceous insects can be controlled by (Avault 1995)

1. Reducing aquatic weeds, which provide a breeding habitat
2. Filling ponds with water just before stocking fish larvae to prevent a buildup of predaceous insects
3. Filling ponds at times of the year when insects are less common
4. Stocking larger individuals into ponds, since larger fish are less likely to be eaten
5. Treating ponds with 0.25 ppm methyl parathion several days before adding fish.
6. Treating the pond surface with oil to prevent the insects from breathing. Spraying along the pond edge when there is just enough breeze to carry the oil slick across the pond. Since some air-breathing insects can remain under water for over 1 hour, several applications may be needed. Diesel fuel has been most commonly used. However, recent data suggest that other, less toxic oils are at least as effective against backswimmer. Linseed oil, unrefined cottonseed oil, or cod liver oil were all effective when applied at 2.2 gallons per surface acre (21 liters per surface hectare).

Avault (1995) provides more details on controlling insects. Some tadpoles can be killed with formalin (Helms 1967). Some data suggests that gouramies will eat hydra (Sugars 1936). Flubendazole is a highly effective treatment for controlling hydra (R. Goldstein, personal communication). *Aiptasia* anemones can be eliminated from a reef tank by individually injecting them with supersaline water, lemon juice or highly concentrated kalkwasser (calcium hydroxide solution). The only reef-safe fish that will also eat *Aiptasia* is the copperband butterfly *Chelmon rostratus*. Peppermint shrimp (*Lysmata wurdemanni*), and the white-spotted hermit crab (*Dardanos mestigos*) also will usually selectively prey on them but the only completely reef-safe organism is the nudibranch *Berghia verrucicornis*. However, the latter must be removed from the tank when all are consumed as it will eat nothing else and will starve to death (Fatherree 2004).

Polychaete worms (bristleworms) can be removed by (Knop 2004a)

- Placing clam meat in pantyhose on the substrate overnight (the bristles get caught in the hose)
- Using a commercial bristleworm trap (perforated PVC tube with clam meat (the worms enter but do not leave)
- Using natural shrimp predators (*Stenopus hispidus*, *Lysmata grabhami*, *L. amboinensis*)

MISCELLANEOUS TRAUMATIC LESIONS

Fish with sunburn lesions usually respond quickly to shading. To avoid iatrogenic trauma, routine procedures (e.g., grading, weighing, vaccination) should be done at one time, so that fish can be handled as few times as possible.

DENSITY INDEX

Density is important because, even at densities that are considered adequate in terms of oxygen consumption, fish can get sick (Piper et al. 1982). For example, rainbow trout should be kept at densities measured in lb/ft^3 that are no greater than one-half their length in inches (e.g., 2-inch fish should not be kept at a density greater than $1 lb/ft^3$); 4-inch fish should be kept at a density of no more than $2 lb/ft^3$, etc.). Thus, a density index can be calculated. This assumes that the density index stays constant as the fish increase in length, but larger fish often tolerate higher densities relative to their length; however, this is still a good rule of thumb. Note that maximum optimal density varies with fish species and culture environment.

PROBLEM 99
Genetic Anomalies

Prevalence Index

WF - 2, WM - 4, CF - 3, CM - 3

Method of Diagnosis

History and clinical signs

History

Excessive inbreeding

Physical Examination

Skeletal abnormalities; cataracts; poor growth; other anomalies may be present

Treatment

Introduce better genetic stock into the population

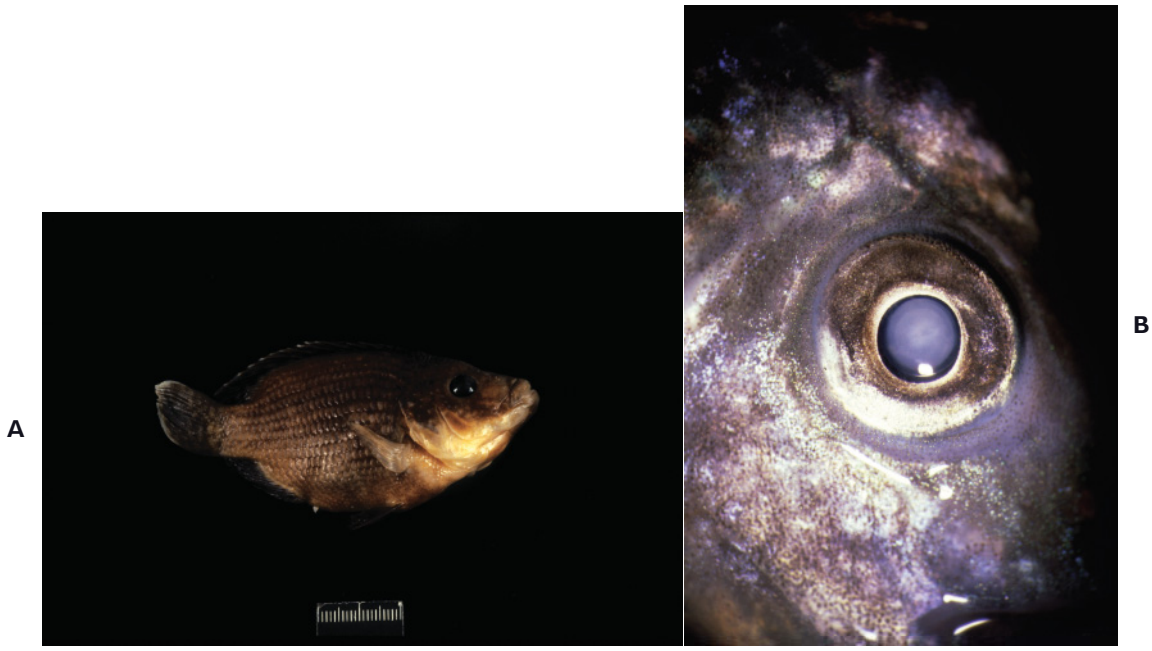


Fig. II-99. A. Stumpbody in tilapia, an inherited malformation (Tave et al. 1982). Compare with fish having a normal conformation (see Fig. I-15, *C*). B. Inherited cataract in Mozambique tilapia. (*A* photograph by J. Stevens and E. Noga; *B* photograph by D. Wolf and E. Noga.)

COMMENTS

Epidemiology/Clinical Signs

Genetic anomalies are most common in captive-bred fish, where such defects can escape natural selection. Twins, cross-bites, pugheads, stumpbody (Fig. II-99, A), spinal curvature (see Fig. I-3, F), double fins, and opercular deformities are some of the most common anomalies. Ophthalmic defects may also occur (Noga et al. 1981; Fig. II-99, B). Malformations are most likely to appear in young fish and may lead to early death. A certain number of malformations will occur in even normal broods, but the incidence of malformed individuals should be low (often less than 1%). Some anomalies are desirable, such as longer fins or albinism. These traits are often selected for in-aquarium fish. Relatively little is known about the genetic predisposition of various strains of fish to develop specific diseases, although there are some data on this phenomenon (Breck et al. 2005).

Diagnosis

There are many possible causes for developmental anomalies, and determining a genetic link is often accomplished by ruling out other possible causes: improve the husbandry conditions that may be predisposing to these problems; pay special attention to proper types and amounts of feed (see PROBLEM 89), proper density, adequate biological filtration and oxygenation, and frequent water changes. Exposure to teratogenic chemicals, such as malachite green and organophosphates, should also be ruled out.

Treatment

Stocks carrying deleterious genes should be replaced or out bred to dilute the undesirable gene. See Tave (1993) and Lutz (2001) for details on selecting for or eliminating certain genetic traits.

CHAPTER 14

PROBLEMS 100 through 102

Rule-out diagnoses 3: *Presumptive* diagnosis is based on the absence of other etiologies combined with a diagnostically appropriate history, clinical signs, and/or pathology. *Definitive* diagnosis is not possible since the etiology is unknown (idiopathic).

100. Lateral line depigmentation
101. Senescence
102. Miscellaneous important idiopathic diseases

PROBLEM 100

Lateral Line Depigmentation (LLD; Freshwater Hole-in-the-Head Syndrome, Freshwater Head and Lateral Line Erosion [FHLLE], Marine Hole-in-the-Head Syndrome, Marine Head and Lateral Line Erosion [MHLLE])

Prevalence Index

WF - 1, CF - 4, WM - 1, CM - 4

Method of Diagnosis

History and clinical signs

History/Physical Examination

Various numbers of pin-head size to larger depigmented foci, especially near the lateral line of head or flanks; cachexia; chronic, low mortalities

Treatment

1. Improve environment and nutrition
2. Appropriate antibiotic

Comments

Epidemiology/Pathogenesis

Lateral line depigmentation (LLD), previously referred to under a number of terms including freshwater hole-in-the-head syndrome, freshwater head and lateral line erosion (FHLLE), marine hole-in-the-head syndrome, and marine head and lateral line erosion (MHLLE), is a chronic dermatopathy affecting mainly tropical freshwater and marine aquarium fish. LLD most commonly affects certain tropical aquarium fish, especially members of the freshwater families Anabantidae, Belontidae, and Cichlidae (especially discus, oscars, and other large South American cichlids [e.g., jurupari]), as well as members of the marine families Acanthuridae (tangs) and Pomacentridae (angelfish). A grossly identical lesion has also been rarely observed in some cultured or wild food fish (Möller and Anders 1986; Baily et al. 2005; Corrales et al. 2009).

This important, chronic, idiopathic syndrome presents as mild to severe, focally depigmented skin along the lateral line of the head and/or flank. Lesions usually begin on the head as shallow, pinpoint foci that expand in size, depth, and surface area (Fig. II-100, A). Advanced lesions may be deep. Lesioned fish can behave normally for quite some time but usually eventually become anorexic and lethargic.

Most descriptive accounts of this syndrome are based on the popular aquarium literature, and there are very few published scientific reports. LLD has been linked to many possible etiologies. The aquarium literature often attributes LLD to diplomonad flagellates (Bassleer 1983a) (PROBLEM 73). One hypothesis is that a latent, intestinal diplomonad infection spreads by both extension and hematogenously to the gall bladder, peritoneal cavity, spleen, kidney, and associated vasculature. In later stages, the classical hole-in-the-head lesions appear, first as pinpoint lesions (Fig. II-100, A) that may discharge small, white "threads" of material containing the parasites. The lesions then expand and coalesce, producing large crateriform lesions (Fig. II-100, B) that may become secondarily infected with bacteria or fungi. The ultimate cause of death is then presumed to be secondary microbial infections. Diplomonad flagellates are common gut parasites of many freshwater fish species that are affected by LLD (e.g., cichlids and anabantids) and can spread to other tissues from the gut (Ferguson and Moccia 1980). However, while *Spironucleus vortens* has been isolated in some discus and freshwater angelfish from both LLD lesions and the intestine (Paull and Matthews 2001), its relationship to LLD is open to question.

The only other microbe that has been associated with LLD is a reovirus-like agent isolated from the viscera of a moribund marine (Koran) angelfish (Varner and Lewis 1991), but there is no evidence that any infectious agent is responsible for the LLD lesion (Blasiola 1989; Baily et al. 2005; Corrales et al. 2009). Some claim that LLD may be caused by a mineral imbalance that results in skeletal damage leading to the pitting lesions. They further speculate that heavy concentrations of flagellates

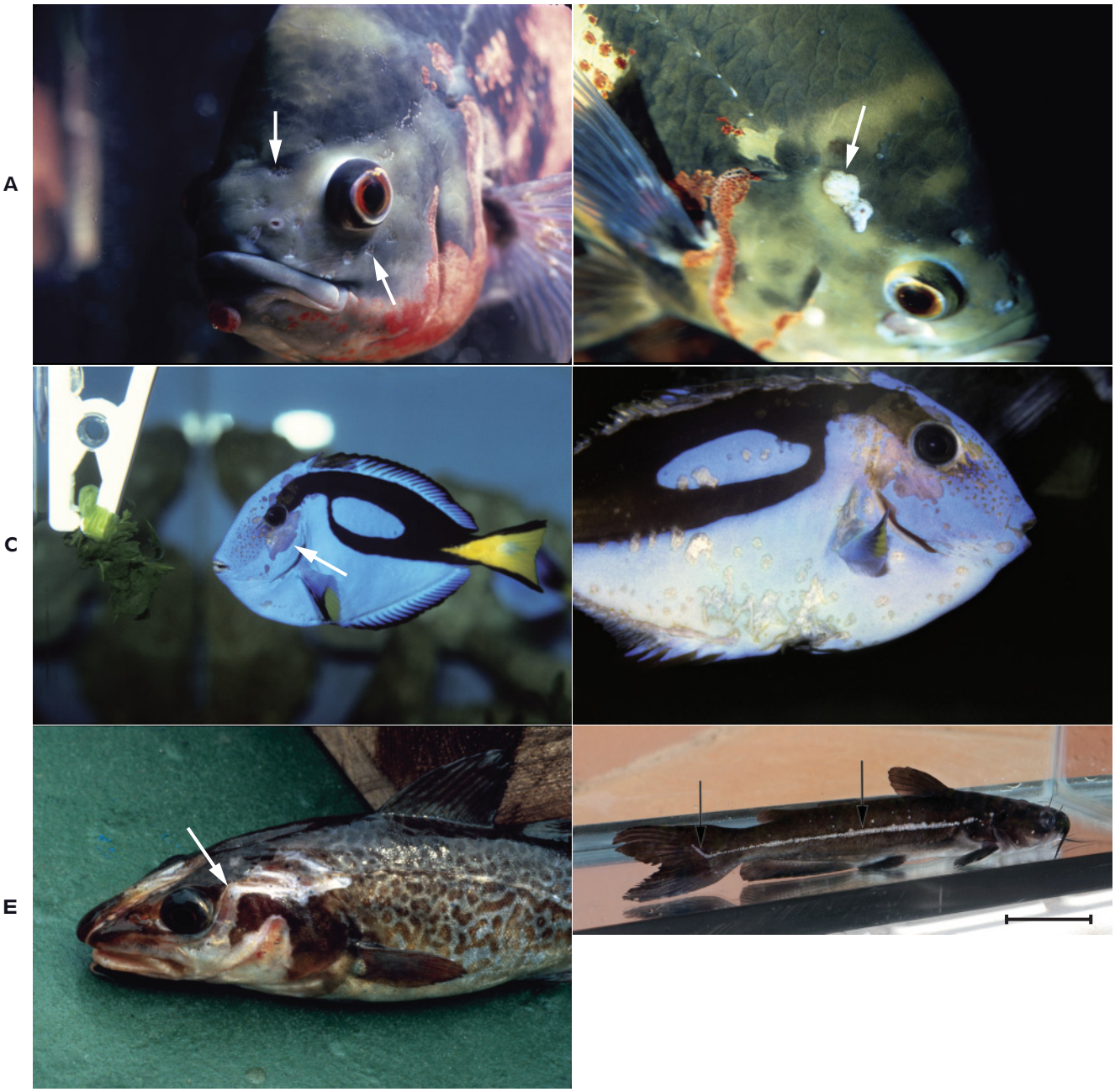


Fig. II-100. Lateral line depigmentation (LLD). A. Oscar with early stage of LLD (*arrows*). B. Oscar with more advanced stage of LLD (*arrow*). C. Powder blue tang with mild to moderate LLD (*arrow*). D. Powder blue tang with severe LLD affecting entire body. E. Atlantic cod with LLD (*arrow*). F. Channel catfish with LLD showing wide, overlapping foci of depigmentation overlying the lateral line of the flank and the caudal peduncle (*arrows*).

Continued.

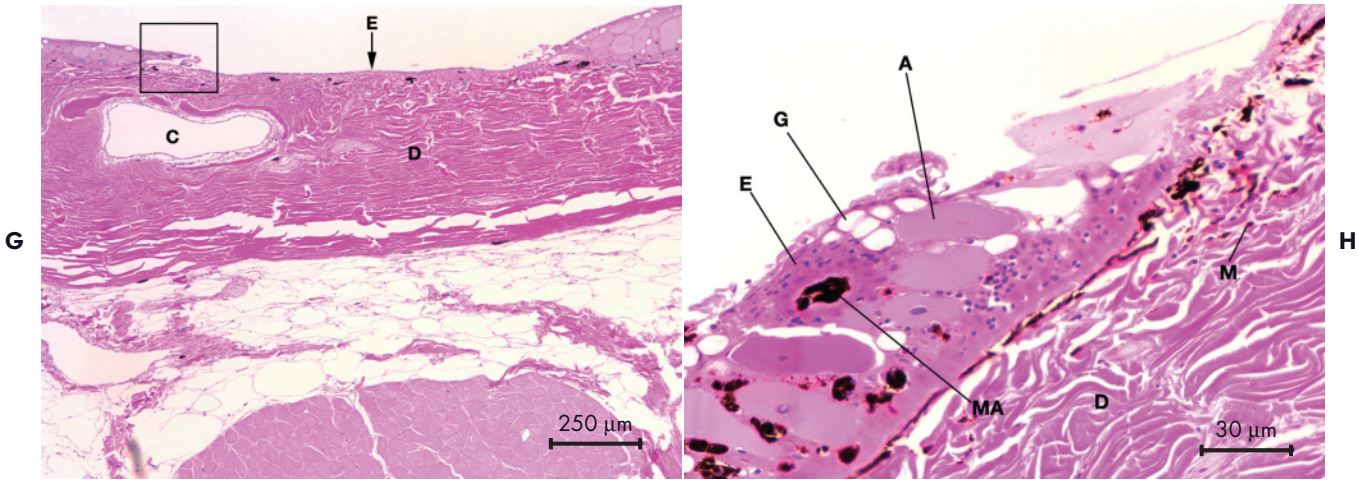


Fig. II-100.—cont'd. G. Skin above the lateral line canal (*C*) of an LLD-affected channel catfish showing extreme thinning of epidermis (*E*). *D* = dermis. Hematoxylin and eosin. H. Close-up of the box in *G* showing periphery of the LLD lesion with degenerate alarm cells (*A*) and melanocyte aggregation (*MA*). *G* = goblet (mucus) cell, *M* = melanocyte, *E* = epithelial cell. *D* = dermis. Hematoxylin and eosin. (*A* and *B* photographs courtesy of T. Wenzel; *C* and *D* photographs courtesy of S. Johnson; *E* photograph courtesy of H. Möller; *F*, *G*, and *H* photographs from Corrales et al. [2009].)

in the intestine can cause maladsorption, leading to the mineral imbalance (Untergasser 1991). Better documented scientific studies have linked inadequate nutrition with some LLD lesions, including inadequate vitamin C (Blasiola 1989) and fasting (Corrales et al. 2009). Induction of LLD is also linked to water quality (Baily et al. 2005), and there is evidence that either ozonation or certain types of activated carbon can induce LLD in marine fish (Stamper 2009).

While the pathogenesis of LLD is poorly understood, the anatomical distribution of the lesion (i.e., intimate association with the lateral line) suggests that it might be linked to changes in the function of this neurosensory organ. A change in intercellular signaling from neuromasts to epidermis might result in thinning of the skin due to lack of nerve input, also suggesting that decreased epidermal thickness in some LLD lesions might result from atrophy rather than erosion (Corrales et al. 2009). Interestingly, grossly similar lesions (termed lateral line necrosis) have been seen in both feral and cultured Atlantic cod, a cold water species (Fig. II-100, C) (Möller and Anders 1986); nerve fibers running from the destroyed lateral line to the brain were inflamed, and their medullary ganglia were degenerated (Naeve 1968).

Diagnosis

Diagnosis of LLD is based on the observance of typical, depigmented (often pitted and symmetrical) lesions associated with the lateral line or flanks (Fig. II-100, A and B). It is advisable to culture the lesions for bacteria, since some fish respond at least partially to antibiotic therapy (E.J. Noga, unpublished data).

While the gross presentation of LLD is highly similar among various fish (Fig. II-100), the histopathology of LLD lesions is highly variable. In some cases, such as palette tang and channel catfish, the epidermis is thin and often reduced to a one-cell-thick layer over the lateral line. Melanocytes are depleted at the dermo-epidermal junction, forming aggregates in the epidermis, but there is little if any inflammation (Blasiola 1989; Corrales et al. 2009). However, in Murray cod, the epithelium is severely hyperplastic, with inflammation and necrosis overlying the sensory canals.

The variable pathology of LLD, along with the diversity of risk factors associated with the development of the LLD lesion, indicates that the clinical signs presented by these various fish are probably not due to a single disease, nor even a single syndrome. Also, since some lesions are not erosive but rather are hyperplastic, it is more appropriate to refer to fish as being affected by LLD, since this is a more accurate description that encompasses the definitive diagnostic lesion of all affected fish. Thus, LLD is a clinical sign in response to any of a number of stressors that can lead to this gross lesion, and LLD appears to be analogous to a skin ulcer (i.e., a gross manifestation of a general host response) rather than being a specific disease or even a syndrome.

Treatment

Since LLD is a clinical sign rather than a specific disease entity, making recommendations for treatment is difficult. Treatment of LLD is purely empirical and relies on elimination of possible initiating causes. While most published treatments in the freshwater aquarium literature

have focused on controlling diplomonad flagellates, there is no proof that this agent is even present in most lesions (Ferguson 1988; E.J. Noga, unpublished data). Some types of stress, such as overcrowding, poor water quality, or poor nutrition, appear to play a role in some LLD lesions. Thus, there should be a thorough evaluation for possible nutritional and/or environmental stress, followed by improvements, such as reducing overcrowding, performing frequent water changes, and providing a varied and balanced diet. Some claim that providing a calcium/phosphorus/vitamin D supplement to the diet can cure fish (Untergasser 1991).

Changing the brand of activated carbon used in filters for marine aquaria may help (T. Frakes, personal communication), and stopping ozonation might also be advisable. Stray voltage is also suspected to be a cause of LLD in marine aquarium fish (Johnson 1993a); it may also cause other neurological signs, including disorientation. It is corrected by determining if stray voltage is present (i.e., an electrical device is not properly grounded) and eliminating the cause. Instead of trying to find the stray current, a grounding device to correct the problem can be installed (e.g., Solution Ground™, Sandpoint). If such manipulations are unsuccessful, another cause should be suspected. Since larger, older fish are often afflicted, LLD may sometimes be a sequela of decreasing immunocompetence in aging individuals. Marine fish can recover from LLD but often have permanent scarring (Hemdal 1989) (Fig. II-100, C).

PROBLEM 101
Senescence

Prevalence Index
WF - 4, WM - 4, CF - 4, CM - 4
Method of Diagnosis
History and clinical signs
History/Physical Examination
Cachexia, possibly other clinical signs
Treatment
None

COMMENTS
Epidemiology/Pathogenesis
Unless maintained as broodstock, food fish are normally harvested well before the end of their natural life span. However, while there are no firm data, most aquarium fish held in captivity probably also do not usually survive the length of their natural life span; in the latter case, it is probably due to succumbing mainly to infectious disease. The natural life span of fish varies tremendously (Table II-101).

Tetras, livebearers, and minnows are relatively short-lived (usually not more than 5 years and maybe much shorter). Certain members of the minnow family Cyprinodontidae, known as annual fish, live naturally in temporary pools and ponds that completely dry up during the dry season. These fish only live for less than 1 year even in the aquarium. Cichlids, catfish, and freshwater "sharks" (*Labeo* and related genera) are relatively long-lived (10 years is not unusual for many species). At the other end of the spectrum, koi can probably live at least 50 years, and there is at least one report of an individual supposedly living over 200 years (Boruchowitz 2004).

Table II-101. Reported maximum life spans of various tropical freshwater and marine fish (compiled from www.aquariacentral.com, http://animaldiversity.ummz.umich.edu, and http://freshaquarium.about.com).

Family	Examples	Life span (years)
Freshwater		
Cyprinodontidae	Killifish	1–2
Anabantidae	Gouramies, bettas	2.5–7
Poeciliidae	Guppies, platies	3–5
Small Cyprinidae	Small barbs, danios	4
Small Characidae	Tetras	4–6
Melanotaeniidae	Rainbowfish	5
Large tropical Cyprinidae	"Sharks," flying fox	7–10
Large tropical Characidae	Pacus, *Leporinus*, piranha	7–10
Callichthyidae	Armored catfish	7–15
Cichlidae	Oscars, convicts, angelfish	7–18
Cobitidae	Loaches	10
Coldwater Cyprinidae	Goldfish, koi	10–30+
Marine		
Syngnathidae	Sea horses	2–5
Gobiidae	Gobies	2–7
Labridae	Wrasses	3–5
Pomacentridae	Clownfish, damselfish	5–10
Chaetodontidae	Butterflyfish, angelfish	5–12
Scaridae	Parrotfish	5–20
Acanthuridae	Tangs	5–30
Osctraciidae	Boxfish, cowfish	6–12
Balistidae	Triggerfish	6–10
Scorpaenidae	Lionfish	11–16
Platacidae	Batfish	11–17

PROBLEM 102
Miscellaneous Important Idiopathic Diseases

Prevalence Index
See specific disease
Method of Diagnosis
Rule-out of other problems
History/Physical Examination
See specific disease
Treatment
See specific disease

COMMENTS
See Table II-102 and Figure II-102.

Table II-102. Miscellaneous important idiopathic diseases of fish. Also see PROBLEM 76 for idiopathic lesions associated with epidermal hyperplasia.

Disease / pathogen	Hosts	Geographic range	Morbidity / mortality	Diagnostic features	References
Ulcerative dermal necrosis	Atlantic salmon, brown trout	Great Britain, Ireland, France, Sweden, Portugal	Acute / subacute / chronic	Shallow grey area that progresses to deep ulcers; progressive focal pemphigoid to bullous lesions; often secondary bacterial, fungal pathogens, virus-like particles in some lesions	Lounatmaa and Janatuinen (1978) Roberts (1989a)
Pacific cod ulcerative epidermal hyperplasia	Pacific cod	Bering Sea	Chronic	1–50 mm ulcers of raised, circular ring-shaped lesions; hypertrophied epithelial cells; herpesvirus-associated	McArn et al. (1978) McCain et al. (1978)
Atlantic cod ulcus syndrome	Atlantic cod	Baltic Sea	Subacute / chronic	2 mm papules producing 2–8 skin cm ulcers leading to depigmentation and scarring; iridovirus-associated; peak prevalence in fall; pollution-associated	Larsen and Jensen (1982)
Ambicoloration (Fig. II-102, B)	Turbot, halibut, other flatfish	Europe, United States	None	Various-sized areas of skin depigmentation; possibly nutrition-related; appearance reduces value	Möller and Anders (1986)
Gill necrosis	Common carp, eel, trout	Europe, Soviet Union	Acute to chronic	Associated with high unionized ammonia; Acute: neurological signs; Chronic: gill edema progressing to epithelial necrosis and hyperplasia; branchitis; iridovirus isolated but unlikely cause; often secondary invaders; diagnosed by measuring blood ammonia and ruling out other causes of gill damage	Kovacs-Gayer (1984) Schäperclaus (1991b)
Cardiomyopathy syndrome (CMS)	Atlantic salmon	Norway, Scotland, Denmark, Faroe Islands	Chronic	Adult fish after 12 months in seawater; often good body condition, showing few clinical signs before sudden death; transmissible and suggestive of a viral etiology; Gross: edema (raised scales, exophthalmos, ascites), fibrinous peritonitis, blood clots on liver and on / in heart, cardiac tamponade; Histopathology: thickening of myofibers with loss of striation; mononuclear epicarditis and endocardial cell proliferation; heart lesions tend to be severe with widespread degeneration with inflammatory cell and macrophage invasion of the entire spongy ventricular muscle; liver lesions are typical of congestive heart failure with significant sinusoidal congestion and necrosis; nodavirus-like particles sometimes observed; Dx: typical gross and histopathological lesions; Ddx: heart and skeletal muscle inflammation (PROBLEM 102), alphavirus diseases (PROBLEM 87), infectious salmon anemia (PROBLEM 82)	Brun et al. (2003)
Heart and skeletal muscle inflammation (HSMI)	Atlantic salmon	Norway	Chronic	Occurs up to 9 months posttransfer to sea. Epi-, endo-, and myocarditis and myocardial necrosis, as well as myositis and necrosis of skeletal muscle; transmissible via cohabitation Dx: typical gross and histopathological lesions DDx: cardiomyopathy syndrome (PROBLEM 102), alphavirus diseases (PROBLEM 87)	Kongtorp et al. (2004)
Idiopathic gastric distension (bloat)	Salmonids (rainbow trout Chinook and coho salmon most susceptible)	Norway, British Columbia (Canada), New Zealand, Chile	Chronic	Usually fish in seawater; enlarged abdomen and dilated stomach with varied amount of water or oil; distended stomach may cause peritoneal wall atrophy and compressed viscera; increased serum sodium and osmolality; may also be swim bladder distention and inflammation Tx: reduce food intake, modify diet, reduce stress	Anderson (2006)

Continued.

Table II-102. Miscellaneous important idiopathic diseases of fish. **(cont'd)**

Disease / pathogen	Hosts	Geographic range	Morbidity / mortality	Diagnostic features	References
Idiopathic bloat	Goldfish	United States	Chronic	Especially common in round-bodied goldfish strains. Ddx: swim bladder torsion, swim bladder inflammation, anatomical anomaly of the swim bladder, enteritis, neoplasia; do not confuse with egg-bound fish or fish that have recently eaten a large meal. Tx: discontinue floating feeds (flakes or pellets) as might contribute to bloating; feeding one lightly crushed green peas (canned or cooked) once daily is curative in some fish.	Lewbart (2000)
Rainbow trout gastroenteritis (RTGE) / summer enteritis	Rainbow trout	France, United Kingdom, Spain, Italy	Chronic	Disease at >15°C in freshwater; swollen appearance, light color, striping of flanks, gastric dilation, congestion of pyloric cecae; infectious agent suspected but not proven (large Gram-variable rods ["*Candidatus arthromatus*"] in gut, especially pyloric cecae); adding salt to feed may be palliative due to protein-losing enteropathy.	del Pozo et al. 2009
Red fillet syndrome	Channel catfish	United States	Acute	Punctate to ecchymotic to diffuse pink or red foci in muscle; associated with acute, sublethal hypoxia, such as due to environmental hypoxia (PROBLEM 1) or PGD (PROBLEM 64); appearance causes rejection by processors	Johnson (1993b)
Systemic granuloma / visceral granuloma / Malawi bloat (Fig. II-102, A)	Salmonids gilthead sea bream African Rift Lake cichlids	United States, Canada, Israel	Chronic	Multiple granulomas in viscera, often surrounding calcium deposits; may be associated with mineral deposition in kidney in salmonids (nephrocalcinosis, see PROBLEM 90); cases in gilthead sea bream associated with hypertyrosinemia; nutrition appears to influence disease, but precise cause unknown; most cases in cichlids probably due to protozoan infection (see PROBLEM 75)	Paperna et al. (1981a) Landolt (1975) Noga (1986a)
Net-pen liver disease (NLD)	Atlantic salmon	Washington (United States) British Columbia (Canada)	Chronic	Affects fish in first year at sea, usually summer; mortality to 90%; liver small, friable, yellow; megalocytosis (diagnostic) and other hepatic lesions suggest hepatotoxin as a cause (algal toxin from resident natural foods?)	Kent (1990)
Osmotic cataracts	Atlantic salmon	Norway	Acute	Associated with rapid environmental change; possibly due to disturbance in osmoregulation ; lesions are reversible.	Breck and Sveier (2001)
Permanent cataracts	Atlantic salmon	Norway	Chronic	Usually in yearling (S1) and subyearling (S0) smolts 2–3 and 8–10 months, respectively, after transfer to seawater; associated with elevated temperature and rapid growth; affected by nutrition status	Wegener et al. (2001) Waagbø et al. (2003)

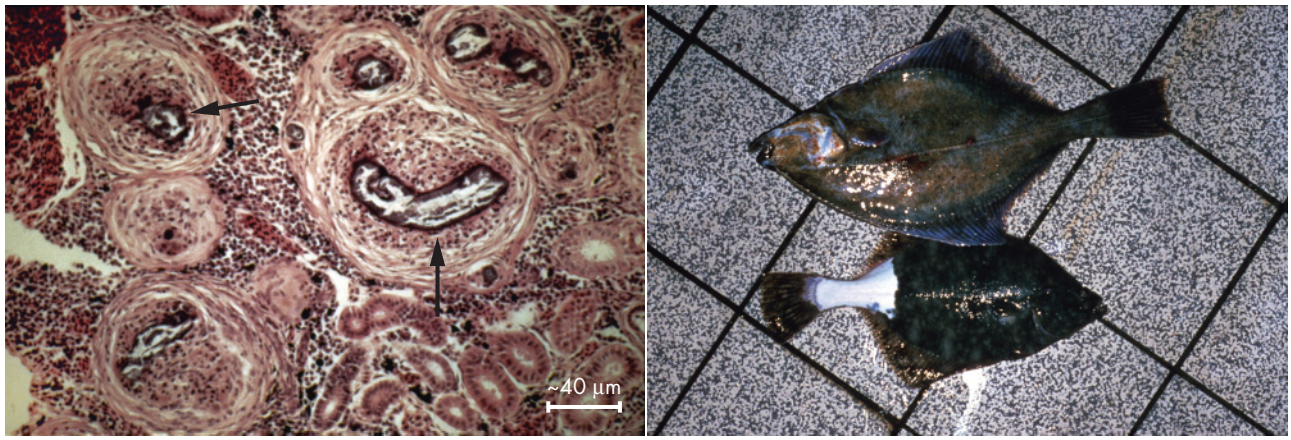


Fig. II-102. A. Visceral granuloma lesion in a salmonid. Note calcification and necrosis (*arrows*) in the center of the granuloma. Von Kossa. B. Ambicoloration in a flounder. (*A* photograph courtesy of C.L. Davis Foundation for Veterinary Pathology; *B* photograph courtesy of H. Möller.)

CHAPTER 15

PROBLEM 103

Diagnoses made by examination of eggs

103. Egg diseases

PROBLEM 103
Egg Diseases

Prevalence Index
See specific disease.

Method of Diagnosis
See specific disease.

History/Physical Examination
See specific disease.

Treatment
See specific disease.

COMMENTS
See Table II-103 and Figure II-103.

Table II-103. Diseases commonly affecting eggs. Note that this list does not include pathogens that may be transmitted asymptomatically in or on eggs.

Disease or condition	Host range	Diagnostic clinical signs	Prophylaxis/treatment	References
Gelatinous egg mass	Cyprinids, ictalurids, other species	Eggs stick together; increased secondary bacterial or water mold infection	Treat with sodium sulfite ("**Pharmacopoeia**") or other appropriate chemical. (see Table III-3).	
Dead eggs (bacterial infection, water mold infection, infertile egg)	All fish	Entire egg(s) opaque and/or soft (Figs. II-103, A, B); premature hatching; rupture of yolk sac	Address pertinent problem.	See PROBLEMS 34 and 37
Bacterial infection (*Pseudoalteromonas piscicida*)	Staghorn damselfish Clark's anemonefish	Embryos with opaque brain and spinal cord	None proven	Nelson and Ghiorse (1999)
Soft egg disease	Salmonids	Eggs become soft and flaccid during incubation because perforations in shell allow loss of fluid. May be due to bacterial infection or amoebic infestation	Antiseptic treatment may help. Maintain good hygiene in hatchery.	Warren (1981)
Parasite infection (*Kudoa ovivora*)	Tropical wrasses			See PROBLEM 69
Parasite infection (*Pleistophora ovariae*)	Golden shiner, fathead minnow			See PROBLEM 70
Parasite infection (*Ichthyodinium chabelardi*)	Atlantic sardine European anchovy Atlantic mackerel, gilthead sea bream, blue whiting, Atlantic cod, turbot, yellowfin tuna, leopard coral grouper	Primarily seen in sardines (Fig. II-103, C); parasitizes the yolk sac, filling it with parasites; depletes energy reserves and ruptures yolk sac after hatching; sardine eggs mainly infected during colder months; can be very high prevalence; complicated life cycle	None proven	Meneses et al. (2003) Stratoudakis et al. (2000) Mori et al. (2007) Skovgaard et al. (2009)
Parasite infection (*Polypodium hydriforme*)	Sturgeon, paddlefish	One of the few metazoan parasites that is intracellular; problem in caviar; may affect reproduction	None proven	Raikova (1994)
Blue sac disease	Salmonids; other species?	Yolk sac swollen and discolored	Reduce/prevent high ammonia or pH change.	
Coagulated yolk disease	Salmonids; other species?	White foci in the yolk sac	Probably water-quality problem	
Smothering	Salmonids; many other species	Eggs covered with debris	Excess turbidity	See PROBLEM 95
Physical shock	Salmonids; probably other species	Movement of eggs between just after fertilization to eyed stage	Avoid moving eggs during this time.	Post et al. (1974)
Light	Salmonids; probably other species	Too bright direct sunlight; exposure to short wavelength (blue white) emissions from artificial lighting	Minimize exposure.	Leitritz (1976)
Temperature shock	Salmonids; probably other species	Mortality	When acclimating shipped eggs, don't increase temperature more than 0.5°C/minute.	
Premature hatch	Salmonids; probably other species	Medication of eggs just prior to hatching	Don't medicate eggs at least 24 hr prior to hatching (at least 3 days for salmonids).	Post (1987)
Constricted yolk disease	Salmonids	Yolk sac becomes constricted, almost splitting in two	Idiopathic	
Gas bubble disease	Salmonids; many other species	Gas emboli in egg		See PROBLEM 11
Electroshock	Trout, probably other eggs susceptible	Damages eggs and reduces egg viability when female broodstock are shocked	Do not shock female broodstock close to spawning time.	Dwyer et al. (1993); also see PROBLEM 98

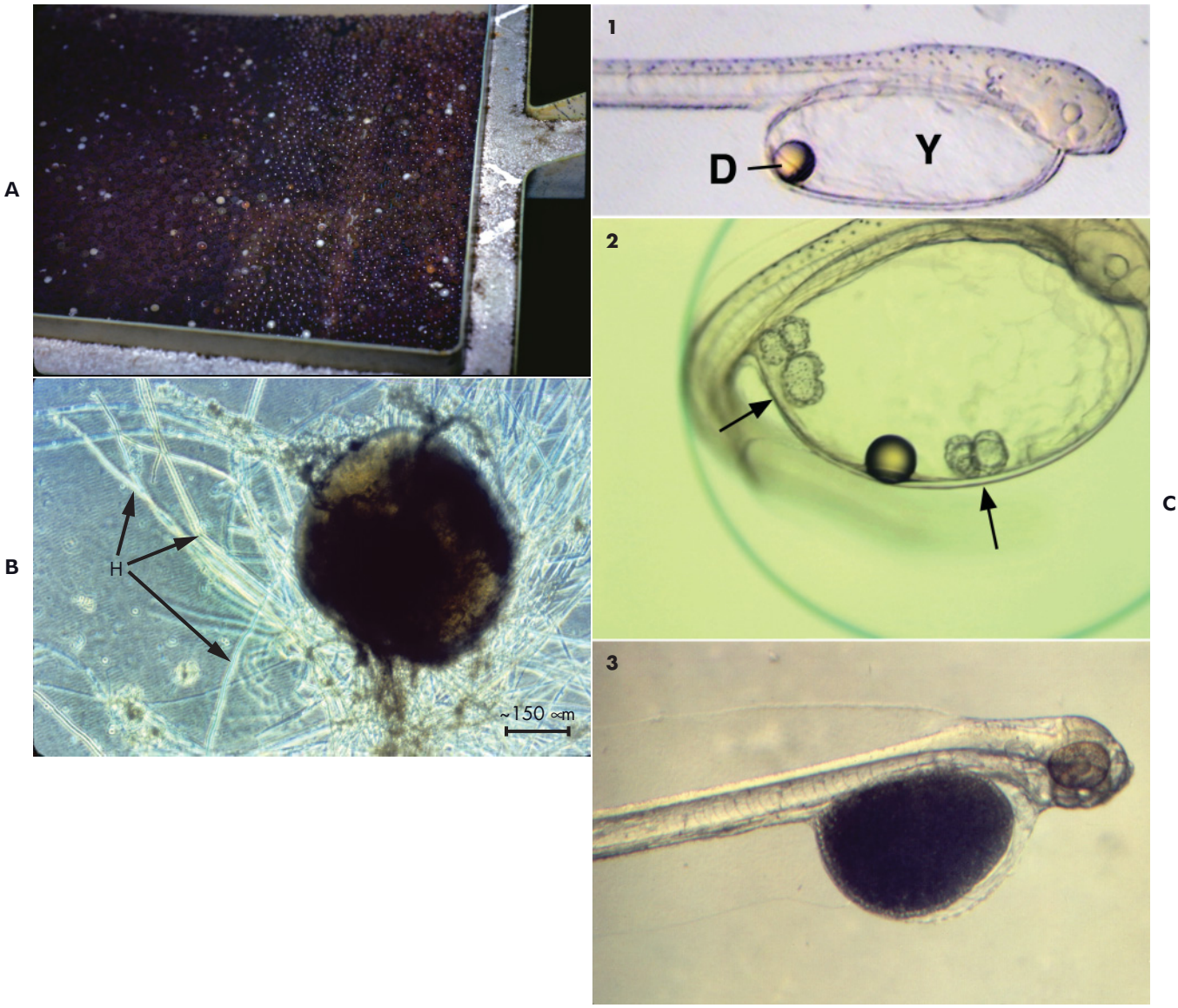


Fig. II-103. A. Rainbow trout eggs in an incubator tray. Viable eggs are brown; dead eggs are white because of precipitation of egg protein. B. A dead, water mold–infected egg of a Madagascar rainbow fish. Many hyphae (*H*) grow in and through the egg. C. *Ichthyodinium* infecting Atlantic sardine. (1) Normal larva (*Y,* yolk sac, *D,* oil droplet); (2) Early stage infection of embryonating egg having several primordial schizonts in the yolk sac (*arrows*); (3) Advanced infection of larva, with parasites filling the yolk sac. (*B* photograph courtesy of T. Wenzel; *C1* and C2 photographs courtesy of I. Meneses; *C3* photograph from Stratoudakis et al. 2000.)

PART III

METHODS FOR TREATING FISH DISEASES

CHAPTER 16

General Concepts in Therapy

TREATMENT GUIDELINES

When and How to Treat

The isolation or identification of a certain pathogen does not always warrant treatment. The practitioner should assess all relevant variables, including pathogens involved, possible treatment sequelae, mortality rate, dollar value of the population, cost of the treatment, effect of the disease on the animals' welfare, and legal considerations. The intended outcomes of these variables are often contradictory, requiring that the clinician make decisions about which is most important under a particular circumstance.

For example, the owner of a recreational pond or an individual pet fish owner may be willing to spend several hundred dollars to save just a few fish because the animals' importance transcends their commercial value. However, cost of treatment versus cost of continued losses is an important consideration for commercial fish producers. If ten 1-kilogram fish are dying daily and if the fish are worth $2.00 per kilogram, a $1,200 treatment of potassium permanganate may not be warranted, especially if nonmedical treatments can be used (e.g., reduce stress). However, not treating such fish might be considered inhumane. Conversely, drug treatment to compensate for poor hygiene is also considered inhumane (ECPAKFP 2006), so improving the environment should be a priority.

Water-borne antiparasite treatments are often contraindicated if primary viral or bacterial infections are in progress. Caution is also recommended if ponds are treated during hot, summer weather when phytoplankton blooms are dense, since chemical treatment could precipitate severe oxygen depletion (Allison 1962).

Treatment Options in Various Aquaculture Systems

Another important factor influencing treatment is the type of culture system (Table III-1). Culture systems may be classified according to the degree of control that the culturist can exert on the environmental conditions prevailing in the system. The four major types of culture systems include aquaria, ponds, cages, and flow-through systems.

Aquaria are the most highly controllable culture systems, since they typically have supplementary methods for maintaining temperature, biological filtration, and oxygen. They are also most amenable to various water-borne treatments because of the relatively small water volume in the system and thus the ease of manipulability.

Ponds, which are more influenced by natural factors such as light, temperature, and rainfall and thus natural biological cycles (e.g., algal growth, nitrification), are less controllable by the culturist. Also, interventional strategies are more limited compared with aquaria.

Cages are even more susceptible to the vagaries of natural environmental changes. Water-borne treatments are doable in such systems, but are much more difficult (see above). Fish that need to be treated in such systems must have the cage enclosed using specially designed skirts or tarpaulins because drugs added to water quickly diffuse away and do not maintain therapeutic concentrations. Alternatively, the fish must be treated in a closed system (e.g., bath treatment) or medications must be delivered orally.

Raceways and other flow-through systems are the least manipulable systems by virtue of the constant and rapid water turnover; similar adverse environmental consequences can follow such treatments. Flow-through systems are even more limited than cages in the ability to use water-borne treatments (although water flow can sometimes be temporarily halted for short-term treatments).

Treatment of Marine versus Freshwater Fish

Treatment modalities for marine fish are similar to those for freshwater fish. Important differences in treating marine fish primarily relate to use of water-borne medications (Noga 1992). The chemistry of saltwater influences the toxicity of many substances. The first important factor to consider is whether the agent is effective in saltwater. Some antibiotics, notably the tetracyclines, chelate divalent cations (calcium and magnesium), which

Table III-I. Some characteristics of the major types of fish culture systems.

	Aquaria	Ponds	Cages/net-pens	Raceways and other flow-through systems
Water turnover*	None	None to low	Moderate to high	High
Ability to manipulate environment	Considerable	Some	Little	Virtually none
Water-borne treatments available	Many	Some	Few	Few

*Defined as the amount of water exchange that normally occurs over time (not including water changes intentionally made by the culturist during therapy).

are present in high concentrations in seawater; this can reduce their uptake into the fish, especially if given as a water-borne treatment. The activity of many other drugs, such as copper and organophosphates, is affected by seawater. While many of these drugs can be used in seawater, the dosage is usually higher than that used in freshwater. Second, many medications for marine fish are toxic to invertebrates, so their use in aquarium reef systems must be avoided. This is especially true for copper, formalin, and organophosphates. Antibiotics, even when they are not directly toxic to invertebrates, may still be harmful. Anemones, corals, and some other invertebrates have symbiotic bacteria and algae that are required for their survival. If these microorganisms are susceptible to an antibiotic, it could indirectly kill the invertebrate host. For these reasons, it is best to avoid exposure of invertebrates to any fish medications.

Third, drug metabolism (pharmacokinetics) varies tremendously, depending on whether a fish is in saltwater or freshwater. In freshwater, teleost fish are hypertonic relative to their environment and regulate osmotic balance by actively taking up ions, especially via the gills (Evans and Claiborne 2006). They also drink almost no water and reabsorb as many ions as possible in the kidney, producing a dilute urine. In full-strength seawater, fish face the opposite problem, a hypertonic environment (10–14 ppt salinity is close to isosmotic for most species). Thus, fish in full-strength seawater drink large amounts to regain lost water and excrete the excess ions via the gills and kidney (Prosser 1973b, 1991).

Whereas similar types of pathogens affect freshwater and marine fish, relatively few pathogens are transmissible from freshwater to marine fish, or vice versa (i.e., most pathogens affect either marine or freshwater fish, but not both). This is the rationale for why many freshwater pathogens can be treated with salt and many marine pathogens can be treated with freshwater.

Fish Pharmacology

Proper use of drugs for fish diseases depends not only on a thorough knowledge of the disease under consideration but also on the properties of the pharmacological agent used, the species under treatment, and the environmental conditions. A detailed discussion of the pharmacology of drugs used to treat fish is given in Treves-Brown (2000). Almost all data on drug pharmacology and environmental fate are from temperate environments; relatively little is available on tropical species.

Fish can vary greatly in their response to the same medication given under exactly the same conditions. Important variables include species and age of fish. Younger fish, especially larvae, are much less tolerant of drug treatments than older fish. The drug availability and excretion dynamics of diseased fish can be very different from that of healthy individuals (Uno 1996). The doses mentioned in the "**Pharmacopoeia**" should be administered with this in mind.

Uptake of Drugs in Relation to Route of Exposure

In contrast to the more traditional routes of administration of therapeutic agents used in treating terrestrial animals, aquatic species are most often treated by adding drugs directly to the water. This mode of therapy is used not only for external problems, such as ectoparasites, but for systemic diseases as well. This method of administration adds another complex variable to the factors that must be considered when attempting to establish the proper therapeutic dose for treating fish under particular circumstances. The following several factors must be considered when treating fish via water-borne administration:

1. The epidermis of fish is not keratinized. Living, dividing cells extend throughout the entire epidermis. This lack of keratinization may increase the ability of drugs to penetrate the epidermis and is especially important in small fish (Kleinow et al. 2008). Conversely, when fish are removed from a therapeutic bath to untreated water, systemic concentrations may decay rapidly because of rapid movement down a concentration gradient.
2. The gill, a highly vascularized organ with a vast blood supply near the epithelial surface, may also be important in drug uptake and excretion.
3. The relative importance of uptake across the gastrointestinal tract in water-borne administration will be influenced by the physiological state and environmental conditions. For example, fish in seawater drink significant amounts of water and may absorb signifi-

cant amounts of a drug via the gastrointestinal tract. Freshwater fish drink little water; thus this route may not be as important in this environment. For example, water-borne uptake of sulfas by rainbow trout in seawater is much greater than uptake in freshwater, probably because of differences in drinking rates (Bergjso and Bergjso 1978). Conversely, oxytetracycline is not absorbed as well in seawater because it chelates divalent cations; the charged complex is not available for uptake across cell membranes. Uptake for many other drugs is less efficient in seawater than in freshwater (Lunestad 1992).

4. The relative importance of drug uptake across the gastrointestinal versus respiratory versus epidermal epithelium in fish is unknown (Shepherd 1993). Indeed, for many drugs, the relationship between concentration in the water and systemic levels has not been determined and dosage levels are based on empirical data.
5. When drugs are added to the water, their half-life within the environment must be considered in addition to their half-life within the fish. The chemical activity and rate of uptake may be influenced by pH, temperature, light, water hardness, and many other factors (Lunestad 1992).
6. The water is the life-support system of aquatic species and adding any substance to it must be done with full consideration made to the potential consequences of that chemical on environmental quality. For example, formalin, commonly used as a parasiticide, is a strong reducing agent and will rapidly reduce oxygen levels if adequate aeration is not provided. Other drugs, such as methylene blue and certain antibiotics, inhibit the ability of nitrifying bacteria to detoxify nitrogenous wastes, resulting in the accumulation of these toxic metabolites.

Drug Metabolism in Fish

As in mammals the liver is the primary organ for detoxification of drugs in fish. Available evidence indicates that many qualitative similarities exist in the metabolism of drugs by fish and mammals (Franklin et al. 1980; James 1986; Treves-Brown 2000; Schlenk et al. 2008). Fish can carry out many, and possibly all, of the Phase I (oxidation, reduction, and hydrolysis) reactions utilized by mammals to detoxify or activate drugs. Although qualitative differences exist among various fish species, oxidation reactions have been demonstrated in representatives of the most primitive to the most advanced fish groups. These reactions are carried out in the microsomal fraction of liver and require reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen, making them similar to the enzymatic reactions performed by the mammalian mixed function oxidase (MFO) system (e.g., cytochrome P450 enzymes). Some of the oxidative enzyme systems of fish are inducible by substances, such as DDT, that are known to induce mammalian enzyme systems. Procarcinogens, such as aflatoxin, can be activated by fish to their active carcinogenic form, producing tumors such as hepatomas in the case of aflatoxin. Some fish can also perform azo- and nitro-reductions, or hydrolyze compounds, such as succinylcholine and organophosphates.

Phase II reactions involve conjugate formation and subsequent excretion via urinary and biliary routes. Fish can conjugate drugs with a number of compounds, including glucuronic acid, glycine, glutathione, acetate, taurine, and sulfate. Excretion appears to be mainly via the urine and bile, but some conjugates are also excreted across the gills.

Fish appear to metabolize drugs at about one-tenth the rate in mammals. Also, the temperature optimum for many of these reactions is lower than that of mammals, usually approximating the temperature of the fish's natural environment (see "**Estimating Withdrawal Time**," **p. 357**).

Legal Use of Drugs

Policies regarding the enforcement of regulations on the use of drugs have changed significantly over the past several years; the use of drugs in fish is receiving increased regulatory scrutiny worldwide. In order to illustrate the legal issues surrounding drug use in fish, details regarding U.S. regulations are mainly discussed here. Some significant differences with other countries are also mentioned. However, the clinician should be aware that regulations vary considerably among countries. Each country has specific regulations regarding drug use. For example, in many European countries, it is regulated by the Committee for Medicinal Products for Veterinary Use (see "**Estimating Withdrawal Time**," **p. 357**); and in Japan, it is regulated by the Ministry of Agriculture, Fisheries and Food (http://www.maff.go.jp/e/index.html). A list of agencies in other countries is available at http://www.fda.gov/oia/agencies.htm.

As is true for other animals in the United States, the legal use of drugs in fish is regulated by the Food and Drug Administration (FDA). This includes all fish, not just those intended for human consumption. All drugs, including animal drugs, must be approved by the FDA (note that pesticides must be registered by the United States Environmental Protection Agency [EPA], and animal biologics/vaccines must be licensed by the United States Department of Agriculture [USDA], www.aphis.usda.gov/animal_health/vet_biologics). In all countries, the use of drugs in fish is coming under increasing scrutiny, so the practitioner should determine the current status of a drug before recommending its use. Any thera-

pies must also comply with national/state/provincial regulations that govern the discharge of chemically treated effluent into waters (Meyer 1989).

The Use and Abuse of Drugs in Aquaculture

In the United States, the FDA regulates the use of drugs in all fish, regardless of whether they are raised for human consumption (e.g., trout, salmon, catfish) or for other purposes (e.g., as pets, in zoos, etc.). In those instances where a drug has been approved, legal restrictions on its use are often much narrower than the use to which it has been applied by aquaculturists.

A great many agents have been used to treat diseases in food fish. Not all of these drugs have been legally approved for use in food fish; to be licensed for use in fish produced for human consumption, a drug must pass rigorous testing for efficacy in treating a specific disease in a specific species at a specific dosage and route of administration. Extensive data must also be obtained on residue dynamics, as well as toxicology and microbial food safety.

Previously, the requirement for extensive and rigorous testing for each particular application of a drug has discouraged the pursuit of new drug approvals by pharmaceutical companies, because the relatively small aquaculture market often would not allow them to recoup their expenses. The problem of a lack of legal drugs has been compounded by the resistance of many of the common pathogens to the presently licensed drugs because of injudicious use of those drugs, especially the antimicrobial agents. However, it is often impossible to avoid repeated use of an antimicrobial agent because of the small number of legally approved antibiotics available.

But, recently enacted legislation has greatly eased the ability to get approval for drugs to be used for treating food fish in the U.S. (see MUMS Act in the "**INADs**" section below). In the United States, a food fish is defined as any individual that is used for human consumption at some time in its life. The treatment of nonfood fish, including pet fish and fish for research, is under much less regulatory scrutiny, but increasing regulatory oversight is also occurring in this sector. Regarding pet fish, many pet fish remedies (e.g., numerous antibiotics) are still sold as over-the-counter preparations in many countries, including the United States. While there is little objective scientific evaluation of these commercial remedies, the few studies done have found many preparations to be ineffective at the recommended dosages (Trust 1972). Also, prolonged exposure to suboptimal concentrations of antibiotics has caused increased incidence of resistant strains of fish-pathogenic bacteria (Dixon et al. 1990; Herwig et al. 1997). It is the ethical duty of the clinician to use any drug judiciously and to avoid unauthorized use if at all possible.

Legal Use of Drugs in the United States

A legally usable drug (e.g., formalin, oxytetracycline) is approved for use in a specific fish (e.g., channel catfish) and for treating a specific pathogen (e.g., motile aeromonad infection). The precise duration and method of dosing (e.g., in water, in feed, etc.) are also indicated. However, drugs are classified and regulated in several different ways and the clinician should be aware of the exact legal status of any drug that will be used.

The recent passage of the Animal Medical Drug Use and Clarification Act of 1994 (AMDUCA) and of the Animal Drug Availability Act of 1996 (ADAA) has expanded the authority of veterinarians to use drugs in fish. In the United States, drugs may be used legally in one of three ways: over-the-counter (OTC), veterinary feed directive (VFD), or prescription (www.fda.gov/cvm; Table III-2). Classification is based upon whether a layperson (i.e., non-veterinarian) can use the drug safely and effectively. In addition, certain drugs may be used in an INAD (see "**INADs**" below). Some unapproved drugs are under less regulatory scrutiny and thus may be used even though they are not approved (see Table III-3); other compounds are not considered to be drugs (Table III-4), while other drugs are totally illegal for food fish use (Table III-5). Finally, use of certain pesticides may also be allowed, under the jurisdiction of the EPA.

Be aware that the status of all drugs is in a constant state of flux. Regulations and drug use also vary significantly among nations (Alderman and Michel 1992; Michel and Alderman 1992; Table III-6).

Over–the–Counter (OTC) Drugs

An OTC drug is defined as one that can be sold to a layperson (non-veterinarian) without a veterinarian-client relationship. The majority of drugs used in treating fish are OTC drugs (Tables III-2, III-3).

Veterinary Feed Directive (VFD) Drugs

The Animal Drug Availability Act of 1996 established VFD drug classification (www.fda.gov/cvm/vfd.html) to more closely control new drugs (mainly antimicrobials) and their use in food animals. This new classification applies only to certain new drugs approved by the FDA after 1999 and administered in feed. All products approved before 1999 have maintained their OTC status.

The VFD program is intended to promote judicious drug use and thus reduce antibiotic resistance and lengthen the time that a drug remains useful. "Extra-label" or "off-label" use is not allowed for any VFD drug.

To be able to use a VFD drug, a producer must obtain a signed VFD from a licensed veterinarian. Producers can obtain a VFD as long as there is a "veterinarian-client-relationship". This is met when:

Table III-2. Drugs that are approved for treating food fish in the United States and their approved uses (from www.fda.gov/cvm/drugsapprovedaqua.htm). These drugs are approved for use only with the specific commercial formulation listed. Bulk drugs from a chemical company or similar unapproved labels are illegal. Approval is given only for the indications (disease) and methods of administration given. OTC = over-the-counter drug; VFD = veterinary feed directive drug.

Drug	Approved labels	FDA-approved use	OTC, VFD, or prescription?	Comments
Formalin	Paracide-F® (Argent)	1. Parasiticide for control of external protozoa (*Chilodonella*, *Costia*, *Epistylis*, *Ichthyophthirius*, *Scyphidia*, *Trichodina*) and monogeneans (*Cleidodiscus*, *Gyrodactylus*, *Dactylogyrus*) on salmon, trout, catfish, largemouth bass, and bluegill Salmon and trout in tanks and raceways: >50°F: Up to 170 ppm for up to 1 hour <50°F: Up to 250 ppm for up to 1 hour Catfish, largemouth bass and bluegill: 250 ppm for up to 1 hour Earthen ponds: 15–25 ppm prolonged immersion 2. Control water molds of family Saprolegniaceae on eggs of salmon, trout and esocids: 1,000–2,000 ppm for 15 minutes in egg treatment tanks	OTC	1. Ponds may be retreated in 5–10 days if needed. Do bioassay with new lots of fish. No withdrawal time 2. Do bioassay with new lots of eggs. No withdrawal time
Formalin	Formalin-F™ (Natchez Animal Supply Co.) and Parasite-S® (Western Chemical) and Formacide-B (B.L. Mitchell)	1. Parasiticide (same parasites as Parasite-F) on all fish Salmon and trout in tanks and raceways: >50°F: Up to 170 ppm for up to 1 hour <50°F: Up to 250 ppm for up to 1 hour All other finfish: 250 ppm for up to 1 hour Earthen ponds: 15–25 ppm prolonged immersion 2. Control of water molds of the family Saprolegniaceae on eggs of all fish: Same dose as for Paracide-F® except for order Aciperseriformes (sturgeon) eggs: up to 1,500 ppm for 15 minutes	OTC	Same as Paracide-F®
Hydrogen peroxide	35% PEROX-AID® (Western Chemical)	1. Control of saprolegnosis on eggs of all freshwater fish Cold water and cool water: 500–1,000 mg/L for 15 minutes in a continuous-flow system once per day on consecutive or alternate days until hatch Warm water: 750–1,000 mg/L for 15 minutes in a continuous-flow system once per day on consecutive or alternate days until hatch 2. Control bacterial gill disease due to *Flavobacterium branchiopilum* on freshwater salmonids 100 mg/L (30 minutes) or 50–100 mg/L (60 minutes) once per day on alternate days for three treatments 3. Control external columnaris on channel catfish and freshwater, cool water fish Fingerling and adults (except northern pike and paddlefish): 50–75 mg/L (60 minutes) once per day on alternate days for three treatments Fry (except northern pike, pallid sturgeon, and American paddlefish): 50 mg/L (60 minutes) once per day on alternate days for three treatments Use with caution on walleye	OTC	For all treatments, an initial bioassay on a small number of eggs or fish is recommended before treating the entire group. No withdrawal time
Tricaine methane-sulfonate	Finquel®*(Argent)* Tricaine-S™ (Western Chemical)	Temporary immobilization for fish of the families Salmonidae, Ictaluridae, Esocidae, and Percidae 15–330 mg/L	OTC	Use only when water temperature over 50°F (10°C). 21-day withdrawal time

Continued.

Table. III-2. Drugs that are approved for treating food fish in the United States and their approved uses (from www.fda.gov/cvm/drugsapprovedaqua.htm). These drugs are approved for use only with the specific commercial formulation listed. Bulk drugs from a chemical company or similar unapproved labels are illegal. Approval is given only for the indications (disease) and methods of administration given. OTC = over-the-counter drug; VFD = veterinary feed directive drug, cont'd.

Drug	Approved labels	FDA-approved use	OTC, VFD, or prescription?	Comments
Chorionic gonadotropin	Chorulon® (Intervet)	Improve spawning function in male and female broodfish Males: 50–510 IU/lb Females: 67–1,816 IU/lb	Prescription	Intramuscular injection Up to three doses, with total dose not to exceed 25,000 IU in fish intended for human consumption. Prescription product restricted to use by or on the order of a licensed veterinarian
Sulfadimethoxine/ormetoprim	Romet® 30, Romet® TC (Aquatic Health Resources)	Antibacterial against *Aeromonas salmonicida* of salmonids and *Edwardsiella ictaluri* of channel catfish Feed 50 mg/kg of fish per day for 5 days.	OTC	Salmonids: 42-day withdrawal time Channel catfish: 3-day withdrawal time
Sulfamerazine	Sulfamerazine (Alpharma)	Antibacterial against *Aeromonas salmonicida* of rainbow, brook, and brown trout; feed 10 g/100 lb of fish per day for 14 days.	OTC	21-day withdrawal time Not currently available
Oxytetracycline dihydrate	Terramycin® 200 for fish (Phibro Animal Health)	1. Antibacterial against: *Aeromonas salmonicida*, motile aeromonads, and *Pseudomonas* in salmonids Motile aeromonads and *Pseudomonas* in channel catfish; feed 2.5–3.75 g/100 lb of fish per day for 10 days. 2. Antibacterial against: Cold water disease (*Flavobacterium psychrophilum*) in freshwater-reared salmonids Columnaris in freshwater-reared *Oncorhynchus mykiss* and its subspecies; feed 3.75 g/100 lb of fish per day for 10 days.	OTC	1. Water temperature must be >62°F when treating channel catfish; 21-day withdrawal time 2. No temperature restrictions. 21-day withdrawal time
Florfenicol	Aquaflor® (Schering-Plough Animal Health)	Control of enteric septicemia of catfish and columnaris in channel catfish Control of furunculosis and cold water disease (*Flavobacterium psychrophilum*) in freshwater salmonids. 10 mg/kg/day for 10 consecutive days	VFD	For columnaris in channel catfish, must use Aquaflor®-CA1 product; 12-day withdrawal time for channel catfish; 15-day withdrawal time for salmonids

1. The veterinarian is responsible for making clinical decisions regarding the health of the fish and the need for medical treatment, and the client has agreed to follow the veterinarian's instructions.
2. The veterinarian has sufficient knowledge of the animals to initiate at least a general or preliminary diagnosis of the animals' medical condition. This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animals, by virtue of an examination of the animals, or by medically appropriate and timely visits to the premises where the animals are kept.
3. The veterinarian is readily available for follow-up evaluation, or has arranged for emergency coverage, in the event of adverse reactions or failure of the treatment regimen.

Extra–Label Use of Drugs

Currently, except for Chorulon® (chorionic gonadotropin), the only prescription drugs approved by FDA for use in fish are extra-label drugs. Drugs approved by the FDA for use in other animals or humans may be used in aquatic species when certain criteria are met. These criteria constitute the FDA's extra-label drug use policy. Extra-label use is defined by the FDA as: "Actual use or intended use of a drug in an animal in a manner that is not in accordance with the approved labeling. This includes, but is not limited to, use in species not listed in the labeling, use for indications (disease and other conditions) not listed in the labeling, use at dosage levels, frequencies, or routes of administration other than those stated in the labeling, and deviation from labeled withdrawal time based on these different uses."

Table III-3. Uses of drugs/chemicals that are not formally approved for treating food fish in the United States but are of LOW REGULATORY PRIORITY (from www.fda.gov/cvm/Documents/LRPDrugs.pdf).

Drug	Use
Acetic acid	Treating ectoparasitic protozoa: 1,000–2,000 ppm for 1–10 minutes
Calcium oxide (unslaked lime)	Treating ectoparasitic protozoa of fingerling to adult fish: 2,000 mg/L for 5 seconds
Onion (whole form)	Treating crustacean infestations and deterring sea lice from infesting marine salmonids of all stages
Garlic (whole form)	Treating helminth and sea lice infestations on marine salmonids of all stages
Magnesium sulfate + sodium chloride	Treating monogenean and crustacean infestations on fish of all life stages: 30,000 mg $MgSO_4$ + 7,000 mg NaCl/L for 5–10 minutes
Sodium chloride	1. Osmoregulatory enhancer to relieve stress and prevent shock: 5,000–10,000 mg/L for an indefinite period 2. Parasiticide: 30,000 mg/L for 10–30 minutes
Potassium chloride	Osmoregulatory enhancer to relieve stress and prevent shock: 10–2,000 mg/L for an indefinite period
Calcium chloride	Osmoregulatory enhancer to relieve stress and prevent osmotic shock: raise hardness as high as to 150 mg/L as $CaCO_3$
Calcium chloride	Increase calcium to ensure proper egg hardening: raise hardness level by 10–20 mg/L as $CaCO_3$
Sodium sulfite	Treating eggs to improve their hatchability: 15% solution for 5–8 minutes
Papain	Removing gelatinous matrix of fish egg masses to improve hatchability and reduce disease incidence: 0.2% solution
Fuller's earth	Reducing adhesiveness of eggs and improving hatchability
Urea + tannic acid	Denaturing the adhesive component of fish eggs: add about 400,000 eggs to 5 liters of water with 15 g urea + 20 g NaCl and incubate for 6 minutes, then transfer eggs to 0.75 g tannic acid in 5 liters of water for another 6 minutes
Ice	Reduce metabolic rate of fish during transport
Carbon dioxide	Anesthetic: 200–400 ppm for 4 minutes
Sodium bicarbonate	Anesthetic: 142–642 mg/l for 5 minutes to introduce CO_2 into the water
Thiamine hydrochloride	Prevent or treat thiamine deficiency in salmonids: immerse eggs in a solution of up to 100 mg/l for up to 4 hours during water hardening. Immerse sac fry in a solution of up to 1,000 mg/l for up to 1 hour
Povidone iodine	Egg antiseptic: 100 mg/L for 10 minutes during or after water hardening of eggs

Table III-4. Chemicals that are not considered to be drugs by the U.S. FDA for the uses given. These compounds are not regulated by the FDA when used as specified below.

Drug/chemical	Use
Calcium hydroxide (slaked lime)	To raise pH of water or pond bottom to 10
Calcium carbonate	To alter pH and/or total alkalinity of water
Sodium hydroxide	To raise the pH of water
Tris buffer	To buffer pH changes in freshwater or saltwater
Ozone	To disinfect and remove organic compounds from hatchery water
Oxygen	To maintain saturated dissolved oxygen levels in water to ensure fish survival

Table III-5. Uses of drugs that are unapproved for treating food fish in the United States and are considered HIGH REGULATORY PRIORITY by U.S. FDA. Note that some of these drugs may be used in the United States under an approved INAD.

Drug	Use
Chloramphenicol	Antibiotic
Nitrofurazone	Antibiotic
Furazolidone	Antibiotic
Nifurpirinol	Antibiotic
Quinolones	Antibiotic
Fluoroquinolones	Antibiotic
Glycopeptides	Antibiotic
All nitroimidazoles (including dimetridazole, metronidazole, and ipronidazole)	Parasiticide
Malachite green	Parasiticide; fungicide
Methylene blue	Fungicide
Acriflavine	Parasiticide
Central nervous system stimulants and depressants (benzocaine, quinaldine sulfate, 2-phenoxyethanol)	Anesthetic; sedative
Hormones and steroids (human chorionic gonadotropin, pituitary extracts, 17 alpha-methyltestosterone, etc.)	Induce reproduction; change sex; induce sterility

Table III-6. Examples of drugs used in various countries for disease treatment and management in food fish aquaculture. Modified from Alderman et al. (1994) with additional data from Treves-Brown (2000), Costello et al. (2001), NIFES (http://www.nifes.no), Bravo et al. (2005), and Reimschuessel et al. (2005). Note that this is not intended to be a comprehensive list. Also, regulations change constantly and this list is only intended as a general guide to the types of compounds that have been used in the past, although most are still being used at some level. Note that a number of drugs that are not approved for food fish may be commonly used for nonfood (aquarium) fish. The clinician should be sure that any drug that is used in a jurisdiction fully complies with all legal, humane, safety (human and fish), and environmental regulations.

CAUTION: Some drugs not approved or even "banned" from use in a certain country may nevertheless still be used to some extent in that country. This is especially true in lesser developed countries. With changing regulations, the official view of drugs that may be used will change rapidly over the next few years. Actual use will also change, but not always in synchrony.

	U.S.	Japan	Canada	Chile	Denmark	Finland	France	Germany	Ireland	Netherlands	Norway	Spain	Sweden	UK	Iceland
Acetic acid	+		+												
Acriflavin						+			+						
Antibiotics															
Amoxicillin		+		+										+	
Ampicillin		+										+			
Chloramphenicol	B		B		B			B	B	+			+	B	
Chlortetracycline			+									+			
Doxycycline		+													
Enrofloxacin				+											
Erythromycin		+	+	+					+						
Florfenicol	+	+	+	+	+						+	+		+	
Flumequine				+			+		+	+	+				
Furaltadone										+					
Furanace	+ (nf)														
Furazolidone					+		+	+		+					
Josamycin		+													
Kanamycin												+			
Nalidixic acid												+			
Neomycin										+					
Nitrofurans							+					+			
Nitrofurazone										+					
Novobiocin		+													
Oxolinic acid		+		+	+		+	+	+		+	+	+	+	+
Oxytetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Penicillin			+												
Potentiated sulphonamides	+	+	+		+		+	+	+	+	+		+	+	
Prefuran			+												
Sarafloxacin				+								+			
Spiramycin		+													
Streptomycin											+	+			
Sulphonamides								+		+					
Sulphamerazine	+		+							+					
Sulphamethazine					+							+			
Thiamfenicol		+													
Azamethiphos				+										+	
Benzalkonium chloride						+						+		+	

Benzocaine				+		+		+ (e)	+	+	+			+					
Bronopol											+			+					
Carbarsone														+					
Carbon dioxide	+				+	+		+	+				+	+					
Chloramine T or B			+		+	+	+		+	+				+					
Chlorbutanol					+			+					+						
Clove oil		+		+															
Copper sulfate			+		+		+		+	+			+	+					
Cypermethrin											+			+					
Deltamethrin				+							+								
Dichlorvos			+						+				+	+					
Dimetridazole	B				+		+												
Diquat	+																		
Enamectin			+	+							+								
Enheptin										+									
Fenbendazole										+	+								
Formaldehyde (A)	+		+		+	+	+	+	+	+	+		+	+					
Formaldehyde (D)	+				+	+		+ (e)	+	+				+					
Hypobromide (D)							+												
Hydrogen peroxide	+		+																
Hypochlorite (D)	+		+			+	+	+	+	+				+					
Iodophores (D)	+		+		+	+			+	+			+	+					
Iodophores	+		+		+	+	+		+					+					
Ivermectin									+ (e)										
Levamisole											+								
Malachite green	+ (nf)		+(nf)		B									B					
Mebendazole					+					+									
Methylene blue						+ (e)													
Metomidate			+																
Tricaine	+		+	+		+			+		+		+	+	+				
Natamycin											+								
Olaquindox										+									
Phenoxyethanol			+											+	+				
Potassium permanganate	+		+		+		+			+			+	+					
Praziquantel											+								
Quatenary ammonium compounds (A)							+		+					+					
Quatenary ammonium compounds (D)	+ (nf)					+	+		+					+					
Ronidazole										+									
Sodium bicarbonate	+																		
Sodium chloride	+		+		+	+				+	+		+	+					
Teflubenzuron			+	+										+					
Tert amyl alcohol			+																
Trichlorfon	+ (nf)						+				+	+							

(e) = for experimental use only, (nf) = for nonfood fish use only, B = specific ban on use, A = used as antiseptic, D = used as disinfectant.

Note that only drugs that are approved by the FDA for some other species (including humans) can be used in an extra-label manner. Extra-label use is limited to circumstances when the health of an animal is threatened, or suffering or death may result from failure to treat. This means that extra-label use to enhance production is prohibited. Extra-label use of drugs may be considered by food fish veterinarians only when:

1. There is no approved new animal drug that is labeled for such use and that contains the same active ingredient in the required dosage form and concentration, except where a veterinarian finds, within the context of a valid veterinarian-client patient relationship, that the approved new animal drug is clinically ineffective for its intended use.
2. Before prescribing or dispensing an approved new animal or human drug for an extra-label use in food fish, the veterinarian must:
   a. Make a careful diagnosis and evaluation of the conditions for which the drug is to be used,
   b. Establish a substantially extended withdrawal period prior to marketing of edible products supported by appropriate scientific information, if applicable,
   c. Institute procedures to assure that the identity of the treated animals is carefully maintained,
   d. Take appropriate measures to assure that assigned time frames for withdrawal are met and no illegal drug residues occur in any food fish subjected to extra-label treatment, and
   e. Ensure that the prescribed or dispensed extra-label drug (prescription legend or over-the-counter) bears labeling information that is adequate to ensure the safe and proper use of the product.

Because extra-label use of animal and human drugs in non-food-producing animals (e.g., pet fish) does not ordinarily pose a threat to the public health, the FDA permits extra-label use of animal and human drugs in non-food-producing animals except where the public health is threatened.

However, in food fish, its use must be accomplished in accordance with an appropriate medical rationale; and if scientific information on the human food safety aspect of the use of the drug in that food fish is not available, the veterinarian must take appropriate measures to assure that the animal and its food products will not enter the human food supply.

Extra-label use of an approved human drug in a food fish is not permitted if an animal drug approved for use in food-producing animals can be used in an extra-label manner for the particular use. Also, use of any drugs that are approved for use in humans is strongly discouraged. Extra-label use does not allow the use of an unapproved drug in the feed. Only other routes (e.g., via injection or via water-borne methods) are allowed.

Animal drugs may be legally compounded from FDA-approved animal drugs and FDA-approved human drugs if the compounding practices are in conformance with the provisions of the regulation on the extra-label use of FDA-approved drugs.

There are legal limitations to extra-label use, and the following are prohibited:

1. Extra-label use by a lay person (except when under the supervision of a licensed veterinarian),
2. Extra-label use in an animal feed,
3. Extra-label use resulting in any residue that may present a risk to the public health, and
4. Extra-label use resulting in any residue above an established safe level, safe concentration, or tolerance.

Examples of drugs listed in the "**Pharmacopoeia**" that could be used in an extra-label manner for food fish in the United States include fenbendazole (Panacur), levamisole (Levasol), mebendazole (Telmintic), monensin (Rumensin), piperazine, praziquantel (Droncit), and some antibiotics. Note that VFD drugs cannot be used extra-label.

INADs

Certain unapproved drugs may be used in aquaculture in the United States in certain circumstances under an Investigational New Animal Drug (INAD) exemption from the FDA. The holder of the INAD (the sponsor, i.e., drug user) has the authority to use the unapproved drug for clinical investigation. It also authorizes the slaughter of treated fish for human consumption and assigns an investigational withdrawal period. The FDA grants INAD exemptions for the investigational use of drugs. While FDA's purpose in granting an INAD is to generate research data to support eventual FDA approval of the drug, an INAD exemption may also allow, in certain limited situations, legal treatment of fish with an unapproved drug. It is important to note that INADs are granted by the FDA's Center for Veterinary Medicine (CVM) with the expectation that data to support an approval of the drug will be generated and submitted to CVM. The purpose of the INAD is to produce data that are ultimately intended to generate a New Animal Drug Application (NADA), which FDA will then consider for possible approval of the drug.

Recent legislation, the Minor Use and Minor Species Animal Health Act of 2004 (the "MUMS Act"), helps make more medications legally available to veterinarians and animal owners to treat fish. This legislation has allowed the approval of a wider array of drugs than were possible under previous regulations. The U.S. Fish and Wildlife Service has acted as a coordinator for gathering data necessary for the registration of drugs considered essential to aquaculture, which has reduced the regulatory burden placed on the developing aquaculture indus-

try in the United States. Information on the status of many drugs currently having INADs is available at www.fws.gov/fisheries/aadap.

Drugs of "Low," "Not Low," and "High" Regulatory Priority

"Low regulatory priority" means a drug not officially approved by the FDA for the use given, but the FDA is "unlikely to object" to use of the compound if it is used under the conditions specified (Table III-3). This enforcement position is considered neither approval nor affirmation of the product's safety or efficacy. These drugs are considered low regulatory priority only if used under the exact conditions specified. The FDA is unlikely to object to the use of these substances if the following conditions are met:

1. The substances are used for these indications,
2. The substances are used at the prescribed levels,
3. The substances are used according to good management practices,
4. The product is of an appropriate grade for use in food animals, and
5. There is not likely to be an adverse effect on the environment.

Drugs that are considered "not low regulatory priority" are ones in which regulatory action is being deferred pending further study. "Not low regulatory priority" drugs should never be used in the United States without an INAD exemption (see **p. 356**).

"High regulatory priority" drugs are agents that pose the greatest public health concern and ones with which the FDA is most likely to take regulatory action (Table III-5). These drugs are forbidden to be used in food-producing animals. Many of these drugs are also forbidden for food fish use in other countries.

Use of EPA–Registered Pesticides

Germicide preparations for use in inanimate objects (i.e., disinfectant uses), as well as rodenticides and most insecticides, are regulated by the U.S. Environmental Protection Agency (EPA). Information about EPA's pesticide program can be found at www.epa.gov/pesticides. Regarding the use of EPA-registered pesticides for drug purposes (e.g., diquat to treat bacterial gill disease [BGD]), the FDA's position is that the agency will not object to the use of a registered pesticide when used in accordance with the EPA-registered labeling, if the pesticide has a secondary therapeutic benefit, provided that the conditions for which the pesticide is registered actually exist in the treatment situation. An example would be the use of an EPA-registered algicide in a situation where an algae problem actually exists and where the chemical happens to have a secondary therapeutic benefit to the fish (e.g., controls a parasite infestation).

Legal Withdrawal Times

When food fish are harvested for human consumption, it is the legal responsibility of the person prescribing the treatment (in most countries, the veterinary clinician) to ensure that illegal residues are not present in edible flesh. Withdrawal times are recommended and in many countries legally enforced for some drugs, especially antibiotics. However, these withdrawal times are based on studies mainly performed on fish held in temperate freshwater. The excretion of a drug by a fish can vary greatly with environmental conditions, especially temperature. For example, oxytetracycline persistence in tissues of rainbow trout increases 10% for every 1°C decrease in temperature (Salte and Liestol 1983). This is intuitively logical, since many metabolic processes in poikilothermic animals generally decrease twofold for every 10°C decrease in temperature (Q_{10} effect) (Prosser, 1973a). In practical terms, this led Salte and Liestol (1983) to recommend that for rainbow trout, there should be a 60-day withdrawal time for oxytetracycline-medicated fish kept at over 10°C compared with a 100-day withdrawal time for fish kept at less than 10°C. The excretion rate at less than 10°C for potentiated sulfonamides was so slow that they suggested that this antibiotic not be used when temperatures are this low. Additional information on pharmacokinetics and residue dynamics of drugs used to treat fish are available at the Food Animal Residue Avoidance Databank (www.farad.org) and in Reimschuessel et al. (2005).

Estimating Withdrawal Time

Because of the variability in drug excretion, especially with temperature, a rule of thumb called degree days has been advocated for estimating the required withdrawal time (Debuf 1991). Degree days are calculated by adding the mean daily water temperatures (measured in degrees centigrade) for the total number of days measured. Thus, if the mean temperature was 11°C for the 50 days immediately after stopping drug treatment, the degree days would be 550. If the withdrawal time for the drug used was 500 degree days, the fish would probably be safe to slaughter. Note that there is only limited scientific data on temperature's effect on excretion of most drugs and other factors affect excretion rate. The largest problem currently faced with estimating withdrawal times is with antibiotic treatments (see "**Antibiotics**," **p. 377**).

When they are available, suggested withdrawal times based on degree days are provided in the "**Pharmacopoeia**." Also provided are legally mandated withdrawal times for some drugs used in some countries. For example, in the European Union, withdrawal time is based upon the MRLs, which are the "maximum

residue limits" of veterinary medicinal products permissible in food produced by or from animals for human consumption (GESAMP 1997). The Committee for Medicinal Products for Veterinary Use (CVMP; http://www.emea.europa.eu) establishes MRLs, and these limits must be established for all pharmacologically active substances contained in a medicinal product before the product can be sold. Legally approved drugs in the European Union must be listed under either Annex I (has an MRL), II (no need for an MRL), or III (provisional and temporary use). Annex IV drugs are banned from use in food fish (Costello et al. 2001).

When fish are to be released into the wild, they must not be treated with a drug that has any withdrawal time. This prevents the use in many countries of sedatives, such as tricaine, that have a withdrawal period. The only exceptions are in cases where the fish to be released are either not considered edible for human consumption or are smaller than the legally allowed catchable size in that jurisdiction.

Legally approved brands of drugs should always be used for therapy (e.g., Table III-2). Stocks purchased from unapproved chemical supply firms or from other nonethical sources, including aquarium stores, are not regulated for quality as well as pharmaceutical brands. Also, use of an unapproved drug undermines the market for the approved drug, which discourages the manufacturer from renewing the drug's license and thus jeopardizes the future legal use of the drug.

Adverse Drug Events

An adverse drug event is defined as a situation where the drug is ineffective in treating the intended disease or when an issue with its safety (human or fish) has been observed. It is important for the clinician to report adverse drug events to the proper regulatory authority so that it is apprised of these problems.

Human Safety in Using Drugs

Before using any drug or other chemical, the clinician should be fully aware of all possible health risks associated with human exposure to the agent and be aware of the proper means of preventing harmful exposure. This includes being familiar with the Material Safety Data Sheet (MSDS), as well as any user safety warnings on the drug label. The clinician must clearly instruct the intended end user (e.g., farmer, aquarist, etc.) on these risks and the correct methods of use. Specific concerns about various compounds are mentioned in the "**Pharmacopoeia**." Note that various local and national authorities might require special training before some chemicals can be used (e.g., application of certain pesticides).

Environmental Safety in Using Drugs

There are increasing concerns about the unintended entry of aquaculture chemicals into the environment. These concerns are especially intense when they involve coastal marine aquaculture, but all other aquatic environments are also at risk. The clinician should be aware of the potential for a certain chemical to accumulate in the environment and/or harm nontarget aquatic life. In some cases, this might dictate the choice of drug (or whether drugs can even be used) to treat a particular problem. Specific concerns about various compounds are mentioned in the "**Pharmacopoeia**." Note that various local and national authorities might require special certification or approval for the use of chemicals in certain environments.

ROUTES OF DRUG ADMINISTRATION

The three major routes by which fish may be treated are water-borne, oral, and injection. For methods that involve handling (e.g., bath, injection) of healthy fish (e.g., vaccination), it is often best to withhold food for 24 hours before treatment. However, for sick fish, whether this is done depends upon if the fish appear strong enough to withstand this fast. After any therapy, it is best to verify if possible that the treatment has been successful. For example, after an anti-ectoparasite treatment, confirm the absence of parasites in a wet mount examination of skin and gills from a representative number of fish. Items needed for treatment are simple (Box III-1). Conversion factors for calculating treatments and for converting from English to metric units are given in Tables III-7 and III-8.

Water-Borne

The water-borne route is the most common method of administering treatments to fish and has distinct advantages, such as being relatively nonstressful and easy to administer. However, there are disadvantages. Relative to other treatment routes, dosing is less precise (too little or too much). Most drugs added to water are unstable and quickly degrade; this method may require repetitive dosing and removal of inactive (and possibly toxic) byproducts of the drug with water changes.

Water-borne treatments are mainly used for surface-dwelling (skin and gill) pathogens, including parasites, bacteria, and water molds. Except for antibiotics and a few anthelmintics, virtually all agents act as antiseptics (see "**Antiseptics**") and nonspecifically kill pathogens. Thus, they often have a low therapeutic index and must be closely monitored for ichthyotoxicity during treatment. Certain species, such as scaleless fish (e.g., catfish, loaches), are often especially sensitive to water-borne treatments.

❧ Box III-1 ❧

EQUIPMENT NEEDED FOR TREATING FISH IN THE CLINIC

Balance
Various-sized beakers (100–2,000 ml)
Graduated cylinders (50, 250, 1,000 ml)
Weighing papers
Spatulas
1, 3, 5, 10, 20 cc syringes
21, 22, 23, 25 GA needles

Table III-7. Conversion factors for calculating treatments.

CONVERSION FACTORS FOR DRY MEDICATIONS			
No. mg/l	×	3.785	= No. mg/gallon
No. mg/l	×	0.001	= No. grams/liter
No. mg/l	×	0.0038	= No. grams/gallon
No. mg/l	×	1	= No. grams/m^3
No. mg/l	×	0.0283	= No. grams/ft^3
No. mg/l	×	100,000	= No. grams/hectare-meter
No. mg/l	×	1230	= No. grams/acre-foot
CONVERSION FACTORS FOR LIQUID MEDICATIONS			
No. ppm	×	0.001	= No. ml/liter
No. ppm	×	0.0038	= No. ml/gallon
No. ppm	×	1	= No. ml/m^3
No. ppm	×	2.83	= No. ml/ft^3
No. ppm	×	100,000	= No. ml/hectare-meter
No. ppm	×	1230	= No. ml/acre-foot

Table III-8. Conversion factors from English to metric units.

No. pounds	x	0.454	= No. kilograms
No. kilograms	x	2.20	= No. pounds
No. gallons	x	3.785	= No. liters
No. liters	x	0.264	= No. gallons
No. U.S. fluid ounces	x	0.0296	= No. liters
No. liters	x	33.8	= No. U.S. fluid ounces
No. dry ounces	x	0.0284	= No. kilograms
No. kilograms	x	35.3	= No. dry ounces
Degrees Celsius		= 5/9 (°F − 32)	= 0.55 (°F − 32)
Degrees Fahrenheit		= 9/5 (°C + 32)	= 1.8 (°C + 32)

The methods used for water-borne treatment range from high drug concentration–short exposure time (bath) to low drug concentration–long exposure time (prolonged immersion). Agents that are intended to treat systemic diseases must reach therapeutic levels in target tissues. Few drugs administered in water can do so. Finally, medications can strongly inhibit nitrifying bacteria in aquaria, killing fish with ammonia or nitrite poisoning. Bath treatments are most toxic to biological filters, but some medications (e.g., erythromycin, neomycin, or methylene blue) are toxic even when used as prolonged immersion. If both short- and long-term exposures are probably equally feasible and effective, it is preferable to use a short-duration drug exposure for the following reasons:

1. It may be less expensive because a smaller amount of drug is needed.
2. Because fish are usually moved to a treatment container, drugs do not have to be added to the system that holds the fish; thus, there is less of a problem with side effects, including toxicity to the biological filter, buildup of drug residues or metabolites in the environment (sediment, etc.), and/or development of resistant pathogens.

It is always advisable to perform a bioassay of a small number of individuals before treating any fish species without a known history of response to the treatment. Most water-borne doses are based on studies of well-established food fish species (e.g., salmonids). When treating other species, idiosyncratic or hypersensitivity reactions can occur. Obviously, bioassay is not feasible before treating an individual pet fish. Note also that even in fish species where drug dosages are well established, relatively small differences in dosage or exposure time can have a major effect on toxicity (Heinen et al. 1995); thus, bioassay is often advisable even for species in which dosages are well established, especially since environmental conditions (e.g., temperature) have a great influence on toxicity. Fish should never be left unattended during treatment; and if an adverse response occurs, the drug should be immediately removed by transferring the fish to clean water or diluting the treatment water.

Adequate plans for detoxification/removal/disposal of used drugs must be in place before treatment is begun. Used drugs must be disposed of responsibly. Disposal procedures depend on the type of drug and local government regulations. Proper disposal is especially important for flush and continuous flow treatments (see "**Activated Carbon**" in "**Pharmacopoeia**"). Know the environmental regulations before using any treatment, especially if effluent may enter public waters.

Bath Method in a Small Volume of Water

Fish are exposed to a concentrated drug solution for a short time (less than 24 hours). One to many fish can be treated simultaneously. The concentration required for effective bath treatment is often toxic to nitrifying bacteria; so, when treating fish that are housed in aquaria or other systems that have biological filters, either treatment should be done in a separate container or the biological filter should be turned off during treatment, followed by replacement of the treated water with clean water. Alternatively, the system can be immediately reseeded with nitrifying bacteria (see "**Nitrifying Bacteria**")

when treatment is finished, but this runs the risk of causing ammonia/nitrite poisoning if inadequate nitrifying bacteria are added. All drugs should be completely dissolved and mixed in the treatment water before adding fish, unless this is not possible. In weak individuals or sensitive species, it is best to give the lower recommended dose. If needed, this might be able to be repeated, depending upon how the fish respond.

1. Add water to a clean container (Fig. III-1). Add a maximum of about 5–10 grams of fish/liter of water used for treatment (this will vary with species). Use lower density if a long-term bath (several hours) is anticipated. The amount of water added should be carefully measured, so that an exact drug concentration can be calculated (see step 2). For larger baths, a water meter (available from Aquatic Ecosystems) can be used to determine the amount of water in the container. It is desirable to place an airstone in the container and it is essential if the fish will be crowded or treated for a long period.
2. Use a syringe or other volumetric container, and add exactly the amount of drug needed for treatment. Mix well by swirling. See Boxes III-2, III-3, and III-4 for calculating the amount of drug to add.

Fig. III-1. Bath method of water-borne treatment in a small volume of water. A. Add a known volume of fresh, dechlorinated water to a small container using a graduated cylinder. Note the container with untreated, aerated water nearby. B. Add a known volume of drug to the treatment container using a 1cc syringe. The drug should then be thoroughly mixed. C. Add the fish to be treated to the treatment container. D. Overdose, as indicated by listing of the fish to one side. Fish should be removed immediately if this occurs. E. Overdose, as indicated by dyspnea (mouth and opercula open wider than normal).

❧ Box III-2 ❧

SAMPLE CALCULATION NO. 1: PROLONGED IMMERSION TREATMENT WITH A DRY MEDICATION

A fish pond is to be treated with potassium permanganate prolonged immersion. After determining the permanganate demand of the pond water, the farmer needs to give a dose of 2 mg/L. The pond volume is 3 acre-feet. How many grams of potassium permanganate are needed for treatment?

1. Using Table III-6, convert the concentration of chemical to be used to the volumetric units of the culture system to be treated. Since the pond volume was measured in acre-feet, the correct formula to use is:

No. mg/L × 1,230 = No. grams/acre-foot

2 mg/L × 1,230 = No. grams/acre-foot
= 2,460 grams potassium permanganate/acre-foot

2. Determine the number of grams of drug needed to treat the pond by using the following formula:

Drug = volume water × concentration of drug × 100/%AI* (g)
= 3 acre-feet × 2,460 grams/acre-foot × 100/100
= 7,380 grams = 7.38 kg potassium permanganate

*Note that potassium permanganate is considered 100% active

3. If the drug is to be weighed out in pounds, it can be converted to English units by using the conversion chart in Table III-7:

No. kg × 2.2 = No. lb

7.38 × 2.2 = 16.2 lb of potassium permanganate

*%AI = percent active ingredient in the drug. Note that potassium permanganate is considered 100% active.

❧ Box III-3 ❧

SAMPLE CALCULATION NO. 2: PROLONGED IMMERSION TREATMENT WITH A LIQUID MEDICATION

An aquarium is to be treated with formalin prolonged immersion. The desired dose is 25 ppm. The volume of water in the aquarium is 185 gallons. How much formalin is needed for treatment?

1. Using Table III-6, convert the concentration of drug to be used to the volumetric units of the culture system to be treated. Since the aquarium's volume was measured in gallons, the correct formula to use is:

No. ppm × 0.0038 = No. ml/gallon

25 ppm × 0.0038 = No. ml/gallon
= 0.095 ml formalin/gallon

2. Determine the number of ml of drug needed to treat the aquarium by using the following formula:

Drug = volume of water × concentration of drug × 100/% AI* (ml)
= 185 gallons × 0.095 ml/gallon × 100/100
= 17.6 ml of formalin

*%AI = percent active ingredient in the drug. Note that undiluted formalin is considered 100% active.

3. Net out the fish to be treated, and place them in the treatment solution for several seconds to several hours. The "**Pharmacopoeia**" gives exact times needed for specific drugs. For any treatments over 1 minute, vigorous aeration of the water is mandatory to maintain adequate oxygen levels. Fish should be monitored constantly. If fish become distressed (excitable, attempt to jump out of the water, depressed, lose equilibrium, and/or begin to list to one side), immediately place them in untreated water, even if the full time course of treatment is not complete. Toxicity with bath treatments is most common when antiseptics are used.
4. After exposure to the bath, immediately net out the fish and return them to unmedicated, aerated water. Observe closely over the next several days to see if a second treatment is needed.

❧ Box III-4 ❧

SAMPLE CALCULATION NO. 3: PROLONGED IMMERSION TREATMENT WITH A COMMERCIAL DRUG SOLUTION

An aquarium of freshwater pet fish is to be treated with 50 mg/L kanamycin sulfate prolonged immersion. The commercial preparation of kanamycin sulfate contains 250 mg kanamycin sulfate/ml of fluid. The aquarium has 5 gallons of water. How much of the kanamycin sulfate commercial preparation must be added to the aquarium?

1. Using Table III-6, convert the concentration of active ingredient to be used to the volumetric units of the culture system to be treated. Since the aquarium's volume is measured in gallons, the correct formula to use is:

$$\text{No. mg/L} \times 3.785 = \text{No. mg/gallon}$$

$$50 \text{ mg/L} \times 3.785 = \text{No. mg/gallon}$$
$$= 189 \text{ mg kanamycin sulfate/gallon}$$

2. Determine the mg of active ingredient (AI) needed by using the following formula:

$$\text{Amt. of AI} = \text{volume of water} \times \text{concentration of drug} \times 100/\%\text{AI (mg)}$$
$$= 5 \text{ gallons} \times 189 \text{ mg/gallon} \times 100/100$$
$$= 945 \text{ mg of kanamicin sulfate}$$

3. Determine the volume of commercial drug needed to treat the aquarium:

$$\frac{\text{No. mg needed for treatment}}{\text{No. mg/volume of commercial preparation}} = \text{volume of commercial preparation needed}$$

$$\frac{945 \text{ mg}}{250 \text{ mg/ml}} = 3.8 \text{ ml of commercial preparation needed}$$

5. This procedure can be used to treat large numbers of fish by simply increasing the volume of water used for the bath accordingly.
6. When fish are treated in flow-through systems, the water flow is stopped and the drug is immediately added to the water that holds the fish. If possible, lower the water level before treatment to decrease the amount of drug needed and to allow quicker dilution if toxicity occurs during treatment. Do not add concentrated drug directly onto the fish. If there is a risk of environmental hypoxia during treatment and supplemental aeration is not available, flush or constant-flow treatment may be required instead.

Bath Method in a Cage

Water-borne treatment of cage-cultured fish presents considerable challenges. Water-borne treatments are either administered while the fish remain in the culture cage, or the fish are placed in an adjacent temporary holding system for the treatment (Fig. III-2). When treated in their culture cage, the bottom of the cage is raised, usually to 2 meters (= 6.5 feet), to reduce the volume of water to be treated. A plastic or canvas skirt (tarpaulin) is then placed around the culture cage and the drug is added at several locations in the cage to accelerate even mixing. Supplemental oxygen should also be added. At the end of the treatment period, the skirt is removed, allowing the drug to dissipate. Supplemental oxygen should continue to be supplied for a short time afterward.

This method has several disadvantages, including being very labor-intensive, wasteful of drug, and possibly environmentally damaging. In addition, the actual volume of water to be treated cannot be accurately determined, making over- or under-dosing a significant problem (Treves-Brown 2000). To address this, a small loading dose can be added to the cage, and then a rapid (within minutes) test to measure the drug concentration on site can be performed. Once the concentration is determined, additional drug needed to achieve the desired dose can be added. If a rapid test is unavailable, a lower dose but longer exposure time can be used for some drugs, but this is less reliable. If distress is observed, the skirt should be removed immediately.

In marine environments, treatments should always be performed at slack tide. Thus, when the tide starts to run, the drug will be more rapidly removed from the cage and more quickly diluted in the environment.

A modification of this procedure involves the placement of a smaller treatment cage immediately adjacent to the culture cage; the fish are then netted and placed in the smaller treatment cage to be treated (Beveridge 2004). Alternatively, the culture cage holding the fish may be towed to a treatment cage and the fish pumped into the treatment (Fig. III-2, A). However, these methods of fish transfer are stressful. Another modification is to use a treatment cage that has only three sides attached to the collar that floats the treatment cage (Fig. III-2, B); the adjacent side of the culture cage is then opened and the netting of the culture cage is attached

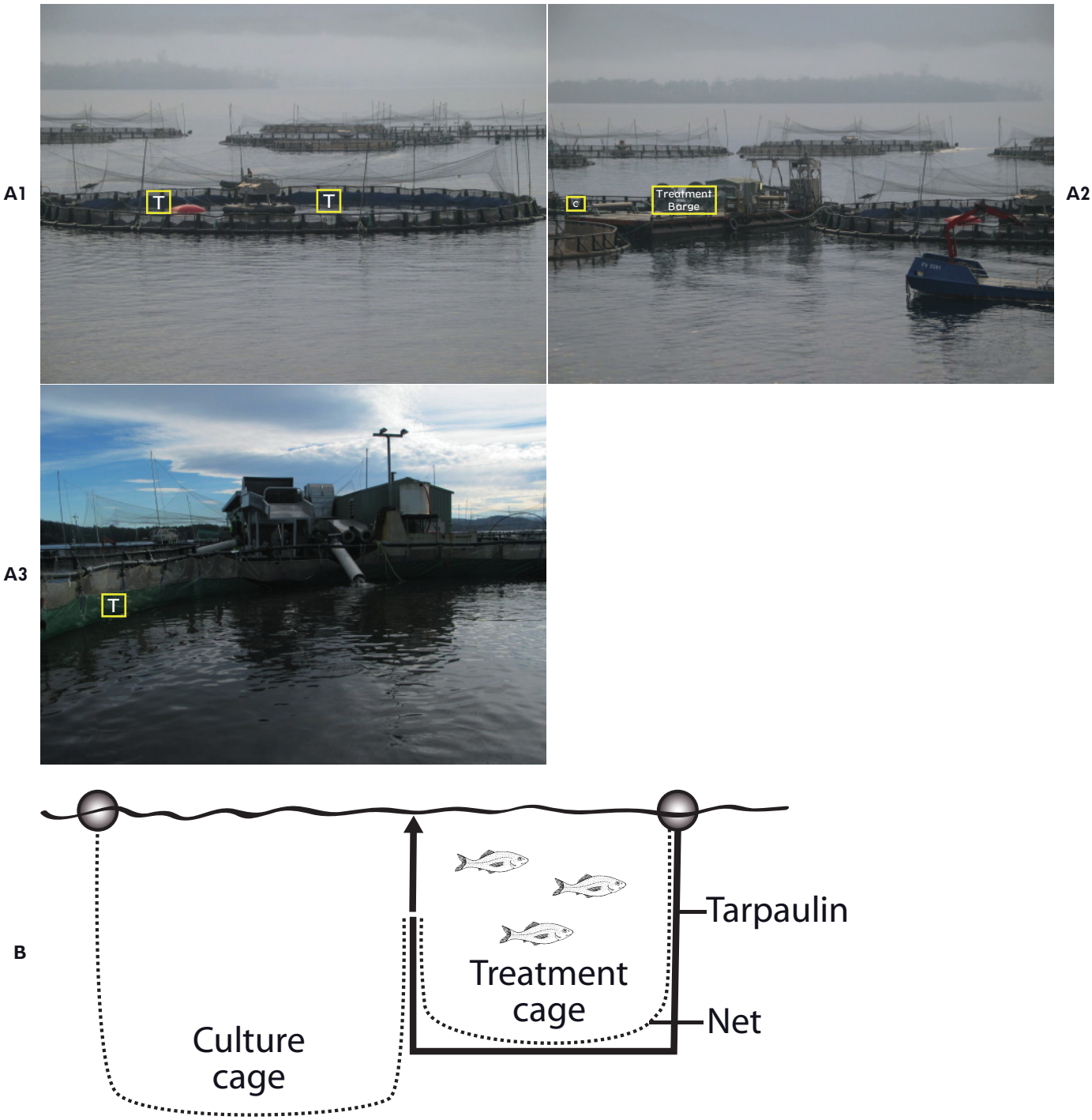


Fig. III-2. Bath method of water-borne treatment in a cage. A. Bath method in a cage. (1) A tarpaulin (*T*) has been placed around the inner perimeter of the treatment cage to be used for treating the fish. (2) A culture cage (*C*) holding the fish to be treated has been pulled alongside a barge (treatment barge) having a pump. (3) The fish are being pumped from their culture cage into the treatment cage. *T* = tarpaulin. B. Drawing of a bath unit for treating caged fish. The fish have swum from the culture cage into the treatment cage. The open side of the treatment cage is being lifted (*arrow*) so the netting and tarpaulin can seal the opening. Treatment then begins. (*A* photographs courtesy of M. Adams; *B* modified from Beveridge 2004.)

to the netting of the treatment cage. The netting at the bottom of the culture cage is then lifted, inducing the fish to swim into the treatment cage. The skirt on the fourth (open) side of the treatment cage is then lifted and the drug is added to the treatment cage. After treatment, this procedure is reversed (Brandal and Egidius 1979). The main advantage of this method is that less drug is released into the environment. However, it is impractical to use with large cages.

Flush Method

Flush is a modification of the bath treatment for flow-through systems. Water flow is not stopped, but a high concentration of chemical is added at the inlet and passed through the system as a pulse. The entire dose should be added in 1–2 minutes. A measured amount of drug is added to the system upstream and allowed to flush through. Flush has been most widely used in salmonid hatcheries. Flush treatment is only feasible for systems that have enough flow to completely flush out the drug within a predetermined time. Highly toxic treatments should not be applied as flush treatments, since a uniform drug distribution within the system cannot be ensured (Piper et al. 1982). Fish will usually retreat from the drug and then rapidly rush through it, reducing the effective exposure. This can be ameliorated by crowding the fish downstream, where mixing of the drug will be most thorough and where the fish cannot escape. It is best to use a reduced flow for flush treatment, so the flow can be increased quickly if needed (adverse side effect, hypoxia). The suggested doses may need to be optimized for different systems.

Constant Flow Method

Constant flow treatments have been used in flow-through systems when it is not possible to shut off the water long enough to use a bath treatment (i.e., even a temporary halt in water supply might cause fish mortality because of oxygen depletion or waste accumulation). Thus, the dosage that is administered with constant flow is exactly the same as that administered with a bath; the only difference is that the water is constantly moving (constant flow) rather than being static (bath). Drugs used in constant flow treatments include formalin, quaternary ammonium compounds, and potassium permanganate. Constant flow treatment is especially good for water mold control on eggs and for treating fish in raceways and small earthen ponds, especially where inflow water turnover rates are less than 1 water change per hour. Treatments are only performed for 1 hour, and "dead spots" must be treated by hand to ensure even chemical concentration (Warren 1981).

The volume of water flowing into the unit must be accurately determined. A stock solution of the drug treatment is precisely metered into the water to obtain the desired therapeutic concentration. Chemical dosimeters are available for metering but are expensive. Commercial poultry waterers (Agri-Pro Enterprises; Ziggity Systems, Inc.), large carboys with spigots, or intravenous drip bags are inexpensive alternatives (Piper et al. 1982). However, flow rate will change with head pressure in gravity-fed devices, so they need constant monitoring.

Before the metering device is started, enough drug should be added to the water that contains the fish to produce the desired final concentration; this reduces the total amount of drug needed for treatment by eliminating the time needed to reach therapeutic concentrations. When the treatment period is completed, the inflow of drug is stopped and the unit is flushed with untreated water. Partial draining of the system will help to speed elimination of the drug. See Piper et al. (1982) for more details concerning constant flow treatments.

The amount of drug needed for constant-flow treatments is computed the same as for bath treatments, except that the flow rate of the water and treatment time must also be taken into consideration.

Constant-flow treatment is less desirable than baths, because of the greater expense and problems with release of toxic chemicals into the environment. Drug concentration in the effluent that is released into natural waters must be in compliance with environmental regulations. In order to do so, the effluent is typically either diluted until an acceptable drug level is reached or the effluent is treated with activated carbon.

Prolonged Immersion Method

Aquaria and Ponds

Fish are left in a low concentration of drug for at least 24 hours. The drug dissipates in the water by natural decay. One advantage to this treatment is that water changes after treatment are usually not mandatory (although still desirable) and many prolonged immersion treatments do not severely impair biological filtration, allowing their use in the system used to maintain the fish.

1. Aquaria: Add the drug to either the display tank or a hospitalization tank having biological filtration. Activated carbon filtration and disinfection equipment (ozonation, ultraviolet filtration) must be stopped during treatment. Some advocate turning off all filtration during treatment. Reducing filtration rate may be useful, but completely shutting off the filter may kill the nitrifying bacteria after several days.
   During and following treatment, ammonia, nitrite, and pH should be monitored at least every few days, if possible. The volume of water to be treated is usually fairly easily estimated if the tank size is known. However, a decremental adjustment should be made for objects in the tank that displace water (e.g., gravel, rocks, and coral). An example is shown in Box III-3.

Add a filter that has activated carbon at the end of the treatment to remove residual drug.

2. Ponds: Add the drug either at the pond bank or by boat. The proper application of chemicals to fish ponds requires correct calculation of the amount of chemical to be applied. One must always know the exact volume of water to be treated.

Calculation of Pond Treatments

Fertilizers and liming materials are usually applied to ponds on an areal basis (e.g., 2,000 kg of agricultural lime/hectare). All other agents must be applied more accurately and are thus calculated on a weight per volume (w/v) basis (e.g., mg/ liter of copper sulfate) or volume per volume (v/v = ppm). It is satisfactory to estimate the amount needed to treat by +/− 10%, but an overestimate of 10% is potentially toxic, as well as wasteful and expensive when large ponds are treated (Boyd 1990).

It is easiest to calculate pond volume in units of acre-feet (1 acre-foot of water is equal to 1 surface acre, 1 foot deep) or hectare-meters (1 hectare-meter of water is equal to 1 surface hectare, 1 meter deep). If a pond's volume is not known, its dimensions should be measured and its volume calculated. Measure the length and width of the pond at the water line and convert the area to surface acres or surface hectares. One surface acre equals 43,560 ft^2. One surface hectare equals 10,000 m^2. Volume is equal to the product of surface area (acres or hectares) and average depth (feet or meters). Average depth can be calculated by making multiple depth measurements and calculating an average. If maximum depth is known, average depth can usually be estimated to be 40% of the maximum depth (Tucker 1984); however, this is less accurate than taking multiple depth readings and may be inaccurate in ponds that have highly uneven bottoms. More accurate methods of determining pond volume (e.g., surveying) are available (Boyd 1990) but are not needed for successfully applying pond treatment, although more accurate estimations may be economically justified for treating large ponds.

Once the volume of water to be treated is known, the amount of drug to apply is calculated as shown in Box III-2. Jensen and Durborow (1984), Piper et al. (1982), and Wellborn (1978) provide more details on calculating treatments.

Application of Drugs to Ponds

Once the exact amount of drug to apply is known, an application method is selected. Application must be uniform to avoid forming "hot spots" of excess drug, which can overdose the fish (Boyd 1990). Supplemental aeration should be readily available (Noga and Francis-Floyd 1991).

Large farms can afford specialized chemical applicators. Boat-mounted tanks can be used to spray dissolved drug over the pond. Liquids can also be dispensed through a pipe having small-diameter holes in its underside (Burkhalter et al. undated). The pipe is hung over the edge of the boat. Solution flow can be regulated either by using a valve on a gravity-fed device or by using a water pump if more uniform release is desired. Drug can be released under the water surface by attaching small vertical tubes of the desired length to the holes in the pipe. Crystals and granules can be spread by using dispensers similar to fertilizer distributors. These dispensers are usually hoppers with adjustable dispensing holes in the bottom. An auger is used to prevent clogging of the holes with coarse particles (Schoenecker and Rhodes 1965). A simple modification of this device was designed by Boyd (1990). An outboard motor propeller mixes the drug with the water as it is released from the siphon. This type of dispenser is available commercially.

Owners with a single pond usually cannot justify purchasing application equipment. In such cases the drug can be dissolved in a large container of water and applied to the pond surface using a garden sprayer. Otherwise, it can be dispensed with a bucket from a boat. Caution owners about the toxicity of the drug to be handled. It may not be advisable to have owners use this technique for highly toxic drugs (e.g., formalin). Granules can be broadcast by hand or with a small "cyclone" seeder. However, heavy crystals (e.g., copper sulfate and potassium permanganate) sink quickly and can be quickly inactivated in the sediment if simply broadcast over the water. Crystals can be placed in a burlap bag and towed behind a boat until they have completely dissolved (Boyd 1990). More details on drug applications are provided by Boyd (1990).

Prolonged Immersion Bioassay

It is especially advisable to perform a bioassay before using some prolonged immersion treatments because prolonged immersion cannot easily be stopped if toxicity develops during treatment. While the safe therapeutic range has been established for most drugs used for commonly cultured food fish (e.g., salmonids, channel catfish), caution is advised when using any drug on a fish species for which no data are available on that species' susceptibility to the drug. Bioassays are also advisable if a particular farm has not previously used copper, since copper's toxicity is often unpredictable.

A bioassay can be performed by placing five or six fish in an aquarium that has treated pond water. Aerate the water to prevent hypoxia. When holding tanks or aquaria are not available, fish can be placed in large polyethylene bags that are filled with pond water and secured to the pond bank (Burress 1975). Fish are seined from the pond and placed in the bags at a density of not more than 1 g/l. The fish should be observed for 1–2 days before treatment to be sure that none have died from

stress of collection. Fish should not be fed while in the bags. The test drug is added to the bags (it is best to test three doses within the desired range), and mortality is compared with fish held in bags without the drug. Fish should be held in the bags for at least 96 hours, unless the drug is known to degrade more quickly.

These procedures can also be used to determine when it is safe to stock fish in a treated pond, such as after liming to disinfect (Boyd 1990). In the latter case, control fish should be placed in bags that have water from a known nontoxic pond for comparison.

Swab and Ointment

The swab is not commonly used because few skin diseases are localized enough to allow this to be effective; it is probably most useful in treating local traumatic wounds that are secondarily infected by bacteria or water molds. Dip a cotton swab in a drug solution and gently touch the swab to the lesion, allowing the solution to soak the lesion via capillary action.

Oral Medications

Oral medications are one of the best ways to administer drugs to fish because they are the least stressful, yet if consumed in the proper amounts and absorbed by the gastrointestinal tract, they can be very effective. However, they can also be cumbersome if a commercially prepared oral medication is not available. Also, sick fish will often not eat, rendering this therapy useless. Force-feeding can be an option but is not often used (see below). Withholding food for 12–24 hours may increase the acceptance of a medicated feed. If fish still refuse the medicated feed after a 24-hour fast, they can be fasted longer if their health status allows this. However, withholding feed is not routinely done and must be done with caution, since this might make them weaker and exacerbate the disease.

The dosage can vary within limits, depending on the feeding rate. For example, in the United States, oxytetracycline (OTC) can be incorporated into the feed at 2.75–3.5 g/100 lb of feed. At the lower concentration, fish will get 52 mg OTC/kg if eating 1% of their body weight per day. If the fish are eating 3% of their body weight per day, the lower dose can be used. It is usually best to use a feed that has enough medication so that feeding at a rate of 1% of body weight per day will give the needed dosage. This helps to ensure that the fish consume enough medication even if their appetite is decreased. The remainder of the daily ration can then be given as a nonmedicated feed.

Commercially Medicated Feeds

Antibiotic-medicated feeds are available for food fish. These feeds can also be fed to aquarium fish directly or can be incorporated into gelatin. A small, crumble-type feed is small enough to be eaten by most aquarium fish. Pellets can also be crushed into smaller pieces for smaller fish by using a mortar and pestle. Medicated feeds for food fish are usually sold only in large quantities (e.g., minimum of 50 lb for many feeds) but are much less expensive than aquarium medications and, if frozen, will last for well over 1 year in storage. Some commercial aquarium feeds are also medicated with antibiotics, but there are no published data on their efficacy.

Injection of Individual Food Items

Injection of food items is a relatively easy way to give oral medications to small numbers of fish. The required dosage is injected into a small fish, which is then fed to the sick fish. This method has limited usefulness, since not all sick fish will accept such preparations, only large fish can be treated, and there is risk of introducing other diseases with the medicated fish.

Loading Food with a Medication

Small food items (e.g., brine shrimp) can be "loaded" with therapeutic levels of drug by soaking in a drug solution (for examples, see "**Sulfadimethoxine-Ormetoprim**" under "**Antibiotics**" and see "**Metronidazole**").

Preparation of a Medicated Artificial Diet

The key to making a successful, medicated artificial diet is to prepare one that will be readily eaten by the sick fish; this can often be difficult, since even healthy fish often initially refuse any change in their normal diet. The most common way to prepare a medicated diet for pet fish is to mix food with gelatin and then add the proper dose of medicine just before hardening the gelatin by refrigeration. A key factor in success is palatability. Regularly feeding fish the same artificial diet (without drug) will acclimate them to the diet, reducing their reluctance to eat it if they ever need to be fed the medicated diet (R. Floyd, personal communication). Gelatin is high in calcium and thus may bind some antibiotics, such as tetracyclines and quinolones. However, this has never been reported to be a problem in diet preparations. Three suggested formulae follow.

Preparing a Gelatin Diet for Aquarium Fish

1. Dissolve 30 grams of unflavored gelatin in 500 ml of boiling water (= 30 grams in 17 U.S. fluid ounces).
2. Thoroughly suspend about 300 grams of commercial fish feed (Purina Trout Chow® or equivalent) in about 150 ml of water. The feed should be in as fine a suspension as possible.
   Note: A gelatin diet is also available from Mazuri.
3. Pour the gelatin solution into the wet food mixture.
4. Mix well, and add more fish feed; try to get as much feed added to the liquid suspension as possible. When the suspension has cooled to room temperature,

❧ Box III-5 ❧

SAMPLE CALCULATION NO. 4: ORAL MEDICATION

A group of trout are to be fed a medicated diet that contains Tribrissen® (40% active sulfadiazine-trimethoprim). The dose to be used is 50 mg sulfadiazine-trimethoprim/kg of body weight. If the fish are to be fed the medicated diet at a rate of 1% of body weight (BW) of fish/day, how much sulfadiazine-trimethoprim must be added to 100 lb of feed to provide the correct dosage?

1. Fish are to be fed 50 mg sulfadiazine-trimethoprim/kg BW

   Feeding rate = 1% of BW/day
   = 10 g feed/kg BW/day

   Fish are to be fed 50 mg sulfadiazine-trimethoprim/10 g feed
   = 5,000 mg sulfadiazine-trimethoprim/kg feed
   = 5 g sulfadiazine-trimethoprim/kg feed

2. Tribrissen® is 40% sulfadiazine-trimethoprim

   Fish must be fed: 5 g/0.4 = 12.5 g Tribrissen®/kg feed

3. If the commercial preparation is to be added to pounds of feed, it can be converted to English units using the conversion chart in Table III-7:

   No. g/kg × 0.454 = No. g/lb

   12.5 g Tribrissen®/kg feed × 0.454
   = No. g Tribrissen®/lb feed
   = 5.7 g Tribrissen®/lb feed
   = 570 g Tribrissen®/100 lb feed

4. To add to the feed, mix with 0.5 kg of soybean oil/25 kg of feed:
   = 0.02 kg of soybean oil/kg of feed
   = 20 g of soybean oil/kg of feed
   = 12.5 g Tribrissen®/20 g soybean oil/kg of feed

dissolve the appropriate amount of drug (Boxes III-5 and III-6) in the water, and mix the solution into the food/gelatin mixture.

5. Line a large plastic dish pan with aluminum foil, and pour the food/gelatin mixture into the pan, spreading it evenly over the entire pan to a thickness of about 1/2 inch (~1 cm).
6. Place the pan in the refrigerator for 2–4 hours until it has gelled; then cut it into blocks, place it in an airtight bag, and freeze.
7. Remove bags as needed, cut into appropriately sized square blocks, and feed to fish.
8. Food fish feeds will often taint aquarium water a yellowish-brown. Substituting an aquarium-type pelleted feed or a flake food for the food fish ration will avoid this problem. More complex gel diet formulae are available (Bower 1983; Spotte, 1992) but are not needed if the above diet is eaten.

Preparing Gelatin Coating of Pellets for Large Fish (Piper et al. 1982)

This treats 100 lb of pellets:

1. Slowly dissolve 125 grams of gelatin in 3 quarts of boiling water (= 25 g/2.8 liters).
2. Allow the gelatin to cool to room temperature, and then stir the appropriate amount of drug (Table III-5) into the gelatin until there are no lumps.
3. Slowly add the drug-gelatin mixture to the pellets (stir by hand, or use a cement mixer). Stir only long enough to mix (don't break the pellets).

Preparing Oil Coating of Pellets for Large Fish

Use a wt:wt ratio of 2–3 parts oil:100 parts feed (Piper et al. 1982):

1. Heat 2–3 lb (or 2–3 kg) of soybean oil to 100–120°F (40–50°C).
2. Quickly mix the drug evenly into the warm oil.
3. Quickly pour or spray the drug-oil mixture over 100 lb (or 100 kg) of pellets (keep exposure of antibiotics to high temperature as short as possible).

Note that lipid carriers may prevent oral uptake of some antibiotics in salmonids, especially macrolides (Austin 1985). This phenomenon has not been examined in other species.

The amount of drug needed to be placed in a diet is given in the "**Pharmacopoeia**." Note that normal feeding rates decrease with lower temperature. The method for formulating a medicated feed is shown in Box III-6.

Force–Feeding

Oral medications can be given via stomach tube (Andrews and Riley 1982; Lewbart 1998).

1. Attach a stomach tube made from a dog catheter (3 mm outer diameter) to a 5 cc syringe, using cyanoacrylate glue (Super Glue®, Loctite). Fill the tube and the syringe with liquid medication. For fish that are larger than 40–50 cm long, a larger diameter tube is usually needed (a horse catheter, with 6 mm outer diameter tubing, attached to a 20 cc syringe has been used). Any tube used should have a smooth anterior

Box III-6

SAMPLE CALCULATION NO. 5: ORAL MEDICATION

The amount of drug that must be added to a feed can be easily calculated by using the dosage (D) of drug to be administered orally and the medication rate (R% of body weight [BW])/day:

D mg/kg BW fed at R% of BW/day requires the addition of the following:

$$\frac{(0.01)(D)}{R}\ \%\ \text{of drug in the feed}$$

Or

D mg/lb BW fed at R% of BW/day requires the addition of the following:

$$\frac{(0.022)(D)}{R}\ \%\ \text{of drug in the feed}$$

Example No. 1: If fenbendazole is to be fed at a rate of 25 mg fenbendazole/kg BW/day and the food will be fed at a rate of 1% of BW/day, the following amount of fenbendazole must be added:

$$\frac{(0.01)(D)}{R} = \frac{(0.01)(25)}{1}\ \%$$

= 0.25% fenbendazole in the diet, or 0.25 g fenbendazole added to every 100 g of feed

Example No. 2: If the same dosage of fenbendazole is fed as mg/lb (i.e., at a rate of 11 mg fenbendazole/lb BW/day) and the food will be fed at a rate of 1% of BW/day, the following amount of fenbendazole must be added:

$$\frac{(0.022)(D)}{R} = \frac{(0.022)(11)}{1}\ \%$$

= 0.24% fenbendazole in the diet, or 0.24 g fenbendazole added to every 100 g of feed (the value is slightly different from the result in Example No. 1 since the 25 mg/kg dose is slightly greater than the 11 mg/lb dose)

end to avoid damage to the gill tissue and gastrointestinal mucosa.
2. Anesthetize fish, and place fish in a lateral recumbency on a smooth, nontraumatic surface.
3. Insert the tube into the stomach (or the anterior intestine of cyprinids, since they do not have a stomach; Fig. III-3).
4. Administer at a rate of about 1.0–1.25 ml/kg body weight (= 0.45–0.56 ml/lb). Both solutions and suspensions can be administered.
5. Observe closely after recovery for possible regurgitation.

Injection

Injection of drugs has the advantage of delivering a precise dosage. Disadvantages include the stress imposed by capturing the fish and, for aquarium fish, the need to bring the fish to the clinic for every injection, since the owner is usually unable to perform the treatment. The weight of the fish must be closely estimated; this is best done by using a scale and weighing by displacement. A container with aquarium water is placed on a scale. The fish is then added, and the change in weight is determined; however, this is only feasible for small fish, unless a large scale is available. Large fish (>~200 g) are more easily weighed by placing them directly on the scale. Fish should be sedated during weighing, unless they are weak and it does not appear that they may tolerate sedation. Most veterinary preparations must be diluted considerably in sterile diluent (saline or water) to administer the proper dosage to aquarium fish.

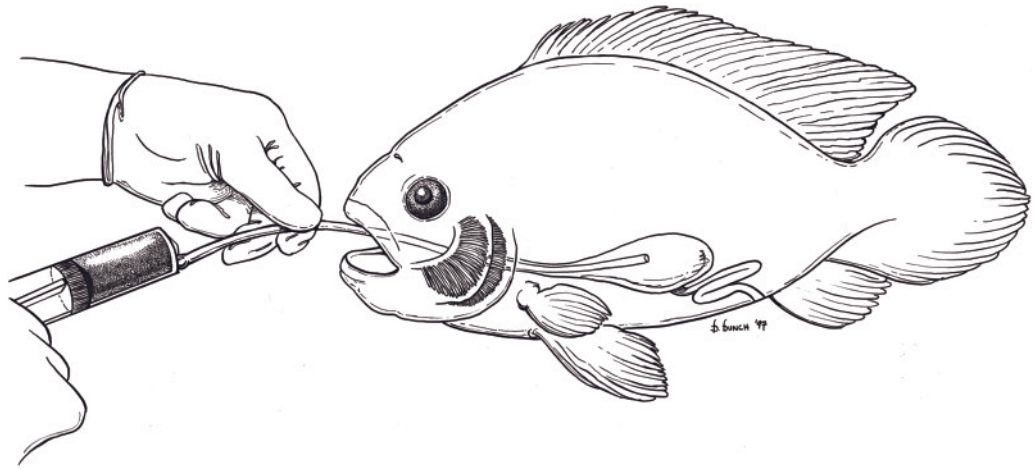
Intraperitoneal (IP)

Fish should be fasted for 24 hours before injection. Failure to do so runs the risk of causing peritonitis caused by puncture of the stomach or bowel. The landmarks for an IP injection are the pelvic fins and the anus. In the more primitive teleosts (e.g., salmonids, goldfish, catfish) the pelvic fins are located in the posterior portion of the body. In the advanced teleosts (i.e., the great majority of fish species), the pelvic fins have evolutionarily migrated anteriorly and the pectoral fins have migrated dorsally. An IP injection can usually be given anywhere from midway between the pectoral and pelvic fins to just anterior to the anus (Akhlaghi et al. 1993; Fig. III-4, A). However, it is best to avoid the area around the pectoral or pelvic girdles.

The injection should be made near the ventral midline. Fish should be held in dorsal recumbency. A proportionately small-gauge needle (25 GA or less) is recommended for fish ~3–4 inches (~8–10 cm), and the needle should not be inserted too far past the body wall to avoid entering the gastrointestinal tract. Presence in the peritoneal cavity is indicated by a lack of resistance to injection and free movement of the end of the needle.

Intraperitoneal injection is a very common method used for administering vaccines, especially to salmonids (Kollevág, 2006). Whenever large numbers of fish are to

A



B

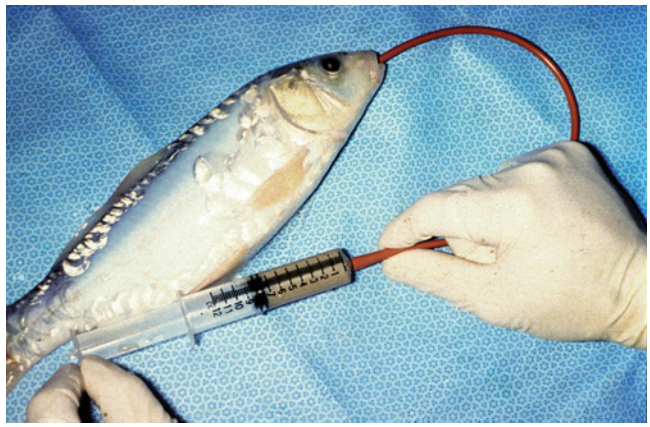


Fig. III-3. A. Method for inserting a stomach tube. B. Administering medicated feed to a koi. (*A* from Lewbart 1998; *B* photograph courtesy of G. Lewbart.)

be injected, one must try to ensure that all fish are consistently injected properly. Improper injection can lead to a number of problems, including mortality from injection, reduced efficacy of the vaccine, side effects (local reactions), reduced carcass quality, and vaccine failure. Even apparently minor deviations from the recommended injection site can lead to decreased vaccine efficacy and adverse reactions.

Common technical problems include incorrect position of the injection site, too shallow an injection (injection goes into the muscle), too deep an injection (injection goes into the viscera), and tears at the injection site. Use of oil-based adjuvants in vaccines is highly prevalent and improper injection can lead to the development of intraperitoneal adhesions.

Deviations from the correct injection site are usually due to one or more of the following:

- Great variation in size of the fish, resulting in too shallow or too deep injections
- Speed of vaccination too high, resulting in errors
- Failure to validate the injection technique
- Failure to regularly adjust the injection equipment for each individual vaccinator
- Failure to fast the fish
- Dull needles

To properly inject salmon and trout fingerlings:

- Inject along the ventral midline, one pelvic fin length anterior to the base of the pelvic fins (Fig. III-4, B).
- Do not inject when the temperature is <15°C (<59°F) from the time of vaccination until several weeks after sea transfer. Note also that a high or rapid increase in water temperature might lead to more local adverse reactions.
- All fish should be at least 35 g.

Details on these guidelines are provided in Kollevág (2006).

Intramuscular (IM)

This type of injection is best used only on fish greater than 5 inches (13 cm) long. The best site is the dorsal musculature just lateral to the dorsal fin (Fig. III-4, C). Only relatively small amounts can be injected (~0.05 ml/50 grams of fish). Fish that are not sedated will have tense muscles and thus are more difficult to inject. Injections should be done slowly to allow maximal deposition of material. This route has the disadvantage of causing damage to carcass quality and the potential of forming sterile abscesses. However, it produces a much

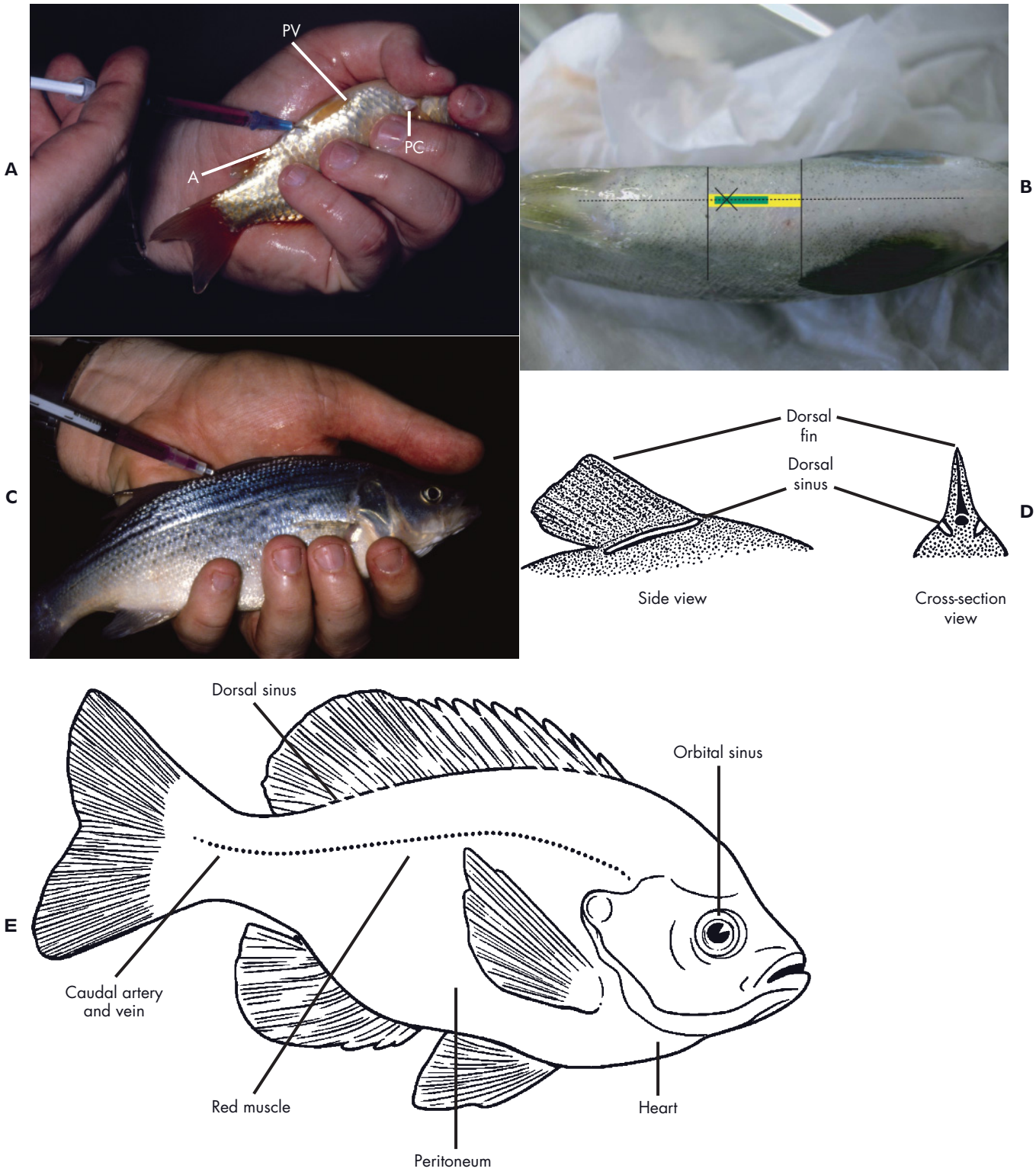


Fig. III-4. Injection of fish. A. Site for IP injection. *PC* = pectoral fin; *PV* = pelvic fins; *A* = anus. B. Proper IP injection site (X) for fingerling salmon and trout. Head is to the right. C. Intramuscular injection of fish. The injection should be made lateral to the base of the dorsal fin. D. Location of the dorsal sinus. E. Various sites available for injection. For injection into caudal vessels, heart, and red muscle, also see Figs. I-15, I-16, I-17, and I-30, *D.* (*B* photograph from Kollevåg 2006.)

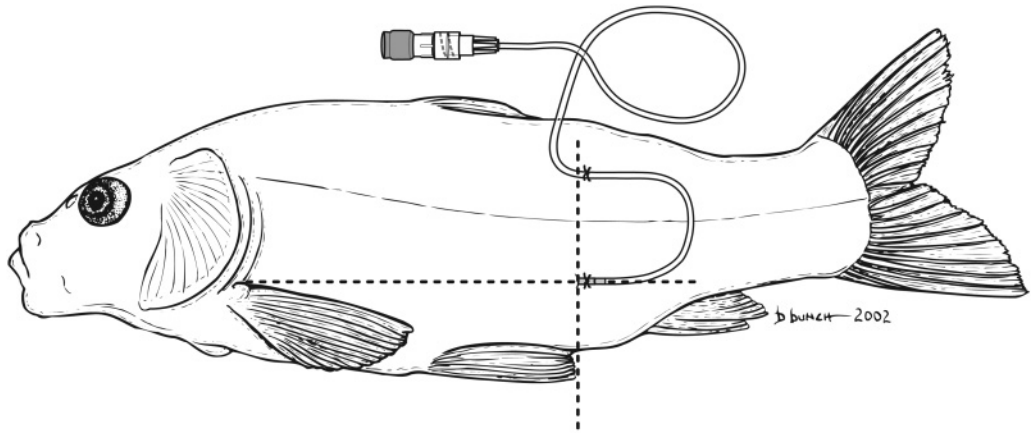


Fig. III-5. Method for inserting an indwelling catheter into the peritoneal cavity. (From Lewbart et al. 2005.)

more reproducible uptake of some drugs and maintains drug levels for a longer time (e.g., see "**Oxytetracycline**" and "**Flumequine**" under "**Antibiotics**") (Nouws et al. 1992).

Dorsal Sinus

This type of injection is mostly used when treating salmonids for bacterial kidney disease (see PROBLEM 54). The dorsal sinus runs near the dorsal fin (Fig. III-4, D). Because it can be difficult to inject, drugs intended to enter the dorsal sinus are often inadvertently deposited subcutaneously (C. Moffitt, personal communication).

Indwelling Intraperitoneal (Intracoelomic) Catheter

A number of relatively complex methods have been developed for inserting indwelling catheters that are used for drug administration or repetitive blood sampling (reviewed in Bakal et al. 1999). These procedures typically involve catheterization of the dorsal aorta as it traverses the roof of the mouth or catheter placement directly into the sinus venosus.

A much simpler method is the placement of an intraperitoneal (intracoelomic) catheter (Fig. III-5; Lewbart et al. 2005). An intraperitoneal catheter is much easier to implant and maintain than an intravascular catheter, making it advantageous in clinical situations where an intraperitoneal drug would require daily or more frequent dosing.

The plastic wings of a 23-gauge, 3/4-inch butterfly catheter from a Vacutainer® 3/4 × 12-inch blood collection set (Becton-Dickinson) are cut off with scissors. The fish is anesthetized with tricaine and placed in right lateral recumbency. The sterile needle of the butterfly catheter is inserted between the scales at about a twenty-degree angle to the body wall in the area just dorsal to the caudal edge of the left pelvic fin (Fig. III-5). The dorsal aspect of the left pectoral fin is used as a landmark for consistent placement of the catheter (Fig. III-5). Once placed, the catheter is tested for patency with 0.4 ml of heparinized saline (100 units of heparin in 250 ml of saline); patency is confirmed by the flush moving easily into the peritoneal cavity.

The plastic hub of the catheter is then secured to the skin using a single, simple interrupted 4-0 nylon suture (Monosof [U.S. Surgical]). The catheter is then looped cranially and secured with a second skin suture above the left dorsal epaxial musculature (Fig. III-5). A single drop of cyanomethacrylate is applied to the needle insertion site and a small amount of 10% povidone iodine ointment is applied to both suture sites using a cotton swab. Finally, an injection port is secured to the free end of the catheter and the entire catheter is flushed with 0.4 ml of heparinized saline. The fish is then returned to its aquarium for recovery.

To keep the catheter patent, it should be flushed daily with heparinized saline and after every drug injection; this can be done without removing the fish from the aquarium. The catheter should remain secure and patent for at least 6 days. There is sometimes mild leakage around the catheter site but this is insignificant. The pharmacokinetics of antibiotic administered via the catheter is identical to individual IP injections (Lewbart et al. 2005). Once the sutures and catheter are removed, epithelial healing occurs quickly and there is negligible scarring.

RECOMMENDED TREATMENTS IN VARIOUS CULTURE SYSTEMS

Aquaria

Treatment in the Display Aquarium versus a Hospitalization Aquarium

For pet fish, whether to treat in the display aquarium is of greatest concern when water-borne medications are

given, but it is also of concern with oral and injectable preparations. In theory, it is always best to treat a sick fish in a hospitalization aquarium because (1) it removes contagion from susceptible tankmates; (2) it is easier to deliver medications to the sick individual (e.g., easier to capture the fish if repeated baths or injections are to be given); and (3) it avoids exposing both the (presumptively) healthy fish and the other organisms in the display aquarium to unnecessary and potentially toxic medications. A smaller amount of drug can often be used because of the relatively small size of the hospitalization aquarium.

However, in practice, there are several limitations to treatment in a hospitalization aquarium. Capturing the fish and placing it in a foreign environment can stress the fish (Schreck 1981; Elsaesser and Clem 1986). It may also be nearly impossible to capture a fish in a large aquarium with many rocks or plantings. Adequate biological filtration must also be provided so that the fish does not die from ammonia poisoning. Most aquarists do not maintain hospitalization or quarantine aquaria that can be used to treat sick fish. If such an aquarium is to be set up the day that treatment begins, it must be seeded with commercially available nitrifying bacteria or gravel or other filter material from an aquarium that has an established biological filter (see PROBLEM 4). There must be an active, functioning biological filter (e.g., in a sponge, box, undergravel, or other filter; nitrifying bacteria cannot simply be dropped into a bare aquarium). Note that nitrifiers from freshwater cannot be used to condition marine aquaria (Bower and Turner 1981), probably because the bacteria are shocked by the rapid salinity change. The converse is also true. Ammonia and nitrite levels must be monitored closely during therapy.

Clinic Hospitalization

If many clients are seen, it may become advisable to set up hospitalization systems. Inpatients can be routinely housed if one has the ability to rapidly set up aquaria that are conditioned with the bacteria needed to detoxify ammonia and nitrite. The simplest method to accomplish this is to use conditioned sponge filters (Lustar). Sponge filters have good biological filtration capacity and are easily moved from one aquarium to another. Sponge filters do not create a strong water current, so weak fish are not drawn to them like other filters. A separate aquarium should be set up having several conditioned sponge filters; then a filter can be placed in a hospitalization aquarium when needed. If both freshwater and marine fish are to be hospitalized, sponge filters should be established in two separate aquaria because, as noted above, nitrifying bacteria do not adapt well to a large salinity change. The nitrifying bacteria in the sponge filters must be kept active; this can be accomplished by adding some fish feed to each aquarium regularly. The amount of feed needed will depend upon the biological filtration capacity typically needed in that clinic (i.e., how many fish and what size fish are typically quarantined in an aquarium).

One can also use commercially available nitrifying bacteria (Aquacenter, Argent) to seed new aquaria. However, commercial nitrifier preparations are usually not as active initially as are the bacteria from a well-conditioned aquarium. Thus an increase is often seen in ammonia and nitrite when commercial nitrifiers are used. This may stress the treated fish.

Another alternative is to add zeolite to the hospitalization aquarium, which will bind ammonia. However, zeolite does not work in seawater. Alternatively, two display aquaria can be maintained, one with healthy freshwater fish and the other with healthy marine fish. The presence of the fish will maintain an active biological filter that can then be used to seed a hospitalization aquarium when needed. Note, however, that there is some risk of exposing treated fish to pathogens carried by subclinically affected fish in the display aquaria.

The size of the hospitalization system is dictated by the fish that are to be held. Small aquaria (2–10 gallons [= 8–40 liters]) are sufficient for most pet fish, but if larger fish are to be hospitalized (e.g., adult koi, large valuable broodstock, etc.), appropriately larger aquaria or other systems (e.g., wading pool, etc.) should be prepared. During hospitalization, it is often advisable to provide some shelter (e.g., small flowerpot or rocks) and keep the lighting subdued to reduce stress. Also, the water quality should be similar to that to which the fish are adapted (e.g., hard, alkaline water for Rift lake cichlids). When hospitalization is completed, the aquarium, filter, and all other materials in contact with the hospitalization aquarium should be disinfected before re-use (see "**Disinfection**" in "**Pharmacopoeia**").

Ponds

Fish in ponds are best treated using oral medications. However, there are few legally approved oral medications for treating ectoparasites or water mold infections on fish. Thus, most skin and gill pathogens are controlled by adding drug to the water containing the fish. Since it is impractical to gather up pond fish for bath treatment, prolonged immersion is the method of choice.

Cages

Fish in cages are best treated by using oral medications or bath treatments. Fish in large net-pens often cannot be easily removed and are thus treated with water-borne medication in the pen (e.g., sea lice). In the latter case,

the entire pen is enclosed in a tarpaulin, or skirts are placed around only the sides of the pen. However, some are treated by moving them to a separate enclosure with the therapy (e.g., freshwater treatment of Atlantic salmon for amoebic gill disease) (Fig. III-2).

Flow-Through Systems

Fish in raceways or other flow-through systems are best treated by using a bath or oral medication. Flush and continuous-flow treatments have also been used in salmonid hatcheries because of the typically high stocking densities and subsequent problems caused by stopping water flow for a particular time period (Piper et al. 1982).

WHICH DOSAGE TO USE

For many drugs in the "**Pharmacopoeia**," a range of doses is given. For water-borne treatments, water quality can greatly affect efficacy and ichthyotoxicity. Related pathogens can also vary in susceptibility. If you are unsure about the dose to use, it is usually best to start with the lower recommended dose. If the disease does not respond adequately, repeat the treatment with a higher dose.

For oral medications, dosage varies with feed intake. Fish that are eating less need a higher percentage of drug in the diet, but there are limits on the legally allowable amount and practical considerations, since some drugs are unpalatable at high doses (e.g., many antibiotics).

CHAPTER 17

Pharmacopoeia

The following drug formulations are a compendium of what I consider the most useful (and potentially useful) agents presently available for treating the major diseases that affect fish. In this regard, I include a number of treatments that have not yet been widely adopted in clinical practice but have strong scientific data supporting their efficacy. Conversely, not all drugs that have been used to treat fish diseases are included, often because I believe that the omitted treatment has not yet proven effective. However, this does not mean that all treatments not included are ineffective; some are useful (see Hoffman and Meyer 1974; Herwig 1979; Alderman 1988; Treves-Brown 2000 for lists of other treatments); however, I believe that the list provided gives a large range of choices in treatment and will be adequate for tackling the great majority of problems encountered. Indeed, the most important cause of drug failure in treating fish is a lack of proper diagnosis, which, when you use this information, you will hopefully remedy. Note that formulations may not be available indefinitely and are dependent on consumer demand, legal/regulatory constraints, and thus manufacturers' continued production.

There is a large body of anecdotal information that surports the efficacy of many drug formulations for treating fish diseases, especially for aquarium fish. Unfortunately, there is an equally great lack of reliable scientific data supporting the use of many remedies. I have avoided recommending the use of such treatments whenever possible. Most scientifically proven treatments are based on work with food fish. While some treatments require formulating the drug, I have included reliable commercial sources when those are available. Many medications are available over-the-counter in aquarium stores and aquaculture supply firms, including many antibiotics that are usually only available by prescription for ethical veterinary use. The availability of these products varies from country to country. No product should be prescribed without a proper diagnosis.

Regarding over-the-counter aquarium remedies, there is very little valid (i.e., published) scientific evaluation of the many proprietary medicines sold in pet stores. While many are useful (e.g., formalin, salt), others have been previously found to be ineffective, and even toxic (Trust 1972). Whether these facts have significantly changed since the seminal study of Trust (1972) is unknown. However, even today, some remedies are concoctions that contain five or more separate drugs that claim to cure everything from skin flukes to mycobacteriosis. Some formulations also combine antibiotics (sometimes a bacteriostatic with a bactericidal agent). Obviously, the practitioner should advise the client to avoid such complex mixtures. Also, the lack of demonstrated quality control for many of these over-the-counter aquarium products (e.g., lack of an expiration date) abrogates recommending their use in many cases.

Not all listed pharmaceutical brands are legally approved for use in fish, and even brands developed for food fish aquaculture are licensed only in certain countries. Sometimes, multiple brands of a particular drug are listed. The clinician should only use specific products that are approved for treating fish in the clinician's jurisdiction. While there is some limited published information on drugs used in specific countries, this information is likely to be quickly outdated so it is incumbent upon clinicians to familiarize themselves with the specific regulations.

Table III-6 lists drugs that have been used in a number of countries for treating diseases in food fish. Note that regulations change constantly and this list is only intended as a general guide to the types of compounds that have been used in the past, although most are still being used at some level. **The clinician should be sure that any drug that is used fully complies with all legal, humane, safety (human and fish) and environmental regulations in that specific jurisdiction**.

Before using any compound, the clinician should also be familiar with all safety precautions for both fish and the human handler (e.g., material safety data sheet [MSDS]). Personnel handling toxic chemicals should wear appropriate protective gear, which may include protective clothing, gloves, eye protection, and a respirator.

All treatments can be used for both marine and freshwater fish, unless noted otherwise. The withdrawal times listed are general guidelines. One should refer to the specific indications of the commercial product being used for details.

ACETIC ACID

Use: Treatment of ectoparasites in freshwater fish. Smaller fish are more sensitive (Lewbart 1991)

Water-borne formulations:

1. Bath
   a. Add 1–2 ml of glacial acetic acid/l (= 1,000–2,000 ppm of acetic acid = 3.8–7.6 ml/gallon) and treat for 45 seconds to 10 minutes (Schnick et al. 1989; G. Lewbart, personal communication). The longer time period may be toxic. Glacial acetic acid is 96% (Japan) to >99% (United States) acetic acid. Vinegar averages 5.7–6.3% acetic acid, but not less than 4% (Anonymous 1982).

ACRIFLAVIN

Acriflavin is a mixture of euflavine and proflavine. It is potentially mutagenic and is an irritant.

Use: Treatment of bacterial, water mold, or parasitic infections/infestations of aquarium fish. This agent has been used frequently in the aquarium trade, but there is a considerable resistance by common fish bacterial pathogens, and there are other, more effective agents for treating water molds and ectoparasites. At high doses, it might inhibit normal swim bladder inflation in developing fry (Sanabria et al. 2009).

ACTIVATED CARBON

Activated carbon is produced by the carbonization of plant material, where the plant's cell wall is retained as a carbon skeleton, leaving numerous cavities that form a large surface area. This allows activated carbon to adsorb numerous substances, including impurities and toxins, such as colloids (e.g., proteins, dyes, organic acids, water-soluble drugs), as well as gases such as chlorine and ozone. However, ammonia, nitrite or nitrate is not adsorbed.

Use No. 1: Removal of medications and other organics from water (Dawson et al. 1976); may not remove heavy metals or nitrogenous toxins from the water, unless they are present in an organic form (e.g., chelated copper, chloramines; Turner and Bower 1983); note also that some organics are not easily removed (see "**Copper**").

While mainly used in small, closed systems, activated carbon can also be used to remove chemicals or drugs from water in flow-through systems before effluent release into a watershed (Howe et al. 1990; Marking et al. 1990).

Water-borne formulations:

1. Prolonged immersion
   a. Use ~75 g (~250 ml dry volume) of activated carbon (Professional Grade Activated Carbon [Aquarium Pharmaceuticals, Inc.], or equivalent) for every 10 gallons (or every 40 liters) of water for 2 weeks. Carbon may be placed into the filter presently in the tank or added to a separate box filter. Discard the carbon used to remove medication after 2 weeks.

Use No. 2: Removal of pigments and other foreign substances from water, keeping aquarium water clear, odor-free, and low in organics

Water-borne formulations:

1. Prolonged immersion
   a. Use ~75 g (~250 ml dry volume) of activated carbon (Professional Grade Activated Carbon [Aquarium Pharmaceuticals, Inc.], or equivalent) for every 10 gallons (or every 40 liters) of water. Replace carbon approximately monthly.

AGRICULTURAL LIME; SEE "BUFFERS: PONDS," "CALCIUM"

ALUM (ALUMINUM SULFATE, $AL_2[SO_4]_3{\cdot}14H_2O$)

Alum must be mixed into the water to be treated as rapidly and evenly as possible. It is best to dispense it as a solution over the pond. It is dangerous to use in ponds that have low alkalinity, where it can cause a considerable decrease in pH. This pH drop can be counteracted by adding slaked lime at the same time. See Boyd (1990) for more details on the use of alum.

Use No. 1: Decreasing turbidity in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add ~15–25 mg alum/l. Alum is more effective and cheaper than agricultural gypsum (see "**Calcium**") but is less safe to use in low-alkalinity waters and has a shorter effective life (Wu and Boyd 1990).

Use No. 2: Decreasing excessively high pH in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add 1 mg alum/l for every 1 mg/liter of phenolphthalein alkalinity to be removed.

ANESTHETICS

Among the most important factors affecting the response to an anesthetic are fish species, health status, water temperature and pH. Other factors include fish strain, size, sex, sexual maturity, lipid content, body condition, salinity and mineral content (Ross 2001). Also, induction is prolonged in air-breathing fish. When possible (e.g., prior to surgery), feed should be withheld for 24 hours prior to inducing anesthesia. During recovery from anesthesia, if water flow is increased through the buccal cavity, heart rate will increase, speeding drug excretion.

Thus, in an emergency (i.e., overdose), water should be pumped over the gills.

See "**Benzocaine**," "**Carbon Dioxide**," "**Clove Oil**," "**Lidocaine**," "**Metomidate**," "**2-Phenoxyethanol**," "**Quinaldine Sulfate**," "**Sodium Bicarbonate**," "**Tonic Immobility**," and "**Tricaine**" for use of specific agents. See **p. 20** for planes of anesthesia and general guidelines for using sedatives/anesthetics.

ANTIBIOTICS

Use: Treatment of bacterial infections

Most agents listed are effective against Gram-negative bacteria, which are responsible for most fish bacterial diseases. Only a limited number of antibiotics are approved for use in food fish in any single country.

It is best to administer antibiotics orally or by injection. The next best alternative is a bath for antibiotics that are well absorbed via the water. Prolonged immersion treatments are least desirable and are economically unfeasible except in small volumes of water (i.e., aquaria). Most antibiotics used for fish diseases are weakly acidic or weakly basic; thus, pH has an important influence on uptake via water (Endo 1992).

The elimination rate of antibiotics from fish tissues varies greatly with temperature. Specific withdrawal times that are approved for selected oral antibiotic treatments vary among countries. Some examples are mentioned with specific antibiotics, but the clinician should closely follow the regulations in the country where the fish are treated. A good rule of thumb for withdrawal time is 500 degree days. Thus, if the mean daily water temperature after treatment is 10°C, the withdrawal period should be at least 50 days ($10 \times 50 = 500$), while at 25°C, the withdrawal period would be 20 days. This obviously can only be a rough estimate of elimination rate, because temperatures fluctuate diurnally and day-to-day and other factors besides temperature affect elimination rates. Note also that 500 degree days might not be sufficient in some cases (Treves-Brown 2000).

The pharmacokinetics of antibiotics vary tremendously among fish species; thus, doses given are only intended as general guidelines unless the formula has been shown to be effective for that particular species (e.g., Food and Drug Administration-approved in the United States). Whenever antibiotics are used, it is imperative that the treatment be given for exactly the time period specified. Treating for longer or shorter than recommended leads to treatment failure and/or development of antibiotic-resistant bacterial strains (Aoki 1992; Lewin 1992).

Intensive, repeated use of a single antibiotic unequivocally promotes the development of resistant bacteria (Tsoumas et al. 1989; Lewin 1992) and may result in complete resistance of the bacterial population (Sorum 1998). For example, there has been a high prevalence of multiple antibiotic-resistant bacteria in aquarium fish imported to the United States from the Far East, where there was heavy use of prophylactic antibiotics (Dixon et al. 1990). In caged salmon, the sediment near farms that have used a large amount of antibiotics has bacteria with a higher frequency of antimicrobial resistance than farms using much less antibiotics, and some fish harbor bacteria having a much higher level of antibiotic resistance (Herwig et al. 1997; Schmidt et al. 2000). However, aquaculture facilities that use antibiotics judiciously have much less likelihood of encountering resistant organisms (Herwig et al. 1997). Prophylactic use of antibiotics is highly discouraged.

Most bacterial resistance is plasmid mediated, meaning that resistance can be transferred both horizontally and vertically; plasmids typically carry resistance to multiple antibiotics (Lewin 1992).

Unfortunately, fish diseases often present as rapidly fulminating epidemics, and because of logistical and technological problems and/or lack of knowledge by the owner, it may not be possible to determine the probable efficacy of a treatment before an agent is used. Even knowing the causative agent (e.g., a specific bacterium) may not preclude having to change drug therapy once antibiotic sensitivity results are received. Once an outbreak becomes established, typically there is significantly higher mortality in a population, even if the antibiotic rapidly reaches therapeutic levels in tissues (Pearse et al. 1974; Egidius and Andersen 1979). This emphasizes the need for prompt therapy. If the proper therapy is given, fish often respond to treatment (e.g., improve appetite) within 24 hours and should respond within 3–5 days.

The pharmacokinetics of most orally administered antibiotic treatments that are listed are fairly well defined. However, most doses given for water-borne and injection routes are empirical, with not much clinical research to optimize or substantiate the dosage. While there is strong evidence that a single treatment with some antibiotics can cure fish of some bacterial infections (Egidius and Andersen 1979; Pearse et al. 1974; E.J. Noga, unpublished data), fish should be monitored closely to ensure that the treatment is effective. For example, treatment may need to be extended if response is not complete, even though this is not advisable in most cases. However, there is little latitude for legally modifying antibiotic therapy for food fish.

Some antibiotics persist for long periods, especially at low temperature, in the dark, and/or in mud (Jacobsen and Berglind 1988). These conditions are often found under cages used to culture marine fish or in ponds.

The risks to persons handling antibiotics or medicated feeds appear to be low and uncommon, primarily restricted to hypersensitivy reaction to a specific antibiotic (Giroud 1992).

When an antibiotic preparation that was not intended for use in fish is used (e.g., using as an unapproved drug for a pet fish), it is best to use preparations that do not have excipients, since these may be toxic to fish. Additional guidelines on the proper use of antibiotics is provided in Lupin et al. (2003) and Hernández Serrano (2005).

A number of antibiotics are sold over the counter in aquarium stores or by aquaculture supply firms. In the great majority of cases, their efficacy against treating the diseases for which they claim to be useful is uncertain. Considerable caution is warranted before considering using any antibiotic. Note that many antibiotics sold in aquarium stores do not have expiration date, calling into question the reliability of these products.

Amoxicillin Trihydrate (Vetremox® [Vetrepharm], Aquacil® [PH Pharmaceuticals], or Equivalent)

This is a beta-lactam antibiotic. Beta-lactam antibiotics are relatively unstable, being degraded/inactivated by heavy metals, as well as oxidizing and reducing agents. However, unlike several other antibiotics, they do not complex with divalent cations. Beta-lactams should be used as soon as possible after preparing the feed to avoid photodecomposition. It is best to top coat the feed since the antibiotic does not mix well and is heat-labile. They reach tissue levels quickly and are eliminated rapidly, but typically have poor activity against vibrios, *Aeromonas hydrophila* and *Yersinia ruckeri*.

Oral formulations:

1. Feed 40–80 mg of amoxicillin trihydrate/kg (= 18–36 mg/lb) of body weight/day for 10 days. Withdrawal time: 30–80 degree days for Atlantic salmon (Alderman et al. 1994).
2. Feed 80 mg of amoxicillin/kg (= 36 mg/lb) of body weight/day for 12 days for *Streptococcus iniae* in blue tilapia. This treatment appears to also eliminate carriers (Darwish and Hobbs 2005).

Injectable formulations:

1. Inject 12.5 mg amoxicillin/kg (= 4.5 mg/lb) of body weight IM once to treat furunculosis, pasteurellosis, edwardsiellosis, or streptococcosis (Brown and Grant 1992; Scott 1993).

Ampicillin Sodium (Amp-Equine [Smith-Kline], Omnipen [Wyeth-Ayerst], or Equivalent)

This is a beta-lactam antibiotic. It is less expensive than amoxycillin. See the general discussion under "**Amoxicillin Trihydrate**."

Oral formulations:

1. Feed 50–80 mg of ampicillin/kg (= 23–36 mg/lb) of body weight/day for 10 days. Use the higher dose for furunculosis, especially if treating by giving half the dose twice daily, since it is rapidly excreted in salmonids (Treves-Brown 2000). The lower dose range can be used for pasteurellosis and streptococcosis.

Injectable formulations:

1. Inject 10 mg ampicillin/kg (= 4.5 mg/lb) of body weight IM every day to treat furunculosis, pasteurellosis, edwardsiellosis, or streptococcosis (Brown and Grant 1992).

Chloramphenicol (Chloromycetin Sodium Succinate [Parke-Davis], or Equivalent)

Chloramphenicol is highly illegal to use on any food animals in most countries, including the United States, European Union, and Japan. In the United States, it is also banned from use on pet fish because of hazards associated with its handling. Human toxicity from chloramphenicol exposure usually causes a reversible, hypoplastic anemia but in rare cases, it causes an idiosyncratic, aplastic anemia, which is usually fatal. Chloramphenicol is also one of the few antibiotics that is effective against typhoid (*Salmonella typhi*) and it is feared that veterinary use might induce transmissible resistance that could be transferred to *S. typhi*. Florfenicol is a preferred substitute due to both efficacy and human safety considerations.

Enrofloxacin (Baytril® [Miles])

Enrofloxacin is a fluorinated quinolone that is active against *Aeromonas salmonicida* (Bowser et al. 1990) and is also useful for treating aquarium fish (Mashima and Lewbart 2000). Enrofloxacin has a longer half-life than any other quinolone commonly used in fish (Treves-Brown 2000; Della Rocca et al. 2004). In some fish (e.g., red pacu), enrofloxacin is metabolized to ciprofloxacin, another quinolone antibiotic (Lewbart et al. 1997) but this does not occur in others (e.g., gilthead seabream, Atlantic salmon) (Rocca et al. 2004). Half-life varies greatly among fish species and the recommended doses might be much more than needed in some fish. See the general discussion of quinolones under "**Oxolinic Acid**."

Water-borne formulations:

1. Bath
   a. Add 2.5–5 mg of enrofloxacin/l (= 9.5–19 mg/gallon) and treat for 5 days (Lewbart et al. 1997). Change 50–75% of the water between treatments.

Oral formulations:

1. Feed 10 mg of enrofloxacin/kg (= 4.5 mg/lb) of body weight/day for 10 days. This dose has been experimentally effective against vibriosis in rainbow trout (Dalsgaard and Bjeregaard 1991).

2. Administer a feed having 0.1% enrofloxacin for 10–14 days for aquarium fish. The injectable preparation can be used to prepare the feed (Lewbart et al. 1997).
3. Feed 5–10 mg of enrofloxacin/kg (= 2.3–4.5 mg/lb) of body weight/day for 10 days for *Streptococcus iniae* in hybrid striped bass (Stoffregen et al. 1996).

Injectable formulations:

1. Inject 5–10 mg of enrofloxacin/kg (= 2.3–4.5 mg/lb) of body weight IM or IP either every day for 10–14 days or every other day for 15 days (Lewbart et al. 1997) In koi, therapeutic concentrations are maintained for up to 5 days after a single 10 mg/kg dose IP (Lewbart et al. 2005).
2. Inject 5–10 mg of enrofloxacin/kg (= 2.2–4.5 mg/lb) body weight IM as preoperative treatment to prevent infection (Harms 2005). There are no clinical studies to confirm if this is efficacious.

Injectable + oral formulations:

1. Inject 10 mg of enrofloxacin/kg (= 4.5 mg/lb) of body weight IM or IP once followed by 0.05% in the feed for 14 days (Lewbart 2001).

Erythromycin (Erythromycin Base [Aurum], Erythro®-200 [Abbott], Erythromycin Thiocyanate, or Equivalent)

Erythromycin is a macrolide antibiotic, mainly effective against Gram-positive bacteria, that is primarily used for controlling bacterial kidney disease in salmonids. It is used at several stages in the life cycle, including preventing prespawning adult mortality, decreasing infection in eggs, and treating young fish with clinical disease (Armstrong et al. 1989). It is also used to treat streptococcosis (Kitao et al. 1987). Prolonged treatment with erythromycin can seriously impair kidney function in salmonids (Hicks and Geraci 1984). Because of its instability outside physiological pH, it is often used as an ester (e.g., thiocyanate or ethylsuccinate). A dosage of 40 mg/kg IP weekly for 4 weeks was nontoxic to lake trout. Note that erythromycin is commonly sold as an antibacterial agent for aquarium fish but is not recommended as prolonged immersion because of its toxicity to biological filtration.

Use No. 1: Prevention and/or treatment of bacterial kidney disease in salmonids

Oral formulations:

1. Erythromycin is used to prevent and treat clinical BKD (Austin 1985). Palatability problems have occurred with this treatment, especially at low temperature, where feeding rate is reduced.
   a. Feed 100 mg erythromycin thiocyanate/kg (= 45 mg/lb) of body weight/day for 21 days. While Austin (1985) found nearly as good a response after feeding for only 10 days, others have not (C. Moffitt, personal communication). Erythromycin thiocyanate is available as a premix for poultry (Gallamycin 50P, 11%) but has a coarse carrier that does not mix well in fish feeds and can cause esophageal damage. A premix with a wheat flour carrier is preferred (Peters and Moffitt 1996). Use premixes with the highest concentration of drug (least amount of carrier).

Injectable formulations:

1. For treating infected, mature salmonids to prevent mortality, while holding them before spawning, and to reduce infection incidence in eggs (Kiryu and Moffitt 2002)
   a. Inject 10–20 mg of erythromycin base (Erythro® 100 or 200)/kg (= 4.5–9 mg/lb) of body weight via the dorsal sinus or IP 9–56 days before spawning. Armstrong et al. (1989) found that the highest drug levels in eggs are achieved by administering the drug 12–20 days before spawning, although this difference is not large. Moffitt (1992) found that the best time for injection was 15–40 days before spawning.

Use No. 2: Treatment of streptococcosis

Oral formulations:

1. Feed 25–50 mg erythromycin/kg (= 11–23 mg/lb) of body weight/day for 4–7 days in yellowtail (Kitao et al. 1987). This was more successful than ampicillin or oxytetracycline (Shiomitsu et al. 1980).

Florfenicol (Aquaflor® Type A Medicated Article [Schering-Plough Animal Health])

Florfenicol is related to chloramphenicol but chemically modified so that it apparently does not induce aplastic anemia like chloramphenicol. It has a relatively short half-life in water (Boxall et al. 2004).

Oral formulations:

1. Feed 10 mg florfenicol/kg (= 4.5 mg/lb) of body weight/day for 10 days for treatment of furunculosis (Nordmo et al. 1994) or rainbow trout fry syndrome (Branson 1998).
2. Feed 6–25 mg florfenicol/kg (= 2.7–11 mg/lb) of body weight/day for treatment of pasteurellosis (Fukui et al. 1987).
3. Feed 10 mg florfenicol/kg (= 4.5 mg/lb) of body weight/day (400 ppm in feed if fed at 2.5% bw/day) for 10 days for treatment of enteric septicemia of catfish (Gaunt et al. 2004, 2006) or columaris in channel catfish; feed at the same rate for furunculosis or coldwater disease in freshwater salmonids. In the United States, extra-label use of florfenicol in feed is prohibited. Withdrawal time is 12 days for channel catfish and 15 days for salmonids. Expiration date for the Veterinary Feed Directive must not exceed 15 days from day of issuance.

Flumequine (Apoquin Aqualets® [Alpharma])

See the general discussion under "**Oxolinic Acid**." Flumequine is replacing oxolinic acid in aquaculture because of its more appropriate pharmacokinetic profile and lower effective doses (Treves-Brown 2000). Intramuscular injection produces high antibiotic levels for a reasonably long time (probably several days for most fish) (Nouws et al. 1992). The susceptibility of aquarium fish pathogens to quinolones makes this an attractive candidate for treating individual pet fish.

Water-borne formulations:

1. Bath
   a. Add 50–100 mg of flumequine/l (= 190–380 mg/gallon) of freshwater that has a pH of 6.8–7.2 and treat for 3 hours. This has been experimentally effective in treating *Aeromonas salmonicida* (O'Grady et al. 1988). Uptake via bath is less in hard water, high pH (sevenfold less uptake in pH 8.0 water compared to pH 7.0 water) and low temperature (tenfold less uptake at 3°C vs. 15°C [37°F vs. 59°F]) (O'Grady et al. 1988). It is probably best to increase the dosage when treating marine fish (Endo 1992).

Oral formulations:

1. Feed a *total* of 100 mg of flumequine/kg (= 45 mg/lb) of body weight for 5–8 days in freshwater. The dose rate is often given as a total to be divided by the period of treatment. In freshwater, 100 mg/kg total is recommended for 5–8 days. Thus, this can be given as 12.5 mg/kg/day for 8 days or 20 mg/kg/day for 5 days (Treves-Brown 2000).
2. Feed a *total* of 125–200 mg of flumequine/kg (= 56–90 mg/lb) of body weight for 5–8 days in seawater. This dose rate is given as a total to be divided by the period of treatment. Thus, a 200 mg/kg dose for 5–8 days can be administered as 25 mg/kg/day for 8 days or 40 mg/kg/day for 5 days (Treves-Brown 2000).

Injectable formulations:

1. Inject 30 mg of flumequine/kg (= 14 mg/lb) of body weight IP. This dose has produced effective serum levels in presmolt Atlantic salmon (held in freshwater) for over 10 days (Scallan and Smith 1985). IM injection is probably also effective (Nouws et al. 1992).

Furaltadone

See the general discussion under "**Nifurpirinol**."

Water-borne formulations:

1. Prolonged immersion
   a. Add 20–50 mg of furaltadone/l (= 76–190 mg/gallon), and treat fish for 24 hours (Debuf 1991).

Furazolidone (NF-180, Furox-50, Furazolidone [Aurum], or Equivalent)

See the general discussion under "**Nifurpirinol**." Furazolidone decays quickly in wet diets (e.g., whole fish, moist salmonid diets).

Water-borne formulations:

1. Prolonged immersion
   a. Add 1–10 mg of furazolidone/l (= 3.8–38 mg/gallon), and treat fish for at least 24 hours.

Oral formulations:

1. Feed 50–100 mg of furazolidone/kg (= 23–45 mg/lb) of body weight/day for 10–15 days. Palatability problems have occurred at higher oral doses.

Kanamycin Sulfate ([Fort Dodge] Injectable, [Aurum], or Equivalent)

This aminoglycoside antibiotic is relatively stable in water and fairly well absorbed (Gilmartin et al. 1976) but may not be safe to use for some species.

Water-borne formulations:

1. Prolonged immersion
   a. Add 50–100 mg kanamycin sulfate/l (= 190–380 mg/gallon) every 3 days for 3 treatments, changing 50% of the water after every treatment.

Oral formulations:

1. Feed 50 mg of kanamycin sulfate/kg (= 23 mg/lb) of body weight/day.

Injectable formulations:

1. Inject 20 mg kanamycin sulfate/kg (= 9 mg/lb) of body weight IP every 3 days for 14 days. This dose is toxic to some species. Administering only 10 mg/kg once weekly caused nephrotoxicity (acute tubular necrosis) and liver damage in steelhead trout (McBride et al. 1975).

Nalidixic Acid (NegGram® [Upjohn] Oral or Tablets)

This quinolone inhibits many aquarium fish pathogens (Samuelsen 2006). It may be toxic to some species and higher doses are typically needed compared to other quinolones. See the general discussion under "**Oxolinic Acid**."

Water-borne formulations:

1. Bath
   a. Add 13 mg of nalidixic acid/l of water (= 50 mg of nalidixic acid/gallon), and treat for 1–4 hours. Repeat if needed (Lewbart 1991).

Oral formulations:

1. Feed 20 mg of nalidixic acid/kg (= 9 mg/lb) of body weight/day to treat furunculosis or vibriosis. This regimen is approved for food fish in Japan.

Neomycin Sulfate (Neomycin Sulfate [Aurum], Biosol® [Upjohn])

This aminoglycoside is commonly sold as an antibacterial for aquarium fish but is difficult to use in prolonged immersions because of its toxicity to biological filtration. Since biological filtration must be removed during treatment to prevent killing the nitrifiers, fish densities must be low enough so that ammonia will not reach toxic levels in the system during treatment.

Water-borne formulations:

1. Prolonged immersion
   a. Add 66 mg of neomycin sulfate/l (= 250 mg/gallon). Repeat every 3 days for up to a total of 3 times.

Nifurpirinol (Furanace, P-7138, Auranace [Aurum], or Equivalent)

Nifurpirinol is a nitrofuran. Nitrofurans are an effective group of synthetic, broad-spectrum antimicrobials that are usually bacteriostatic but can be bactericidal at high concentrations. Their potency is relatively low compared to other antibiotics and thus high doses are needed. Some are stable in both freshwater and saltwater and are rapidly absorbed by fish (Anonymous undated; Pearse et al. 1974; Nusbaum and Shotts 1981). They are also effective against many of the common pathogens that affect fish (Anonymous undated). Bacterial resistance is slow to develop; when it occurs, there is complete cross-resistance to all other nitrofurans but not to other drug groups (Treves-Brown 2000). A single bath treatment is often effective against susceptible organisms (Anonymous undated). There have been some palatability problems with oral nitrofurans (Amend 1972). Unfortunately, nitrofurans are carcinogenic, genotoxic, and mutagenic (Yndestad 1992) and are strictly illegal for use on food fish in some countries, including the United States and the European Union.

Catfish, loaches, and other scaleless fish are considered sensitive to water-borne nitrofurans, but this varies with species (Anonymous undated). Nitrofurans are photosensitive and may be inactivated in bright light. By preventing exposure to light, the half-life is greatly increased. Nitrofurans should be handled with appropriate care to avoid human exposure.

Water-borne formulations:

1. Bath
   a. Add 1–2 mg nifurpirinol/l (= 3.8–7.2 mg/gallon), and treat for 5 minutes to 6 hours (Anonymous undated; Piper et al. 1982). A wide range of doses has been used. As little as 5 minutes exposure to a 2 mg/liter solution has treated marine fish at 10°C (50°F; Pearse et al. 1974).
   b. A 6.5-hour bath is effective against some, but not all channel catfish pathogens (Mitchell and Plumb 1980). The 96-hour water-borne LC_{50} for nifurpirinol ranges from 0.30 to 2.0 mg/liter (Anonymous undated).
2. Prolonged immersion
   a. Add 0.10 mg nifurpirinol/l (= 0.40 mg/gallon) and treat for 3–5 days (Piper et al. 1982). Prolonged immersion of channel catfish at 0.50 mg/liter causes skin damage (Mitchell and Plumb 1980).

Oral formulations:

Palatability problems have occurred with oral nifurpirinol (Amend 1972).

1. Feed 4–10 mg of nifurpirinol/kg (= 1.8–4.5 mg/lb) of body weight twice daily for 5 days (Anonymous undated).
2. Feed 0.45–0.90 mg of nifurpirinol/kg (= 1–2 mg/lb) of body weight/day for 5 days (Piper et al. 1982).

Nitrofurazone (Furacyn® = 9.3% Nitrofurazone [Aurum])

See the general discussion under "**Nifurpirinol**." Nitrofurazone is not absorbed via the water in gilthead seabream or Mozambique tilapia in seawater, or common carp in freshwater.

Water-borne formulations:

1. Bath
   a. Add 100 mg nitrofurazone/l (= 380 mg/gallon), and treat for 30 minutes (Anonymous undated).
   b. Add 10 mg nitrofurazone/l (= 38 mg/gallon), and treat for 6–12 hours. Repeat as needed (Lewbart 1991).
2. Prolonged immersion
   a. Add at least 2 mg nitrofurazone/l (= 7.6 mg/gallon) and treat for 5–10 days. Nitrofurazone is toxic to sac-fry and swim-up fry of channel catfish at 15 mg/liter (Piper et al. 1982). Do not use >5 mg/liter for this species (best not to use it for this purpose).

Oxolinic Acid (Aqualinic™ [Vetrepharm] or Aquinox™ [Vetrepharm], Oxolinic Acid [Aurum], Apoxolon Aqualets® [Alpharma], or Equivalent)

Oxolinic acid is a quinolone, a class of synthetic antimicrobials that are highly effective against many Gram-negative bacterial pathogens of fish (Samuelsen 2006). Quinolones inhibit bacterial DNA gyrase, thus inhibiting negative supercoiling of the bacterial chromosome. They can be bacteriostatic but are usually bactericidal. They are well absorbed orally (Alderman 1988).

The widespread use of oxolinic acid has led to the development of a significant amount of chromosomal resistance in organisms from medicated populations (Tsoumas et al. 1989), with cross-resistance against other quinolones. A second generation of quinolones, the fluoroquinolones (e.g., sarafloxacin, enrofloxacin), is also used for treating some fish pathogens (Bowser and Babish 1991). Resistance to quinolones (e.g., oxolinic acid, flumequine, nalidixic acid, piromidic acid) usually does not confer cross-resistance to fluoroquinolones.

All quinolones, especially the fluoroquinolones, chelate divalent cations and thus are inhibited by high hardness and possibly divalent cations in the diet. The half-life of oxolinic acid in fish is ~9–12 hours.

When oxolinic acid is fed to fish, residues enter the environment mainly from antibiotic bound to particulate matter (uneaten food and feces). Feral fish and filter-feeding shellfish near cages can have detectable residues after a treatment regimen, but the antibiotic concentrations in sediment dissipate much faster and are typically much lower than with oxytetracycline. Depuration of the sediment is by dissolution into the water column; there is no chemical or microbiological degradation (Treves-Brown 2000).

Water-borne formulations:

Oxolinic acid can be well absorbed, especially in soft water at low pH. There is less uptake in hard water and pH greatly affects uptake, with over a tenfold greater tissue concentration in fish held in pH 6 versus pH 7.7.

1. Bath
   a. Add 25 mg oxolinic acid/l (= 95 mg/gallon) and treat for 15 minutes. Repeat twice daily for 3 days. This regimen has successfully treated vibriosis in juvenile turbot (Austin et al. 1982).
2. Prolonged immersion
   a. Add 3–10 mg oxolinic acid/l (= 11–38 mg/gallon), and treat for 24 hours in freshwater. Use the higher dose range at pH > 7 (Endo and Onozawa 1987).
   b. Add 200 mg oxolinic acid/l (= 760 mg/gallon), and treat for 72 hours in seawater to treat Atlantic halibut against vibriosis (Samuelsen 1997).
   c. Add 10 mg oxolinic acid/l (= 38 mg/gallon) and treat for 8 hours (Endo and Onozawa 1987).

Oral formulations:

1. Feed 10 mg of oxolinic acid/kg (= 4.5 mg/lb) of body weight/day for 10 days in freshwater. Usually 25–50 mg/kg is needed in seawater (Treves-Brown 2000). Withdrawal time is 500 degree days (Debuf 1991).
2. Produce medicated brine shrimp by placing nauplii (larvae) in a lipid emulsion in seawater containing a 240 mg/liter concentration of oxolinic acid in seawater for 24 hours. Rinse in seawater and immediately feed it to fish for 17 days (Duis et al. 1995). Note that the method for preparing the medicated brine shrimp is complicated and very wasteful (<1% of drug enters the nauplii and the remaining >99% of drug must be disposed of responsibly). See Duis et al. (1995) for more details. This can protect against experimental vibrio challenge.

Oxytetracycline (Terramycin® 200 for Fish [Phibro], Liquamycin-l00® [Pfizer] Injectable, Tetraplex® [PH Pharmaceuticals], Microtet® [Microbiologicals])

Tetracyclines are mostly static inhibitors of bacterial protein synthesis that bind to the 30S ribosome. Oxytetracycline is effective against several important fish pathogens. It is probably most useful for treating columnaris disease (see PROBLEM 38). Resistance by aeromonads, vibrios, and other bacteria is common. All tetracyclines share virtually identical spectra of antibacterial activity; thus, cross-resistance and susceptibility of bacteria are nearly complete. Transmissible plasmid-mediated bacterial resistance is well documented (Michel and Alderman 1992).

Oxytetracycline is light-sensitive and will turn dark brown when decomposing. When used as a prolonged immersion, half of the water should be changed immediately if this happens. Degraded tetracyclines are nephrotoxic to humans (Fanconi syndrome). Avoid contact with the degraded drug (wear gloves). A pure preparation of oxytetracycline should be used for prolonged immersion. Do not use products that have small amounts of active drug (e.g., products having only 5% oxytetracycline), since the large amounts of sugar in these preparations cause a massive bacterial bloom.

Oxytetracycline is fairly stable in water (Nusbaum and Shotts 1981; Nouws et al. 1992), making it suitable for prolonged immersion, but only for species that actively take it up. All tetracyclines chelate divalent cations (Ca, Mg), causing their inactivation (Lunestad and Goksoyr 1990); thus, higher doses should be used in hard water. Magnesium has a higher avidity for oxytetracycline than does calcium. Complexation is probably also responsible for oxytetracycline being less absorbed when it is given as medicated diet to fish in seawater (Lunestad 1992).

Oral bioavailability of oxytetracycline is low compared to other antibiotics. It is very low in common carp, possibly due to absence of a stomach (Treves-Brown 2000) and thus, probably not advisable to use in any cyprinid (e.g., koi, goldfish). Bioavailability is also low in European seabass. The elimination rate of oxytetracycline from fish is typically very slow so the given "daily" dose may actually last 2–3 days (Treves-Brown 2000).

Because of its poor oral bioavailability, >90% of the drug enters the environment in solids (food and feces). Wild fish adjacent to cages can have detectable levels of

oxytetracycline for several days after treating caged fish. All tetracyclines bind to organic matter and clay, and oxytetracycline is persistent in sediments and accumulates under cages. However, the biologically active concentration of oxytetracycline that can accumulate under cages does not seem sufficient to induce microbial resistance, although this has not been fully substantiated (Treves-Brown 2000).

Oxytetracycline is not clinically toxic to lake trout at even 5 times the therapeutic concentration (Marking et al. 1988), but it causes depression of many immune functions at subtherapeutic doses (Rijkers et al. 1980; van der Heijden et al. 1992). The clinical significance of this immunosuppression is unclear, especially in light of oxytetracycline's long-term success in treating fish pathogens.

Water-borne formulations:

A yellow-brown foam may develop in the water during treatment.

1. Bath
   a. Add 10–50 mg oxytetracycline/l (= 38–190 mg/gallon), and treat for 1 hour for surface bacterial infections (Bullock and Snieszko 1970; Piper et al. 1982). A dose of 20 mg/liter (76 mg/gallon) is usually effective against susceptible bacteria. This can be repeated every day for up to 4 days.
2. Prolonged immersion
   a. Add 10–100 mg oxytetracycline/l (= 38–380 mg/gallon), and treat for 1–3 days (Piper et al. 1982). Use higher doses in hard water. If the fish are still sick, retreat on the third day after a 50% water change before treatment. Keep the tank covered during treatment to prevent photoinactivation.
   b. Add 25 mg oxytetracycline/l (= 95 mg/gallon). Then run water flow to produce a 100% water change in ~4.5 hours; treat twice daily for 3 days to treat epitheliocystis (Goodwin et al. 2005).

Oral formulations:

1. Oxytetracycline is palatable. See Table III-2 for specific indications for treating food fish in the United States.
   a. Feed 55–83 mg oxytetracycline/kg (= 25–37 mg/lb) of body weight/day for 10 days. This dose of Terramycin® 200 for Fish (Phibro) is approved for treating *Aeromonas*, *Pseudomonas*, and "*Haemophilus*" (*Aeromonas salmonicida*) infections in salmonids and channel catfish in the United States. Withdrawal times are 21 days (United States), 300–500 degree days (Debuf 1991).
   b. Feed 83 mg oxytetracycline/kg (= 37 mg/lb) of body weight/day for 10 days. This dose of Terramycin® 200 for Fish (Phibro) is approved for treating coldwater disease in freshwater salmonids and columnaris in rainbow trout in the United States. Withdrawal time is 21 days in the United States.
   c. Feed 100 mg oxytetracycline/kg (= 46 mg/lb) of body weight/day for 21 days to treat bacterial kidney disease (Kent 1992).

Injectable formulations:

Intramuscular (IM) injection produces high antibiotic levels for a reasonably long time (probably several days for most fish) (Nouws et al. 1992), but intraperitoneal (IP) injection produces a more rapid increase in plasma levels and reduces necrosis at the injection site (Treves-Brown 2000).

1. Inject 25–50 mg oxytetracycline/kg (= 11–23 mg/lb) of body weight IM or IP once (Piper et al. 1982).
2. Inject 10 mg oxytetracycline/kg (= 4.5 mg/lb) of body weight IM as a preoperative treatment to prevent infection (Harms 2005). There are no clinical studies to confirm if this is efficacious.

Sarafloxacin (Sarafin® [Abbott], Floxasol, Saraflox)

This is a fluoroquinolone that has broad spectrum potency against many fish pathogens, including *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri*, and *Edwardsiella ictaluri* (Wilson and MacMillan 1990; Stamm 1992; Martinsen et al. 1994). It is currently used for treating furunculosis in Atlantic salmon. See the general discussion under "**Oxolinic Acid**."

Oral formulations:

1. Feed 10 mg of sarafloxacin/kg (= 4.5 mg/lb) of body weight/day for 5 days. Withdrawal time is 150 degree days (Treves-Brown 2000).
2. Feed medicated brine shrimp for 17 days. Prepare medicated brine shrimp as described for oxolinic acid (Duis et al. 1995).

Sulfadiazine-Trimethoprim (Co-trimazine: Sulphatrim® [Hand/PH], Tribrissen™ 40% Powder [Coopers Pitman-Moore], or Equivalent)

This is a potentiated sulfonamide consisting of 1 part trimethoprim (a pyrimidine potentiator) and 5 parts sulfadiazine (a sulfonamide). Potentiated sulfonamides inhibit the bacterial dihydrofolate reductase enzyme pathway at two points, causing a synergistic inhibition of folate synthesis. Uptake of water-borne sulfas is much greater in seawater than freshwater (Bergsjo and Bergsjo 1978; Samuelsen et al. 1997). Some sulfas are toxic to fish (Kubota et al. 1970); however, reported toxic side effects with potentiated sulfas are uncommon. Potentiated sulfonamides are also relatively persistent in the environment (Boxall et al. 2004).

Oral formulations:

1. Feed 30–50 mg of sulfadiazine-trimethoprim/kg (= 14–23 mg/lb) of body weight/day for 7–10

days. Withdrawal time is 500 degree days (Debuf 1991).
2. Produce medicated brine shrimp by placing nauplii (larvae) in sulfamethoxazole-trimethoprim in seawater. Rinse in seawater, using a brine shrimp net, and immediately feed it to fish (Duis et al. 1995). Note that this method is very wasteful (<1% of drug enters the nauplii) and the remaining >99% of drug must be disposed of responsibly.

Injectable formulations:
1. Inject 125 mg of sulfadiazine-trimethoprim/kg (= 60 mg/lb) of body weight IP (Debuf 1991).

Sulfadimethoxine-Ormetoprim (Romet® TC [Aquatic Health Resources], Romet® 30 [Aquatic Health Resources], Primor® [Hoffman-LaRoche] Tablets)

This potentiated sulfonamide (a 5:1 combination of sulfadimethoxine:ormetoprim) is commonly used to treat *Aeromonas* and *Edwardsiella* infections in food fish in the U. S.. It is available as a premix powder, Romet® TC, that can be top-coated onto feed at the farm; it is an improved version of Romet® B. Romet is also available already incorporated into medicated feed (Romet® 30). It is more expensive than oxytetracycline. For channel catfish, antibiotic should be incorporated into feed at a rate of 1.65%. Higher concentrations in feed are poorly consumed because of the poor palatability of ormetoprim. It is best to incorporate at least 16% fish meal in the feed to ensure palatability (Robinson et al. 1990). There is considerable resistance by *Edwardsiella ictaluri*. See the general discussion under "**Sulfadiazine-Trimethoprim**."

Oral formulations:
1. Feed 50 mg of sulfadimethoxine-ormetoprim/kg (= 23 mg sulfadimethoxine-ormetoprim/lb) of body weight/day for 5 days. In the United States, withdrawal time is 42 days for salmonids and 3 days for channel catfish.
2. Produce medicated brine shrimp by placing nauplii (larvae) in a 3 mg/liter concentration of Romet® TC in seawater for 4 hours. Rinse in seawater, using a brine shrimp net, and immediately feed it to fish (Mohney et al. 1990). This procedure may also work with adult brine shrimp and other live feeds.

Sulfadimidine-Trimethoprim

See the general discussion under "**Sulfadiazine-Trimethoprim**."

Water-borne formulations:
1. Prolonged immersion
   a. Add 500 mg of sulfadimidine and 100 mg/ml of trimethoprim/l (= 1,900 and 380 mg/gallon) and treat for 72 hours for vibriosis (Samuelsen et al. 1997).

Sulfamerazine (Sulfamerazine in Fish Grade [American Cyanamid Company])

Sulfamerazine is a sulfonamide. Sulfonamides are synthetic, broad-spectrum antibiotics that inhibit the bacterial dihydrofolate reductase enzyme pathway. Sulfamerazine and other sulfonamides have been used extensively in aquaculture but current widespread resistance has made them largely ineffective. However, sulfonamides are often used in combination with pyrimidine potentiators (see the general discussion under "**Sulfadiazine-Trimethoprim**"). Sulfamerazine is approved in the United States for treatment of furunculosis in salmonids, but it is virtually useless because of resistance. The product has been withdrawn by the manufacturer because of lack of sales. Sulfonamides may be toxic when fed at over 220 mg/kg of body weight/day.

Oral formulations:
1. Feed 220 mg of sulfamerazine/kg (= 100 mg/lb) of body weight/day for 14 days.

Sulfamethoxazole-Trimethoprim (Co-trimoxazole: Septra® IV [Burroughs Wellcome], or Equivalent)

See the general discussion under "**Sulfadiazine-Trimethoprim**."

Water-borne formulations:
1. Bath
   a. Add 25 mg of sulfamethoxazole-trimethoprim/l (= 95 mg/gallon) and treat for 6–12 hours. Treat until clinical signs are gone (Lewbart 1991).

Oral formulations:
1. Feed 50 mg of sulfamethoxazole-trimethoprim/kg (= 23 mg/lb) of body weight/day for 10 days (Lewbart 1991).

Injectable formulations:
1. Inject 50 mg of sulfamethoxazole-trimethoprim/kg (= 23 mg/lb) of body weight IP every day for 7 days (Lewbart 1991).

ANTISEPTICS

Antiseptics are germicides that are used on living tissues. Antiseptics are typically used to treat eggs, skin, and/or gills.

See "**Acetic Acid**," "**Chloramine-T**," "**Chlorine**," "**Formalin**," "**Hydrogen Peroxide**," "**Potassium Permanganate**," "**Povidine Iodine**," "**Quaternary Ammonium Compounds,**" and "**Salt.**" For an explanation of the proper use of antiseptics, see "**Disinfection**."

BAYLUSCIDE® (BAYER 73, BILTRICIDE® [BAYER])

The active ingredient in Bayluscide® is clonitralid, the ethanolamine salt of niclosamide. Niclosamide has been used as an anthelmintic for cestodes in mammals. Originally developed for use as a molluskicide to control schistosomiasis in humans, Bayluscide® is selectively toxic for soft-bodied invertebrates, especially mollusks, turbellarians, oligochaetes (including tubificids) and leeches. Its mechanism of action is uncertain, but it might involve uncoupling of oxidative phosphorylation. It is photodegraded and toxicity increases with lower pH.

Use No. 1: Adjunct to TFM to kill lampreys in streams, to survey for lampreys in streams. Bayluscide® can only be applied to streams by certified government officials (Marking 1992).

Use No. 2: Control of aquatic snails

In the United States, this use is only approved in Florida and Arkansas under an Environmental Protection Agency Species Local Needs Label. It has also been legal under single season Section 18 Emergency Exemptions in other southeastern states. Bayluscide® is highly ichthyotoxic and thus cannot be used in ponds with live fish. It is best to treat dry ponds before filling them. Treatments are best done at night, when snails are more active (Francis-Floyd 1993). Unlike with other snail treatments such as copper, snail behavior is unaffected (i.e., snails do not try to avoid the treatment).

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add 1 lb of Bayluscide/surface acre of water (1.1 kg/ha) (Francis-Floyd et al. 1997).

BENZOCAINE (ETHYL AMINOBENZOATE)

Benzocaine and tricaine are both derived from benzoic acid. Benzocaine is used in mammals as a local anesthetic. It is only moderately water soluble, and thus must be prepared as a stock solution in ethanol, and then added to the water. It is best to use the more water-soluble benzocaine hydrochloride. Like tricaine, benzocaine solutions should be neutralized (2:1 ratio of sodium bicarbonate:benzocaine); it is not known if unbuffered benzocaine can cause skin and eye damage as occurs with unbuffered tricaine. It is photolabile. There is faster recovery in warm water. Benzocaine may be more toxic than tricaine for some species. It is no safer or more effective than tricaine, but it is less expensive since it is unapproved for food fish use. Note, however, that in the United States, all ethical sources of benzocaine have complex additives (e.g., lotions, cremes), since benzocaine is approved only as a topical anesthetic for mammals. These preparations are not suitable for fish anesthesia.

Fish may retain some movement during anesthesia, making it less desirable to use during surgery. Benzocaine's activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines. The clinical response of the fish should also be used to ascertain the proper dosage (see **p. 20**).

Use No. 1: Sedation for transporting fish

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add ~10–40 mg benzocaine/l (= ~38–150 mg/gallon).

Use No. 2: Anesthesia

Water-borne formulations:

1. Bath
   a. Add ~50–500 mg benzocaine/l (= ~190–1,900 mg/gallon). This concentration will usually cause anesthesia within 60 seconds.
   b. For large fish, a 1 g/l solution of benzocaine can be sprayed onto the gills, using an aerosol pump sprayer. This can be reapplied if needed during a procedure.

Use No. 3: Euthanization

Water-borne formulations:

1. Bath
   a. Add to effect. This usually requires a slightly higher dose than for anesthesia. Fish should be kept in this solution for 10 minutes after all breathing stops to ensure that they are dead.

BIOLOGICAL CONTROL—SEE P. 75 IN "HEALTH MANAGEMENT"

BITHIONOL (SYVA)

This is a mammalian anthelmitic that has shown efficacy in experimentally treating ichthyobodosis in rainbow trout and amoebic gill disease in Atlantic salmon.

Use: Treatment of ectoparasitic protozoa

Water-borne formulations:

1. Bath
   a. Add 25 mg bithionol/l (= 95 ml/gallon) for 3 hours on 2 consecutive days to treat ichthyobodosis (Tojo et al. 1994)
   b. Add 1 mg bithionol/l (= 3.8 mg/gallon) and treat for 60 minutes in seawater to treat amoebic gill disease (Florent et al. 2007); may be toxic at this dose.

BRONOPOL (2-BROMO-2-NITROPROPANE-1,3-DIOL, PYCEZE™ [NOVARTIS ANIMAL HEALTH])

Bronopol is a thiol-containing dehydrogenase enzyme inhibitor that is believed to cause cell membrane leakage. It was originally developed as a preservative for cosmetics, pharmaceuticals and industrial applications. In the

relatively few fish species that have been studied (mainly salmonids), it has highly efficacious anti-oomycete activity, being similar to that of malachite green (Sudova et al. 2007). It is also safer to use on eggs than malachite green (larger and fewer abnormal fry). However, it should not be used in smolting Atlantic salmon or rainbow trout alevins.

The aquaculture brand Pyceze™ (50% [w/v] bronopol) is permitted by the European Union for treating water mold infections of salmonids and their ova under veterinary prescription. Pyceze™ contains 500 of mg bronopol per ml of inert carrier. It rapidly degrades, especially when exposed to high intensity ultraviolet light (e.g., UV sterilizers). It is approved in Norway, the Faroe Islands and Sweden. There is no withdrawal period.

Use No. 1: Treatment of water mold infection on fish

Water-borne formulations:

1. Bath
   a. Add 1 ml Pyceze™/25 liter (= 20 mg bronopol/l = 0.15 ml Pyceze™/gallon), and treat for 30 minutes. Flow rates must be such that a complete exchange of the water is achieved in 60 minutes or less following the end of the treatment period. Repeat daily for up to 14 consecutive days. This regimen has cured rainbow trout of water mold infection (Branson 2002; Bronopol Product Insert, Novartis).

Use No. 2: Treatment of water mold infection on eggs

Water-borne formulations:

1. Bath
   a. Add 0.1 ml Pyceze™/liter (= 50 mg bronopol/liter = 0.38 ml Pyceze™/gallon), and treat for 30 minutes daily, as necessary, beginning at 24 hours afer fertilization (Bronopol Product Insert, Novartis). Flow rates to the incubator must be such that a complete exchange of the incubator water is achieved in 30 minutes or less following the end of the treatment period. If infected, repeat daily for 15 days.

BUFFERS: FRESHWATER AQUARIA

Use: Adjusting pH to the proper range in freshwater aquaria. Do not adjust pH more than 0.2–0.3 units/day, except in an emergency. Each solution should be added dropwise to the aquarium (start by adding a small amount and measure change in pH after thoroughly mixing; add more as needed).

Water-borne formulations:

1. Prolonged immersion
   a. To lower pH, add commercial buffer (pH Lower [Fritz], or equivalent).
   b. To lower pH, prepare buffer stock solution by adding 1.0 gram of sodium phosphate monobasic (NaH_2PO_4) to 100 ml of water and 1.0 gram of sodium phosphate dibasic (Na_2HPO_4) to 100 ml of water. Add equal numbers of drops/gallon of both solutions to the tank.
   c. To raise pH, add commercial buffer (pH Higher [Fritz], or equivalent).
   d. To maintain pH at 7.0, add mixed commercial buffer (pH Block [Fritz], or equivalent).
   e. To maintain pH, prepare buffer stock solution by adding 1.0 gram of food-grade sodium bicarbonate (Arm and Hammer Baking Soda, or equivalent) and 1.0 gram of sodium phosphate dibasic (Na_2HPO_4) to 100 ml of water.

BUFFERS: MARINE AQUARIA

Use: Adjusting pH to the proper range in marine aquaria. In marine aquaria, do not use buffers that contain phosphate.

Water-borne formulations:

1. Prolonged immersion
   a. Add a salt mixture of carbonates, borates, and trace elements: Liquid pH Buffer (Marine Enterprises). Use as directed.
   b. Add salts of carbonates and borates (Sea Buffer [Aquarium Systems], or equivalent). Use as directed.

BUFFERS: PONDS (LIMESTONE, AGRICULTURAL LIME, CALCITE, DOLOMITE, SLAKED LIME, UNSLAKED LIME)

Use: Adjusting pH and/or alkalinity to proper range in ponds

Agricultural lime (limestone) is calcite ($CaCO_3$), dolomite ($CaMg[CO_3]_2$), or some combination of both. Do not confuse agricultural lime with slaked lime or unslaked lime, which can also be used for buffering ponds; however, these are much more dangerous to use. Agricultural lime is the safest, cheapest, and most effective liming material for ponds (Boyd 1990).

To determine the amount of buffer required, mud should be collected from several locations in the pond. About 10 representative, equal-volume samples are usually needed for sampling a 1 ha (2.5 ac) pond; proportionately more samples are needed in larger ponds (Boyd 1990). Samples can be shipped to a laboratory for determination of lime requirement. A county extension agent can help to find a suitable lab. About 2,000–4,000 kg/ha (1–2 tons/ac) of agricultural limestone will usually be needed; one application usually lasts for several years. Limestone can be applied by shoveling it into the pond from a boat or by placing it along the pond bank and allowing it to wash into the pond. In temperate areas, lime after fertilization ends in late fall or early winter. In the subtropics and tropics, lime a few weeks

before fertilization begins in spring. See Sills (1974) and Boyd (1990) for more details on liming ponds.

Water-borne formulations:

1. Prolonged immersion
   Add lime at a rate sufficient to raise the alkalinity to the desired level (usually >20 mg/liter total alkalinity). Ponds that require lime usually need at least 2000 kg/hectare (= 1 ton/acre) (Boyd 1990). Note that liming rate is calculated based on pond surface area and not on water volume.
   a. Add agricultural lime to effect.
   b. Add slaked lime to effect (see "**Unslaked lime**").
   c. Add unslaked lime to effect (see "**Slaked lime**").

BUTORPHANOL (FORT DODGE ANIMAL HEALTH)

Use: Alleviation of pain just after major surgery

Injectable formulations:

1. Inject 0.4 mg of butorphanol/kg (= 0.18 mg/lb) of body weight IM as a single dose just before recovery from surgery (Harms 2005). There are no clinical studies to confirm if this is efficacious (it depends upon whether fish can perceive pain).

CALCIUM

Use No. 1: Adjusting hardness and/or calcium to proper range

Liming with calcite usually will not raise the calcium concentration to more than 45 mg/liter hardness (Boyd 1990). This is satisfactory for most fish species. For species that require harder water, gypsum or calcium chloride must be used. Gypsum is less expensive and more readily available than calcium chloride. Every 1.0 mg/liter of calcium that is added to water increases the hardness by 2.5 mg/liter as $CaCO_3$.

Water-borne formulations:

1. Prolonged immersion in aquaria
   a. Add Aqua-cichlids (Aquatronics) mineral mix. Use as directed.
2. Prolonged immersion in ponds
   a. Add lime (see "**Buffers: Ponds**").
   b. Add agricultural gypsum at a rate of 1.72 mg pure gypsum/l for every 1.00 mg/liter of total hardness required. Gypsum is available in two forms (anhydrite and dihydrate). Anhydrite calcium sulfate ($CaSO_4$) is available as a powder or as granules (land plaster). Dihydrate calcium sulfate ($CaSO_4 \cdot 2H_2O$, damp agricultural gypsum, peanut mixer) is used mainly as a calcium supplement for peanuts. Approximately 50% more of damp gypsum is needed to provide the same amount of calcium as in anhydrite calcium sulfate. However, the anhydrite form is much more expensive than damp agricultural gypsum and is usually used only for small ponds. Determine the percentage of calcium and other minerals in the preparation before using it, and use a neutralized product.
3. Constant flow in raceways/troughs during egg incubation
   a. Add calcium chloride ($CaCl_2 \cdot 2H_2O$) at a rate of 1.45 mg pure $CaCl_2 \cdot 2H_2O$/l for every 1.00 mg/liter of total hardness required.

Use No. 2: Reducing excessively high diurnal pH rise in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add a concentration of agricultural gypsum equal to twice the difference between total hardness and total alkalinity to roughly equalize hardness (Boyd 1990).

Use No. 3: Decreasing turbidity in ponds

Divalent cations, such as calcium, neutralize charged, suspended particles (e.g., clays), causing them to aggregate (flocculate) and thus precipitate (Stumm and Morgan 1970).

Water-borne formulations:

1. Prolonged immersion in ponds
   a. Add agricultural lime (see "**Buffers: Ponds**").
   b. Add agricultural gypsum at a rate of 250–500 mg/l. Gypsum is more expensive than alum, but it is safer to use and has a longer lasting effect (Wu and Boyd 1990).

CARBON DIOXIDE (CARBONIC ACID, CO_2)

In mammals, carbon dioxide causes direct depression of the cerebral cortex, subcortical structures, and vital centers. It also causes direct depression of heart muscle. Similar effects presumably occur in fish. Carbon dioxide is a poor anesthetic or sedative (slow-acting, stressful, lethal after repeated exposures) (Marking and Meyer 1985). Its major advantage is that it leaves no residue, so fish can be slaughtered immediately for human consumption.

The rate of narcosis is determined by the partial pressure of gas in water and is not easily measured (one can only do so indirectly by measuring pH) (Robb et al. 2000). It is essential to maintain high DO (>5 mg/l). Because of variable results and the difficulty in maintaining proper CO_2 concentrations, it should only be used as a last resort (e.g., when no residues are acceptable or no other anesthetics are available). It is best to measure the free CO_2 (see APHA 1992, 2005). Do not use CO_2 gas in closed areas (>10% causes loss of consciousness in humans).

Also see the general discussion under "**Sodium Bicarbonate**" for more information on CO_2 anesthesia.

Note that activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines, with the clinical response of the fish being used to gauge the proper dosage (see **p. 20**).

Use No. 1: Sedation/anesthesia

In Atlantic salmon, it has been used to anesthetize fish prior to euthanization via exsanguination (see "**Euthanasia**"). However, the time to loss of brain function is slow and fish may still have activity at the time of gill cutting. It is also associated with strong aversive movements (Robb et al. 2000).

Water-borne formulations:

1. Bath
   a. Produce a CO_2 concentration of 200–400 mg/liter by bubbling carbon dioxide gas through water (Takeda and Itzawa 1983). Anesthesia usually occurs within 5 minutes.

Use No. 2: Euthanasia

Water-borne formulations:

1. Bath
   a. Bubble carbon dioxide gas through water until death occurs (no breathing for >10 minutes).

CHLORAMINE NEUTRALIZER

Use: Removal of chloramine from municipal (tap) water supplies

Chloramine neutralizer can also be used to remove chlorine. Note that ammonia is released by the detoxification of chloramine and must also be removed. Use zeolite to remove ammonia.

Water-borne formulations:

1. Prolonged immersion
   a. Use Ammo-Loack® (Aquarium Pharmaceuticals). Use as directed for both freshwater and marine aquaria.
   b. Use Marine Antichlorine and Antichloramine™ (Marine Enterprises) Use as directed. For removal of chloramine and ammonia from water used for marine aquaria.
   c. Add sodium thiosulfate (see "**Chlorine Neutralizer**").

CHLORAMINE-T (N-CHLORO-4-METHYLBENZENESULFONAMIDE SODIUM SALT [WISCONSIN PHARMACAL COMPANY], CHLORASOL™ [INTERVET], HALAMID® [AXCENTIVE])

When dissolved in water, choramine-T acts as a biocidal agent due to the conversion of the chloramine-T anion to hypochlorite ion (OCl^-) and then to the weak hypochlorous acid (HOCl); this in turn releases active chlorine and oxygen. Hypochlorous acid is a strong disinfectant while hypochlorite anion is less so. Thus, chloramine-T is more active at acid pH. Compared to formalin, chloramine-T is more active against bacteria and less active against protozoa.

Chloramine-T is regarded as safer than chlorine because it does not form trihalomethanes with organic matter (see PROBLEM 92). It is a strong oxidizing agent and should not be used with reducing agents such as formalin, in either acid or alkaline solutions, or with benzalkonium chloride. It is less toxic with high organic loading. Overdosing with chloramine-T damages the gill epithelium, causing respiratory distress. At high doses, it might inhibit normal swim bladder inflation in developing fry (Sanabria et al. 2009).

Because it is unstable in aqueous solution, chloramine-T is supplied as a powder. Human contact with the powder can cause burns. Contact or inhalation may also lead to skin or respiratory sensitization. It is injurious to the eyes and harmful if swallowed. Protective clothing should be worn when handling it. Do not dispense the solution in metal containers.

The chlorine, and in particular the chloramine-T ion itself, kills microbes by oxidation and/or irreversibly binding to essential microbial structures. It remains "dormant" until required—the presence of microbes "turn it on." It is biodegradable. It is very stable in powder. Chlorasol is pure (>98%) chloramine-T.

Dose depends on water hardness and pH (Table III-9; Rach et al. 1988; Bullock et al. 1991). High doses may be toxic to koi. There can be increased ventilation frequency and blood CO_2, which is presumably caused by the liberation of hypochlorite. Repeated intermittent exposure of rainbow trout to 10 ml/l chloramine-T causes swollen and vacuolated gills with extensive intercellular edema. There are also fewer mucous cells and an increased number of chloride cells (Powell et al. 1995).

Use No. 1: Treatment of monogeneans and skin/gill bacterial infections

Water-borne formulations:

1. Bath
   a. Add the appropriate dose of chloramine-T to systems with a 4-hour turnover. Treatment can be

Table III-9. Concentration of chloramine-T to use at various pH and hardness levels.

pH	Dose (mg/L)	
	Soft water	Hard water
6.0	2.5	7.0
6.5	5.0	10.0
7.0	10.0	15.0
7.5	18.0	18.0
8.0	20.0	20.0

repeated every 4 hours for a total of 4 times if needed.

b. Administer the appropriate dose of chloramine-T for 60 minutes. Repeat every other day for up to a total of three times for treating columnaris and gill flavobacteria. A dose of 12 mg/liter (= 46 mg/gallon) at hardnesses ranging from 40 to 400 mg/liter (as $CaCO_3$) and pHs ranging from 7.1 to 7.8 was effective against bacterial gill disease; a single dose might be sufficient in some cases (Bowker et al. 2008). This can also be used as a constant flow treatment.

Use No. 2: Disinfectant

Chloramine-T is effective against many viruses, bacteria, water molds and parasites.

Water-borne formulations:

1. Prolonged immersion
   a. Add 10–50 ml chloramine-T/l (= 38–190 ml/gallon = 1–5% chloramine-T). This can be used for disinfection of apparatus and surfaces; use higher doses with more critical applications (eradication of serious pathogens or highly contaminated sites). Rinse apparatus well before use.

CHLORHEXIDINE (NOLVASAN [FORT DODGE ANIMAL HEALTH])

Use No. 1: Antisepsis of wounds

Water-borne formulations:

1. Swab
   a. Add 25 ml chlorhexidine/l (= 95 ml/gallon = 2.5% chlorhexidine). Swab the affected area and return fish back to the water.

Use No. 2: Disinfectant for surgical equipment

Water-borne formulations:

1. Prolonged immersion
   a. Add 100 ml chlorhexidine/l (= 380 ml/gallon = 10% chlorhexidine). This can be used as an indefinite soak for surgical instruments and for disinfecting surfaces. Keep the solution well covered and in a well-ventilated room. Rinse utensils well before using.

CHLORIDE

Use: Treatment of nitrite toxicity

Add enough chloride to produce at least a 6:1 ratio (w/w) of Cl:NO_2 ions:

$$\text{Amount of } Cl^- \text{ needed (mg/l)} = \left(6 \times [\text{NO}_2^- \text{ in water (mg/l)}]\right) - [Cl^- \text{ in water (mg/l)}]$$

Note that [NO_2^-] refers to the amount of nitrite, not the amount of nitrite-nitrogen.

$$\text{Sodium chloride needed (lb)} = \text{Pond volume (acre-feet)} \times 4.54 \times \text{(Chloride needed in mg/l)}$$

PROBLEM 5 describes methods for measuring chloride and nitrite concentrations.

Water-borne formulations:

1. Prolonged immersion
   a. Add rock salt (NaCl) to effect (contains ~60% Cl^-; see "**Salt**").
   b. Add solar salt to effect (contains ~55% Cl^-; see "**Salt**").
   c. Add artificial seawater (Instant Ocean®, or equivalent) to effect (contains ~55% Cl^-).
   d. Add $CaCl_2$ to effect (contains ~64% Cl^-); 1.0 mg $CaCl_2$/l releases 0.64 mg Cl/l.

CHLORINE (BLEACHING POWDER [CALCIUM HYPOCHLORITE, $CA(CLO)_2$, OLIN]; BLEACH [SODIUM HYPOCHLORITE, NAOCL, CHLOROX®]; CHLORINE DIOXIDE [SODIUM CHLORITE, $NACLO_2$, CLIDOX-S®, PHARMACAL], OR EQUIVALENT)

Use No. 1: Disinfectant

Chlorination actually involves the formation of several reactive species that have different disinfecting potencies. Free chlorine residual consists of aqueous molecular chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-). Chlorine is less active at high pH (more exists as $OC1^-$, which is less active than other forms). At the pH of most water, hypochlorous acid and hypochlorite ion predominate (also see PROBLEM 92).

Household bleach is 3–6% sodium hypochlorite. Calcium hypochlorite is more stable than bleach and has greater available chlorine than sodium hypochlorite. Extreme care should be used in handling calcium hypochlorite. Always keep in a cool dry place away from any organic material. When mixing it with water, it is safest to add the calcium hypochlorite to water; it can undergo self-heating and rapid decomposition accompanied by the release of toxic chlorine gas.

Chlorination can also be performed using sodium chlorite. Sodium chlorite ($NaClO_2$) is sodium salt of the unstable chlorine compound, chlorous acid ($HClO_2$). When added to water, sodium chlorite generates chlorine dioxide. An advantage in this application, as compared to the more commonly used chlorine, is that trihalomethanes are not produced from organic contaminants. Mixing the base with water and activator generates the active principal (acidifed sodium chlorite). Chlorine dioxide is also less corrosive than other chlorine disinfectants and is more effective as a disinfectant in most circumstances than other chlorine disinfectants against water-borne pathogenic microbes of humans.

Chlorination is a better overall disinfectant than quaternary ammonium compounds or povidone iodine (see "**Disinfection**"); however, it will destroy netting. Prolonged contact will also rapidly corrode metal and damage many plastics (see "**Quaternary Ammonium Compounds**," "**Formalin**").

Chlorination releases volatile chlorine gas, which irritates mucous membranes. Solutions should only be used in a well-ventilated area. Large amounts of organic matter necessitate a higher dosage. Alkaline (>7.0) pH inhibits chlorination (Boyd 1990). Use extreme caution around areas that hold fish, since chlorine is highly toxic, even in trace amounts. Items must be well rinsed in water before reuse and should be allowed to stand in aerated water for at least 1 day. Chlorine can also be neutralized with sodium thiosulfate (see "**Chlorine Neutralizers**"). See "**Disinfection**" for general guidelines on use.

There is evidence that some pathogens, such as mycobacteria (PROBLEM 55) can become somewhat resistant to chlorine disinfectants (Vaerewijck et al. 2005), but this has not yet been documented for fish pathogens.

Water-borne formulations:

1. Prolonged immersion
   a. Add calcium hypochlorite ($Ca[OCl]_2$) = high test hypochlorite = HTH [Olin]) to produce 200 mg/liter available chlorine for at least 1 hour to disinfect raceways, small aquaria, and utensils. Calcium hypochlorite is a dry powder. It has a longer shelf life than sodium hypochlorite, but it is more expensive. HTH powder may contain either 15%, 50%, or 65% of available chlorine to produce a solution that has 200 mg/liter available chlorine (Leitritz and Lewis 1976).
      i. Add 1.4 g of 15% available chlorine HTH powder/l (= 2 oz/10.5 gallons).
      ii. Add 0.4 g of 50% available chlorine HTH powder/l (= 1 oz/18 gallons).
      iii. Add 0.32 g of 65% available chlorine HTH powder/L (= 1 oz/23 gallons).
   b. Add 10 ml of commercial household bleach (Clorox® or equivalent = 5.25% sodium hypochlorite)/l (1:100 dilution = = 35 ml/gallon) for at least 1 hour (= 200 mg/liter available chlorine).
   c. Prepare sodium chlorite solution (Clidox-S or equivalent) at a 1:5:1 ratio of base:water:activator and treat for at least 1 minute to kill *Mycobacterium marinum* (Mainous and Smith 2005).

Use No. 2: Antisepsis of zebrafish eggs

Water-borne formulations:

1. Bath
   a. Add calcium hypochlorite ($Ca[OCl]_2$) = high test hypochlorite = HTH [Olin] to produce 25–50 ppm for 10 minutes (Kent and Fournie 2007).

CHLORINE NEUTRALIZER

Use: Neutralization of chlorine in water

Chemical neutralization of chlorine uses sodium thiosulfate. Seven mg of sodium thiosulfate neutralizes 1 mg of chlorine. Obviously, large amounts must be used when neutralizing chlorine levels used for disinfecting utensils, compared with the relatively low amounts needed to remove chlorine from tap water. Earler studies have found that some commercial dechlorinating products are unreliable (Kuhns and Borgendale 1980).

Water-borne formulations:

1. Prolonged immersion for municipal (tap) water
   a. Sodium thiosulfate [Super Strength 5-in-1 Water Conditioner™ (Aquarium Pharmaceuticals) or equivalent]. Use as directed.
2. Prolonged immersion for chlorine-disinfected utensils
   a. Prepare a solution that has enough sodium thiosulfate (Argent, Western Chemical, or equivalent) to neutralize the free chlorine in the water. One liter of 200 mg/liter available chlorine is neutralized by 1.5 g of sodium thiosulfate.

CHLOROQUINE DIPHOSPHATE (FISHMAN CHEMICAL, SPECTRUM CHEMICAL)

The therapeutic concentration is nontoxic to fish but is highly toxic to micro- and macroalgae and to various invertebrates (C. Bower, personal communication), especially corals and echinoderms (M.D. Stafford, Wonders of Wildlife Zooquarium, Springfield Missouri, unpublished data).

Chloroquine is expensive. It is used to treat malaria in humans.

Use No. 1: Treatment of *Amyloodinium ocellatum*

This drug is effective in treating *Amyloodinium* and is relatively nontoxic to fish. Chloroquine has no effect on tomont division, but it kills dinospores immediately on their excystment. Experimentally infested false percula clownfish were freed of *A. ocellatum* infestation after a 10-day exposure to a single treatment of 5–10 mg/liter chloroquine diphosphate.

Water-borne formulations:

1. Prolonged immersion
   a. Add 10 mg chloroquine diphosphate/l (= 40 mg chloroquine diphosphate/gallon). Only one treatment is reportedly needed; however, monitor closely for 21 days, and retreat if necessary. Add activated carbon if no relapse is apparent after 21 days.

Use No. 2: Treatment of *Cryptocaryon irritans*

Water-borne formulations:

1. Prolonged immersion
   a. Add 10 mg chloroquine diphosphate/l (= 40 mg chloroquine diphosphate/gallon) every 5 days for

at least 4 treatments. This works best if the salinity is <14 ppt; try to keep at 12–13 ppt (M.D. Stafford, unpublished data). Add activated carbon if no relapse is apparent after 21 days.

CHORIONIC GONADOTROPIN (CHORULON® HUMAN CHORIONIC GONADOTROPIN INJECTABLE [INTERVET])

Use: Induce spawning in male and female broodfish

Injectable formulations:

1. Inject an appropriate dose of Chorulon® for the species to be spawned. Each vial has 10,000 I.U. In the United States, a cumulative maximum of 25,000 I.U. can be injected; see Table III-2 for specifications.

CLOVE OIL; SEE "EUGENOL"

COPPER

Use No. 1: Treatment of ectoparasitic protozoa, monogeneans, water molds and flavobacteria

While copper has been claimed to be effective against columnaris, bacterial gill disease, and water mold infections in cold water species, there are much better treatments available for these problems (Piper et al. 1982). However, copper sulfate is effective against water molds in some warm water fish when treated early in the infection (Li et al. 1996). Copper has a low therapeutic index, making it easy to overdose the fish. It is toxic to gill tissue (Cardeilac and Whitaker 1988) and under certain exposures can be immunosuppressive (Hetrick et al. 1979) but in other situations might be immunostimulatory (see below).

Copper is also algicidal (also see PROBLEM 93), including both filamentous and higher algae. When used as an herbicide, copper concentrates in plant tissues to many times the levels in ambient water. Thus, it can pose a risk to animals (e.g., manatees) that feed on aquatic plants. Copper is toxic to zooplankton, so should not be used in ponds where zooplankton is being used to feed the fish in the pond (e.g., fry ponds).

Besides its antimicrobial and antiparasitic activity, copper also might function in some instances as an immunostimulant in protecting against infection since several studies have shown that exposure to copper can increase resistance of fish to infectious challenge (Griffin and Mitchell 2007).

Copper Sulfate (Bluestone, Copper Sulfate Pentahydrate, $CuSO_4 \cdot 5H_2O$ [Phelps Dodge])

Water-borne formulations:

1. Prolonged immersion in marine aquaria

Prolonged immersion copper is the most common and well-established method for controlling protozoan ectoparasites on marine aquarium fish (Cardeilhac and Whitaker 1988). While copper can be used to treat ectoparasites of freshwater aquarium fish, this is not advisable because safer remedies are available. Copper is absorbed and/or inactivated in marine aquaria because of the high levels of calcareous materials (e.g., seawater, coral, or limestone), which react to form insoluble copper carbonate (Keith 1981). Copper's solubility is also highly dependent on pH, which controls the solubility of tenorite (CuO), the stable, solid phase of copper above pH 7 (Straus and Tucker 1993). Cupric ion concentrations decrease dramatically with increasing pH (up to 100-fold with every 1 unit increase in pH). Copper is also bound and inactivated by organic matter.

Free copper ion levels must be maintained between 0.15 and 0.20 mg/l. A weaker concentration will not kill parasites, while a stronger concentration may kill the fish. Because of the high alkalinity in marine aquaria, large amounts of copper must first be added to an aquarium to reach this dose. The unpredictable nature of this initial dose requires that copper levels be assessed with a commercial kit (e.g., LaMotte Company, Hach Company, Aquarium Systems) and adjusted as needed. Initially, copper levels should be measured and adjusted twice daily. After several days, copper levels will become more stable and daily monitoring is usually satisfactory. Copper is extremely toxic to invertebrates and many algae. Also, the copper that precipitates out of solution may eventually resolubilize under some conditions, such as if the filter stops working for some reason and thus the pH of the water in the filter begins to drop. This may release toxic levels of free copper. Thus, it is best to treat in a separate hospital tank.

a. Prepare a copper stock solution (Bower 1983) by adding 1 gram of $CuSO_4 \cdot 5H_2O$ to 250 ml of distilled water. Mix thoroughly until all of the crystals have dissolved. This stock solution contains 1 mg of copper/ml. Add enough copper to the aquarium to produce a concentration of 0.15 mg/liter (= 0.57 mg/gallon):

$$\#\ \text{liters to treat} \times 0.15 = \#\ \text{mg copper needed} = \#\ \text{ml stock needed}$$

$$\#\ \text{gallons to treat} \times 0.57 = \#\ \text{mg copper needed} = \#\ \text{ml stock needed}$$

For example, if a 40-liter tank is to be treated with copper, one needs 6 mg of copper and thus must add 6 ml of copper stock solution ($40 \times 0.15 = 6$).

Measure copper immediately to confirm that the proper dosage has been added. Use a copper test

kit that measures in increments of at least 0.05 mg/l. Add more copper as needed to maintain a concentration of 0.15–0.20 mg copper/l. When more copper is needed, add 0.19 ml/gallon (= 0.05 ml/l) of copper stock to increase the copper concentration by 0.05 mg copper/l. Copper can be quickly removed with activated carbon (Keith 1982).

2. Prolonged immersion in freshwater ponds
Copper sulfate is usually the treatment of choice for protozoan ectoparasites of pond-reared fish. It can also treat winter kill if caught early. Copper sulfate is approved by the U.S. Environmental Protection Agency for use in fish ponds as an algicide; however, it has not been approved by the U.S. Food and Drug Administration as a drug.

Copper sulfate is relatively economical to use in ponds. It is best to use the finest form of copper sulfate available (i.e., snow) since larger-grained forms are more difficult to dissolve (R. Francis-Floyd, personal communication). The volumes of different forms of copper sulfate vary, which can affect treatment calculations made on a volume basis rather than a weight basis (Jensen and Durborow 1984). Only pure forms (100% copper sulfate) of unoxidized (bright blue, not green) copper sulfate should be used.

The free copper ion is the active form of copper. The amount of free copper present in water depends on many factors, but especially total alkalinity. Thus, the amount of copper sulfate to add is determined from the total alkalinity of the water (Jensen and Durborow 1984):

Total Alkalinity (mg/l)	[$CuSO_4 \cdot 5H_2O$] (mg/l)
20–49	0.25–0.50
50–99	0.50–0.75
100–149	0.75–1.00
150–200	1.00–2.00

While this information provides useful guidelines, it is preferable to calculate a more accurate dosage based on the following formula (Kleinholz 1990):

$$\text{Amount } CuSO_4 \cdot 5H_2O \text{ to add (mg/l)} = \frac{\text{Total alkalinity of water [mg/liter as } CaCO_3\text{]}}{100}$$

It is also advisable to perform a bioassay if copper sulfate has not been used previously in a particular body of water. Many clinicians believe that if the total alkalinity is less than 50 mg/l, copper sulfate is unpredictably toxic and thus contraindicated. Copper should never be used if the alkalinity is less than 20 mg/l. In water with high alkalinity (>250 mg/l), copper sulfate rapidly precipitates as copper carbonate, and thus a single treatment is insufficient to provide a therapeutic concentration (see #1 above).

Guidelines for copper treatment in ponds have been based mainly on experience with channel catfish.

A bioassay is advisable before treating a species with unknown susceptibility to copper.

a. Add copper sulfate to effect.

3. Bath in freshwater aquaria
This treatment has recently been found to be effective in treating ichthyobodosis in hybrid striped bass in high alkalinity (200 mg/l) water and suggests that relatively short-term, repeated copper treatments may be useful against freshwater ectoparasites.
   a. Add 2 mg/liter copper sulfate for 4 hours and then perform a complete water change. Repeat after 2 days (D. Mitchell, A. Darwish, and A. Fuller, personal communication).

4. Constant flow in freshwater
This is used to treat salmonids for bacterial cold water disease.
   a. Administer the appropriate dose of copper sulfate for 1 hour.

Chelated Copper

Water-borne formulations:

1. Prolonged immersion in marine aquaria
Chelated copper compounds consist of copper bound to one of several organic complexing agents, such as citrate, EDTA, or ethanolamine. They have the advantage of being more stable in seawater than copper sulfate, and thus normally require fewer additions of drug.

However, their efficacy and safety have not been thoroughly proven. A special test kit is also needed to measure chelated copper concentrations. It is probably safer to use unchelated copper sulfate. Remove chelated copper with activated carbon at the end of the treatment. Note that some brands of chelated copper are difficult to remove with activated carbon (Keith 1982), so water changes may be needed instead.
   a. Prepare copper-citrate stock solution (Blasiola 1978) by adding 2.23 g of $CuSO_4 \cdot 5H_2O$ and 1.5 g of citric acid to 1 liter of distilled water. This stock solution contains 0.56 mg of copper/ml. Add 1 ml of stock solution/ gallon (= 0.26 ml/l) to produce an initial copper ion concentration of 0.15 mg/l. Immediately test to be sure that copper is at this concentration. If additional copper is needed, adding 0.33 ml/gallon (= 0.087 ml/l) will add 0.05 mg copper/l. Maintain copper ion levels at 0.15–0.20 mg/liter for 14–21 days.

b. Sea Cure Copper Treatment (Aquarium Systems) is to be used as directed.
c. CopperSafe® (Mardel Laboratories) is a proprietary treatment for freshwater and saltwater parasites. Use as directed.

2. Prolonged immersion in ponds
Chelated copper is infrequently used for treating freshwater pond fish. Its main advantage is its greater stability in high-alkalinity water. Chelated copper compounds are purportedly less toxic to fish than an equal amount of free copper, also making them potentially useful in low-alkalinity waters (Skea and Simonin 1979; Straus and Tucker 1993). However, the safety of chelated copper for treating fish under any conditions is not proven. Concentrations as low as 0.21 mg/liter are lethal to some fish species, even in fairly high-alkalinity waters (95 mg/l) (Skea and Simonin 1979). Chelates are also more costly than copper sulfate. Do not treat with more than 0.15 mg/liter total copper, unless the fish species is known to be tolerant of a higher dose. Treatments should only be given once.
a. Add chelated copper (Cutrine®, Applied Biochemist, or equivalent) (Wellborn 1979).
b. Mix two parts copper sulfate with one part citric acid (wt/wt) and spray over the pond (Anonymous 1989).

Use No. 2: Eradication of snails
Water-borne formulations:

1. Prolonged immersion in ponds
a. Copper treatment of ramshorn snails. Apply an aqueous solution of copper at 2.5–5.0 mg copper sulfate/l. This treatment can kill 96% of snails in channel catfish ponds, but may cause some fish mortality since it is very close to the lethal dose for channel catfish. Both alkalinity and hardness must be at least 200 ppm (Wise et al. 2006).
b. Use unchelated copper sulfate at the same dosage as for ectoparasites (Use No. 1, Part 2). It may be best to treat at night since many snails are nocturnal and more susceptible to treatment because they are more active (R. Francis-Floyd, personal communication).
2. Prolonged immersion (shoreline treatment) in ponds. Do not use this procedure in fry ponds because fry may not be able to avoid the treatment area.
a. Shoreline chelated copper treatment of ramshorn snails. Apply an aqueous solution of 589 g of copper sulfate and 58.9 g of citric acid per 10 linear meters in a 2-meter-wide swath along the pond shoreline (=10 lb copper sulfate and 1 lb citric acid per 250 linear feet in a 6-foot-wide band along the pond shoreline). This gives an instantaneous treatment rate of about 59 mg/liter copper sulfate if the water in the 2-meter swath averaged 0.5 meters deep. It is best to only use this procedure on large (>7 acre [>3 hectare]) ponds having at least 150 mg/liter alkalinity. It can be used on smaller ponds if the alkalinity is higher. This can reduce snail populations by at least 95% (Mitchell 2002). This method is often no more effective than copper alone and is more toxic to fish at high temperature (see #2b below); however, it may be preferred to copper alone if levee control grasses or aquatic weeds are present along the margins of the pond (Mitchell and Hobbs 2003). This method is approved by the USEPA.
b. Shoreline unchelated copper sulfate treatment of ramshorn snails. Use the same copper dosage as in #2a above, but omit using citric acid. This is less toxic than citrated copper, especially at high (>87°F [31°C]) temperature. At lower temperature (<70°F [<21°C]), this treatment loses much of its effectiveness (Mitchell and Hobbs 2003).

DEIONIZED WATER (DISTILLED WATER)

Use: Decreasing hardness or salinity
Water-borne formulations:

1. Prolonged immersion for aquaria
a. Add deionized water in an amount inversely proportional to the salinity or hardness desired.

Several brands of home reverse osmosis (R/O) water treatment units (e.g., Sandpoint, Kent Marine, Spectrapure) are available.

DIFLUBENZURON (DIMILIN® [UNION CARBIDE], LEPSIDON® [EWOS])

Diflubenzuron is a benzyl-urea insect growth regulator. It inhibits chitin synthesis and is highly effective against immature but not adult copepods. In order to be effective, it must be present at the time that a crustacean is shedding (undergoing ecdysis) because it kills the parasite by preventing exoskeleton formation. Insect growth regulators are highly toxic to nontarget crustaceans (Burridge et al. 2004) and are persistent in the environment. When used in prolonged immersion, it is not degraded by pond temperatures >27°C (80°F) and over 76% of the dosage persists after 1 week in water (Hoffman 1985). It has a half-life of 29 days at 10°C, pH 7.7.

Use No. 1: Treatment of *Lernaea*
Water-borne formulations:

1. Prolonged immersion
a. Add 0.03 mg of diflubenzuron/l (= 0.11 mg/gallon) (Burtle and Morrison 1987).

Use No. 2: Treating sea lice in salmon

Oral formulations:

1. Feed treatment for sea lice: see general concerns regarding treatment of sea lice under "**Organophosphate**."
   a. Feed 75 mg diflubenzuron/kg body weight (= 34 mg/lb body weight)/day for 14 days. This is equivalent to a feed that has 15 kg of diflubenzuron per ton and is fed at a rate of 0.5% of body weight/day (Alderman 2002).

DIMETRIDAZOLE (EMETRYL [RHONE-POULENC])

Emetryl is 40% dimetridazole. Dimetridazole is banned for use in food animals in many countries (e.g., European Union, United States) but is still widely available for treating pet fish. While dimetridazole is available from some sources (e.g., pigeon feed stores), metronidazole is more readily available for treating nonfood fish.

DIQUAT (SYNGENTA)

Use: Treatment of bacterial gill disease

Diquat is registered by the U.S. Environmental Protection Agency as a herbicide, but it is also effective against bacterial gill disease and columnaris. It is especially useful in earthen ponds; however, it is expensive and has decreased effectiveness in muddy or organically polluted waters (Warren 1981). Avoid use if the treated water may be used to irrigate crops. Use rubber gloves and a respirator to avoid exposure to the chemical; it is highly toxic via skin contact.

Water-borne formulations:

1. Bath
   a. Add 19–28 mg Diquat/l (= 72–106 mg/gallon) and treat for 30–60 minutes for 1–3 treatments on alternate days
   b. Add 2–18 mg Diquat/l (= 7.6–68 mg/gallon) and treat for 1–4 hours for 1–4 treatments on alternate or consecutive days (AADAP 2008)

DISINFECTION; ALSO SEE "CHLORAMINE-T," "CHLORHEXIDINE," "CHLORINE," "FORMALIN," "OZONE," "POVIDINE IODINE," "QUATERNARY AMMONIUM COMPOUNDS," "SLAKED LIME," "UNSLAKED LIME," "ULTRAVIOLET LIGHT," "VIRKON® AQUATIC"

Disinfectants and antiseptics are germicides. Germicides that eliminate microorganisms from inanimate objects are disinfectants. Germicides that are used on living tissue (e.g., eggs or fish) are antiseptics. Many germicides are used for both purposes, but maximum concentrations used for antisepsis are lower than for disinfection to prevent damage to living tissue.

Disinfection eliminates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial endospores) on inanimate objects. The effectiveness of disinfection is reduced by large numbers of contaminating organisms, organic matter, and materials that have small crevices that do not allow rapid disinfectant penetration. The type and concentration of germicide and the time and temperature of exposure also affect disinfection. Thus, the exposure times given for various disinfectants are for relatively ideal conditions, and less effective results may occur if, for example, nets are heavily contaminated with debris or there are many pathogens coating a relatively inaccessible crevice.

Disinfectant activity can be divided into several levels: high, intermediate, and low (Tables III-10 and III-11). Note that many disinfectants commonly used in aquaculture provide only intermediate-level disinfection at best. This is not to say that such disinfectants are not highly effective when used properly, but the clinician

Table III-10. Levels of disinfectant action according to type of microorganism (modified from Favero and Bond [1991], with permission).

Disinfectant level	Killing effect[a]					
	Bacteria			Fungi[b]	Viruses	
	Spores	*Mycobacterium tuberculosis*	Vegetative cells		Hydrophilic (nonlipophilic) and small	Hydrophobic (lipophilic) and medium size
High	+[c]	+	+	+	+	+
Intermediate	–	+	+	+	±	+
Low	–	–	+	±	±	+

[a]+, Killing effect can be expected; –, little or no killing effect.

[b]Includes asexual spores but not necessarily chlamydospores or sexual spores.

[c]The high-level disinfectants hydrogen peroxide and chlorine are capable of killing high numbers of bacterial spores in laboratory tests only with extended exposure times; they are, however, capable of sporicidal activity. However, the only fish pathogens that produce resistant spores are *Clostridium* species (PROBLEM 57).

Table III-11. Efficacy and characteristics of commonly used disinfectants (modified from Widmer and Frei [1999], with permission). + = yes; – = no; V = variable results. The efficacies of the disinfectants are based on exposure time of less than 30 minutes at room temperature.

Germicide	Use dilution	Level of disinfection	Active against					Important characteristics								
			Vegetative bacteria	*Mycobacterium tuberculosis*	Fungi	Hydrophilic viruses	Lipophilic virus	Shelf life >1 week	Corrosive	Residue	Inact. by organic mat.	Skin irritant	Eye irritant	Resp. irritant	Toxic	Environ. concern
Formaldehyde	2–3%	High	+	+	+	+	+	++	–	+	–	+	+	+	+	+
Hydrogen peroxide	3–25%	High	+	+	+	+	+	+	V	–	V	+	+	–	+	–
Chlorine	100–1,000 ppm Cl	High	+	+	+	+	+		+	+	+	+	+	+	+	V
Isopropyl alcohol	60–95%	Intermediate	+	+	+	V	+	+	V	–	V	V	+	–	+	–
Glucoprotamine	4%	Intermediate	+	+	+	+	+	+	–	–	–	+	+	–	–	–
Phenolic compounds	0.4–5% aqueous	Intermediate	+	+	+	V	+		–	+	–	+	+	–	+	+
Iodophors	30–50 ppm	Intermediate	+	V	+	+	+	+	V	+	+	V	+	–	+	–
Quaternary ammonium compounds	Active 0.4–1.5% aqueous	Low	V	–	V	–	+	+	–	+	+	+	+	–	+	–

should be aware of their limitations. Fortunately, it appears that important pathogens affecting fish appear to be eliminated by proper intermediate-level disinfection, with few exceptions (e.g., *Clostridium*). However, some organisms are especially resistant to certain types of disinfectants at levels used routinely in aquaculture (e.g., *Myxobolus cerebralis* is not totally killed by even 1200 mg/liter calcium hypochlorite for 16 hours [Hoffman 1972]). Disinfection is not sterilization; thus, it doesn't guarantee the complete elimination of all pathogens.

Disinfection of water to destroy infectious agents before use in culture systems is usually accomplished by using chlorination, ozonation, or ultraviolet irradiation. Equipment is available from several suppliers (e.g., Aquatic Ecosystems, Prominent Environmental, Aqua Logic). See Piper et al. (1982), Spotte (1992), and Table III-11 for details.

Which disinfectant to use should be based on:

- Efficacy: It is important to know the sensitivity of the pathogens (viruses, bacteria, water molds, parasites) of concern. That is, what disinfectants and at what concentration for what period of time will kill them. For example, IPNV is very resistant to germicides compared to CCV.
- Environmental impact: A good disinfectant must kill pathogens but must not harm nontarget organisms in the environment when released.
- Operator safety: Any products used must be safe for staff employing the product and all safety protocols must be strictly followed.

To optimize disinfection, surfaces and equipment should be cleaned with an appropriate cleaner or detergent in order to remove as much organic material as possible prior to disinfection. They should also be rinsed with water and air dried between cleaning and disinfecting.

Most disinfectants can be irritating to the eyes, skin, and/or mucus membranes. When using dry products (powders), minimize dust release. When mixing solutions, it is usually best to wear goggles, chemical-resistant gloves and a mask; it may also be advisable to do so when applying the disinfectant under certain circumstances (e.g., when applying with a hand-held fogger or with a pressure washer). Be sure to carefully read the product insert and MSDS sheet before using any disinfectant.

Use No. 1: Eradication of infectious agents from a contaminated environment

Euthanized fish should be disposed of using standard biohazard guidelines for infectious waste (see "**Mortality Management**," **p. 78**). Equipment and everything else that has come in contact with contaminated water (aquaria, filters, ornaments, tubing, gravel, etc.) are usually disinfected by using chlorination. If there is a large amount of organic matter in the aquarium, this may require more disinfectant than recommended for "clean" aquaria. In ponds or other large culture systems, slaked or hydrated lime is the method of choice for disinfection.

Use No. 2: Killing of infectious agents in a contaminated water supply

Surface water sources are often contaminated with infectious agents that may cause problems in culture. Treating incoming water can reduce or eliminate these problems, although it is expensive to treat water in this manner. Ozone, ultraviolet irradiation, and chlorination/dechlorination have been used (see "**Ozone**" for more details).

Pathogens are often spread by fomites. If these fomites are properly disinfected at defined critical control points, exposure to pathogens will be greatly reduced. Disinfectants are routinely used for prophylactic disinfection (e.g., net soaks, footwear baths, etc.).

ELECTROSHOCK

Electroshock is used extensively in sampling wild fisheries populations (Redman et al. 1998). It is not an approved method of anesthesia. The response to electroshock is dependent upon the intensity of the electrical field, as well as the water conductivity. High conductivity even in freshwater limits its effectiveness, and it is ineffective in seawater because the water is more conductive than the fish (Coyle et al. 2004). Effectiveness is also influenced by fish size and species. Lower voltage should be used for larger fish. Electroshock can be performed with either alternating electrical current (AC), direct current (DC), or pulsed DC. Fish exposed to low voltage DC are immobilized but only while they remain in the electrical field. AC can render the fish unconscious for a short period, but is the most damaging form of electroshock.

While sometimes called electroanesthesia, fish are stunned, not anesthetized. The induction of tetany may cause severe spinal damage due to vertebral fracture. It might also cause arrhythmias, respiratory arrest and internal hemorrhage. However, most deleterious effects are typically short-lived when DC or pulsed DC is used appropriately.

Extreme care must be taken by the operator to avoid electrocution (neoprene gloves, properly grounded boat, first aid available, never work alone, etc.).

ENAMECTIN BENZOATE (SLICE™ [SCHERING-PLOUGH ANIMAL HEALTH])

Enamectin is an avermectin, a family of macrocyclic lactone antibiotics with potent, neurotoxic, anti-parasitic activity. Avermectins act by irreversibly binding to and opening GABA receptors and glutamate-gated channels, causing paralysis and death of the parasite. SLICE™ is used to treat sea lice in Europe and North America

(Wescott et al. 2004). It kills all parasitic stages of sea lice for up to 10 weeks; it is used as a feed premix. Withdrawal time is 175 degree days in Norway, about 68 days in Canada, and 0 days in Chile and Scotland. It is potentially neurotoxic to mammals.

Use: Treating sea lice in salmon

Oral formulations:

1. Feed for treating sea lice: see general concerns regarding treatment of sea lice under "**Organophosphates**."
   a. Feed 50 µg enamectin benzoate/kg body weight (= 23 µg/lb body weight)/day for 7 days (Hakalahti et al. 2004). This is equivalent to a feed that has 10 kg enamectin benzoate per ton and is fed at a rate of 0.5% of body weight/day (Stone et al. 2000).

EUGENOL (AQUI-S™ [AQUI-S NEW ZEALAND, LTD.], CLOVE OIL [HUMCO])

Eugenol and related compounds are components of clove oil, which is a mixture of aromatic distillates from the clove tree (*Eugenia aromatica*). Eugenol is the major active ingredient of clove oil (85–95%), with the remaining 5–15% being various amounts of isoeugenol and methyleugenol. A synthetic form of isoeugenol (AQUI-S) has been developed for anesthetizing and euthanizing fish. AQUI-S™ contains 54% isoeugenol and 46% polysorbate 80. Another product, AQUI-S E, is a 50% solution of eugenol. This commercial preparation is legal for food fish with no withdrawal time in Australia, New Zealand, Korea, Chile, Costa Rica, and Honduras. However, the United States Food and Drug Administration has recently rescinded authorization for investigational use of AQUI-S™ because it was discovered to be a carcinogen in animal studies.

Over-the-counter preparations of eugenol (e.g., clove oil [Humco]) are also effective. Eugenol is poorly soluble in water; thus, clove oil should be diluted 1:10 with 95% ethanol (1 part clove oil + 9 parts ethanol) to yield a working stock solution of 100 mg/ml (each ml of clove oil contains ~1 g of active ingredients). High concentrations of eugenol are irritiating to skin and contact with eyes and mucous membranes should be avoided. Eugenol is considerably less expensive than tricaine.

Even fish deeply anesthetized with eugenol often react to needle puncture (Sladky et al. 2001) and may retain some involuntary movement, making it less desirable to use during bleeding or surgery. Eugenol's activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines. The clinical response of the fish should also be used to ascertain the proper dosage (see **p. 20**).

Use No. 1: Sedation for transporting fish. With sedating doses, fish typically quickly recover and return to feeding.

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add 0.06–0.10 ml of clove oil (HUMCO, or equivalent) stock solution (100 mg/ml) to 1 liter of water (= 0.23–0.38 ml of stock to 1 gallon of water; = 6–10 mg/liter = 23–38 mg/gallon final concentration).

Use No. 2: Anesthesia. Anesthetic complications (severe cardiorespiratory depression and death) have occurred with members of the tropical marine family Acanthuridae (tangs and surgeonfish) (Harms and Lewbart 2000).

Water-borne formulations:

1. Bath
   a. Add 0.4–1.2 ml of clove oil (HUMCO, or equivalent) stock solution (100 mg/ml) to 1 liter of water (= 1.5–4.5 ml of stock to 1 gallon of water; = 40–120 mg/liter = 150–450 mg/gallon final concentration). This concentration is effective in freshwater and marine species, and results are comparable to tricaine, except that recovery may be prolonged (Sladky et al. 2001). For surgery, use the high end for induction and the low end for maintenance (Harms and Lewbart 2000).

Use No. 3: Euthanization

Water-borne formulations:

1. Bath
   a. Add to effect. This usually requires a slightly higher dose than for anesthesia. AQUI-S™ is used for anesthetizing farmed food fish prior to exsanguination at harvest; it is claimed to significantly improve carcass quality.
   b. For pet fish owners, advise to add a minimum of 10 drops per liter (40 drops per gallon) of hot water (to aid dissolving) and mix well with a whisk (Ross 2001). The water should turn slightly cloudy, indicating that the clove oil has emulsified. After the water has cooled to aquarium temperature, add the fish to be euthanized. Have the owner closely watch the fish until no breathing occurs for at least 30 minutes of observation. Then leave the fish in the solution for another 2 hours and check it again to be sure it is dead.

EUTHANASIA

An accepted method of euthanasia is one that causes death with minimal suffering to the animal. While "acceptable" and "unacceptable" methods are generally similar among various countries, the clinician should be aware that there are differences and the following discussion is intended only as a general guideline. Also, in some cases, it may not be possible to use accepted methods and the best possible alternative must be chosen.

For food animals at slaughter, methods must not leave drug residues and thus physical procedures are most

common. For example, commercial slaughter methods for Atlantic salmon have included either exsanguination (gill cut without prior stunning), carbon dioxide narcosis followed by gill cut, or percussive blow followed by gill cut (Robb et al. 2000).

Reliable indicators of death (ECPAKFP 2006) include:

- Immediate and irreversible respiratory arrest (loss of rhythmic opercular activity). There should be lack of breathing for at least 10 minutes.
- Immediate and irreversible loss of the eyeroll (vestibulo-ocular reflex). This is movement of the eye when a fish is rocked from side to side. It does not occur in a dead fish. It is only useful in larger fish.

Note that the heart continues to contract long after the fish has died.

Accepted methods for humane euthanasia of fish according to the AVMA (Anonymous 2000, 2007c) include anesthetic overdose via use of either

1. Tricaine
2. Benzocaine
3. 2-phenoxyethanol
4. Barbiturates (sodium pentobarbital)
5. Inhalant anesthetics
6. Carbon dioxide

Although not specifically accepted by AVMA (Anonymous 2007c), quinaldine sulfate is probably also satisfactory for euthanization. Clove oil is also not an accepted method of euthanasia (Anonymous 2007c) but is readily available at local pharmacies and thus can be used by a client to euthanize a pet in a relatively humane fashion.

Conditionally acceptable methods of euthanasia (Anonymous 2007c) include:

1. Decapitation followed by either pithing or exsanguination
2. Stunning followed by either decapitation/pithing or exsanguination

Decapitation is assumed to cause rapid unconsciousness by stopping the blood supply to the brain. However, the central nervous system of some poikilothermic vertebrates is tolerant of hypoxic and hypotensive conditions (Cooper et al. 1989); thus, decapitation should be followed by pithing.

Pithing: Pithing is an effective and inexpensive means of euthanasia but requires dexterity and skill. It acts by causing trauma to nervous tissue. Double pithing (pithing both the brain and the spinal cord) is recommended to ensure immediate death.

Stunning (cranial concussion): Stunning delivers a single, sharp blow to the head with sufficient force to produce immediate depression of the central nervous system. Stunning can be done with a club ("priest"), commonly used for farmed fish, but requires considerable skill to perform successfully. Stunning renders a fish unconscious but is not a method of euthanasia; thus, it must be followed by a method to ensure death. The AVMA-accepted method is pithing, but exsanguination is often used as well (see below). Cranial concussion can cause iatrogenic gill telangiectasis and thymic hemorrhages (Herman and Meade 1985).

Other types of concussive blows are used to more severely traumatize the brain, causing unconsciousness (see below). In Atlantic salmon and some other salmonids, two types of concussive blows have been used prior to euthanization:

Percussive blow with a rapidly moving, manually applied club followed by a gill cut (Robb et al. 2000): This causes a differential acceleration of the brain within the skull. In all fish, it is more rapid (and thus more humane) than either only using a gill cut or using CO_2 followed by a gill cut, but for some fish there is a short time interval before loss of brain activity (visual evoked responses [VERS]).

Spiking ("iki jime"): This has been used to kill tuna in New Zealand. A spike is inserted manually into the brain and rotated to destroy it.

For both the percussive blow and spiking, it can be difficult to hit the correct part of the brain every time, prolonging euthanasia (Robb et al. 2000).

Exsanguination ("gill cut"): This causes rapid and extreme hypovolemia, resulting in significant distress. It should only be used in sedated, stunned, or anesthetized animals (Anonymous 2007c; ECPAKFP 2006). Varying the number of gill arches cut (e.g., 2 vs. 4) is likely to cause a variation in bleed out.

When fish are examined for disease, mechanical trauma (e.g., decapitation and pithing) is often preferred to chemical overdose because ectoparasites may detach because of the chemical treatment (see **p. 21**).

Unacceptable methods of euthanasia (AVMA 2000, 2007c) include:

Cooling: Cooling fish to 4°C (refrigerator temperature) decreases metabolism and facilitates handling but probably does not raise the pain threshold. Rapid freezing is also not considered to be humane, unless preceded by anesthetization.

Formalin: This is inhumane.

Rotenone, antimycin, bayluscide, and other poisons are commonly used to kill fish in ponds or other small bodies of water (Marking 1992) but are not approved methods of euthanasia because they cause considerable distress (e.g., rotenone causes asphyxiation).

Decapitation, stunning, or exsanguination, when used alone.

FENBENDAZOLE (PANACUR® [HOECHST])

Use No. 1: Treatment of nonencysted nematodes in the gastrointestinal tract

Water-borne formulations:

1. Prolonged immersion
   a. Add 2 mg fenbendazole/l (= 7.6 mg/gallon) once/week for 3 weeks.

Oral formulations:

1. Feed 25 mg fenbendazole/kg (= 11 mg/lb) of body weight/day for 3 days for aquarium fish (Gratzek and Blasiola 1992). This is equivalent to a feed that has 0.25% fenbendazole and is fed at a rate of 1% of body weight/day.
2. Feed 50 mg fenbendazole/kg (= 23 mg/lb) of body weight once/week for 2 weeks (Langdon 1992a). This is equivalent to a feed that has 0.50% fenbendazole and is fed at a rate of 1% of body weight/day.
3. Intubate 50 mg fenbendazole/kg (= 23 mg/lb) of body weight (Langdon 1992a).

Use No. 2: Treatment of mongeneans

Water-borne formulations:

1. Bath
   a. Add 25 mg fenbendazole/l (= 95 mg/gallon) for 12 hours.

FLUBENDAZOLE

Flubendazole is a benzimidazole carbamate anthelmintic that is active against a range of gastrointestinal parasites in pigs and poultry. The dose used to kill hydra is also toxic to snails.

Use: Treating hydra in freshwater aquaria

Water-borne formulations:

1. Prolonged immersion
   a. Add 1/2 teaspoon of 5% flubendazole powder (5% active ingredient) per 30 gallons of water (= 2 mg/l) (Harrison 2003). First add the dry powder to a container with some aquarium water, shake well to dissolve as much as possible, and then pour it in the aquarium. After 6 days, do at least a 30% water change.

      Alternatively, sprinkle a small amount on the water surface (a light salting). Solubility is extremely low and potency is extremely high and so overdosing is not a problem (it does not appear to affect any larval fish) (R. Goldstein, personal communication).
   b. Add Fluke-Tabs (Interpet) per label directions.

FORMALIN (FORMALIN-F™ [NATCHEZ], PARACIDE-F® [ARGENT], FORMACIDE-B® [B.L. MITCHELL])

Formalin-F™, Paracide-F®, and Formacide-B® are formalin labels approved for food fish use in the United States (see Table III-2 for specific indications), but all uses of formalin have recently been banned in the European Union. Formalin is an aqueous solution of 37–40% formaldehyde gas (which equals 100% formalin). Formalin cross-links proteins, resulting in cell death (van Ham and Hall 1998). It is an effective parasiticide for bath treatment of most ectoparasitic protozoa and monogeneans. It has moderate-to-weak antibacterial activity. It also has moderate-to-strong activity against water molds on eggs but is not antifungal at doses that are nontoxic to fish.

Formalin is not usually recommended for treating commercial fish ponds because each 5 mg/liter of formalin added to a pond chemically removes 1 mg/liter of dissolved oxygen from the water (Allison 1962; Schnick et al. 1989). It is also algicidal, which can further reduce oxygen (Schnick 1973). Finally, it is usually too costly for use in large ponds. Formalin is also toxic to macrophytes (e.g., aquarium plants).

Formalin can be irritating to the gills, and water should be well aerated during treatment. Formalin is more toxic in soft, acid water and at high temperatures. Even slight differences in dosage or exposure time can have a major effect on toxicity (Heinen et al. 1995). Some fish, especially elasmobranchs, are sensitive to formalin, so it is best to do a bioassay before using it on an untested fish species. Idiopathic deaths may occur within 1–72 hours of treatment (Warren 1981). Rainbow trout seem especially susceptible. Typically, fish are piping, have excess mucus and pale color, and die with their mouth agape.

Formalin is contraindicated if fish have been recently stressed (e.g., transported, shipped) or if skin ulcers are present. Used formalin solutions should be diluted to at least 25 ppm before discarding.

Formalin is volatile and irritating. It causes cancer in laboratory rodents and can cause contact hypersensitivity and lung damage in humans; solutions should be tightly sealed during storage and not allowed to contact human skin. Formalin should only be used in well-ventilated areas.

Formalin should be stored in the dark and above 4°C (39°F) to inhibit paraformaldehyde formation, a highly ichthyotoxic white precipitate (Fig. III-6). Formalin should never be used for treating fish if paraformaldehyde is present. Methanol (12–15%) is added to formalin to inhibit paraformaldehyde formation. Formalin should not be mixed with potassium permanganate.

Formalin chemically interferes with the methods used to commonly measure ammonia and thus accurate ammonia readings are not possible with these methods when using formalin (see PROBLEM 4).

Use No. 1: Treatment of protozoan and metazoan ectoparasites

Water-borne formulations:

1. Bath
   a. Add 0.125–0.250 ml formalin/l (= 125–250 ppm = 0.47–0.95 ml formalin/gallon), and treat for up to 60 minutes. This can be repeated two to three times once daily if needed. When temperatures are high (>21°C [= 70°F] for warm water fish or >10°C [= 50°F] for cold water fish), do not use >167 ppm (= 0.167 ml/l = 184 mg/liter = 0.63 ml/gallon) (Warren 1981; Jensen and Durborow 1984). The maximum dose should only be used

Fig. III-6. A white precipitate (paraformaldehyde, *arrow*) in degraded formalin. Note that degradation can occur even when stored under reduced light conditions, as present in this amber bottle. An obvious precipitate may not be present but rather the solution may simply appear cloudy.

every 3 days. Up to 167 ppm can be used on concurrent days (Post 1983). Formalin is usually contraindicated if the temperature is >27°C (80°F).

2. Prolonged immersion in aquaria
   a. Add 0.015–0.025 ml formalin/l (= 15–25 ppm = 16.5–27.6 mg/liter = 0.06–0.09 ml/gallon). For *Ichthyophthirius*, use 25 ppm every other day for 3 treatments (Hoffman and Meyer 1974). Remove all plants before treatment. Change up to 50% of the water on alternate days. Do not use >10 ppm for striped bass fingerlings (Piper et al. 1982). The treatment schedule must be prolonged at low temperature (see PROBLEM 20).
3. Constant flow
   a. Add 0.015 ml formalin/l (= 15 ppm = 16.5 mg/liter = 0.06 ml/gallon) as a constant flow for 24 hours. This can successfully treat ich in trout raceways (J. Hinshaw, personal communication).

Use No. 2: Treatment of water mold infection on eggs. Do not treat eggs within 24 hours of hatching. Formalin will concentrate in the shell, killing the embryo (Jensen and Durborow 1984).

Water-borne formulations:

1. Bath
   a. Add 1–2 ml formalin/l (= 1,000–2,000 ppm = 1,103–2,206 mg/liter = 3.8–7.6 ml/ gallon), and treat eggs for up to 15 minutes. This can be repeated as needed.
   b. Add 0.23 ml formalin/l (= 227 ppm = 250 mg/liter = 0.87 ml/gallon), and treat eggs for up to 60 minutes. This is experimentally effective in treating infections of rainbow trout eggs (Bailey and Jeffrey 1989).

Use No. 3: Killing of tadpoles in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add 0.03 ml formalin/l (= 30 ppm = 0.11 ml/gallon). This dose is most effective for small (2.5- to 5.0-cm [1- to 2-inch]) leopard frog tadpoles. Bullfrogs or larger tadpoles need higher doses that are usually ichthyotoxic (Helms 1967).

Use No. 4: Disinfectant

Water-borne formulations:

1. Prolonged immersion
   a. Add 27–220 ml formalin/l (= 102–833 ml/gallon = 1–8% formaldehyde [Favero and Bond 1991]). This can be used as an indefinite soak for nets and other utensils. Keep the solution well covered and in a well-ventilated room. Rinse utensils well before using in aquaria.

FORMALIN/MALACHITE GREEN (LETEUX-MEYER MIXTURE)

Use: Treatment of *Ichthyophthirius multifiliis*

This combination is synergistic for ich (Gilbert et al. 1979). See separate precautions under "**Formalin**" and "**Malachite Green**." Slightly higher doses of either drug are ichthyotoxic (Leteux and Meyer 1972).

Water-borne formulations:

1. Prolonged immersion
   a. Add 25 ppm formalin + 0.10 mg/liter malachite green (= 0.09 ml of formalin + 0.1 ml of malachite green stock [3.7 mg/liter stock] solution/gallon). Treat every other day for 3 days. Change up to 50% of the water on alternate days. Remove all plants before treatment. The treatment schedule must be prolonged at low temperature (see PROBLEM 20).

FRESHWATER

Use: Treatment for marine ectoparasites

Freshwater can be used for therapy of clinical cases of marine *Trichodina* and other protozoans, Monogenea, and some crustaceans (Langdon 1992a).

Water-borne formulations:

1. Bath
   a. Bathe marine fish in dechlorinated freshwater for 3–15 minutes. Remove immediately if stressed. This can be repeated weekly for an indefinite

number of times. For treating *Caligus elongatus* on euryhaline marine fish, keep fish in freshwater for 20 minutes to kill all parasites (Landsberg et al. 1991).

2. Prolonged immersion
   a. Reduce salinity to freshwater. This is effective but can only be used for treating euryhaline fish (see "**Hyposalinity**").

FUMAGILLIN (BICYCLOHEXYLAMMONIUM FUMAGILLIN, FUMIDIL B® [CEVA; MID-CONTINENT AGRIMARKETING])

Fumagillin is an acyclic polyene macrolide produced by the fungus *Aspergillus fumigatus* and was originally developed for treating microsporidiosis (*Nosema apis*) in honeybees. It has been used to treat several fish microsporidioses (Shaw and Kent 1999). However, its safety margin is narrow, with toxic effects including anorexia, poor growth, anemia, renal tubular degeneration and atrophy of hematopoetic tissue. Toxicity is observed when treating at high doses or for long periods of time (e.g., > ~30–40 days in salmonids).

In virtually all cases, the drug mainly delays parasite development but does not completely cure the infection. It has also been used against some myxozoans, where efficacy has generally been lower than with microsporidians. Fumagillin is heat labile; thus, prepared feed must be sprayed with a solution of fumagillin dissolved in ethanol (it is poorly water soluble) and then the feed is coated with oil. Alternatively, Fumidil B®, a 2% premix, can be used to top-coat the feed using conventional means. This treatment is not approved for food fish. An analogue of fumagillin (TNP-470 [Takeda Chemical Industries, Ltd.]) appears to have similar activity.

Use No. 1: Treatment of microsporidiosis

Oral formulations:

1. Feed 1.5 mg fumagillin/kg (= 0.7 mg/lb) of body weight/day for 21 days for *Nucleospora* (= *Enterocytozoon*) *salmonis* in chinook salmon (Hedrick et al. 1991).
2. Feed 10 mg fumagillin/kg (= 4.5 mg/lb) of body weight/day for 30 days for *Loma salmonae* in chinook salmon (Kent and Dawe 1994). A lower dose, 2–4 mg/kg (= 0.9–1.8 mg/lb) of body weight/day for 30 days has been advocated by Shaw and Kent (1999) to avoid toxicity.
3. Feed 0.1–1 mg TNP-470/kg (= 0.05–0.45 mg/lb) of body weight/day for 45 days for *Loma salmonae* or *Nucleospora salmonis* (Shaw and Kent 1999).
4. Feed 250 mg fumagillin/kg (= 113 mg/lb) of body weight/day for 30 days for *Heterosporis anguillarum* in Japanese eel (Kano et al. 1982). Note that this dose would be lethal to salmonids.

Use No. 2: Treatment of myxosporidiosis (myxozoan infection)

Oral formulations:

1. Feed 5 mg fumagillin/kg (= 2.3 mg/lb) of body weight/day for 42 days for proliferative kidney disease or for whirling disease in salmonids. Higher concentrations for prolonged periods (> ~30–40 days) may cause morbidity or mortality.
2. Feed 15 mg fumagillin/kg (= 6.8 mg/lb) of body weight/day for 56 days for *Sphaerospora testicularis* in European seabass (Treves-Brown 2000). This treatment aided spermiation but was not curative.

GONADOTROPIN RELEASING HORMONE (SALMON GONADOTROPIN RELEASING HORMONE ANALOGUE, sGnRHa [OVAPLANT®, AQUATIC LIFE SCIENCES])

Use: Induce gamete maturation in fish

1. Injectable formulations:
   a. Implant pellets at a dose of 10–75 µg sGnRHa/kg body weight (= 4.5–34 µg/lb). This use is available under an INAD in the United States. All treated broodfish must be maintained indefinitely or destroyed (no release or slaughter for food is allowed).

HYDROGEN PEROXIDE (H_2O_2; SALARTECT® 300 AND 500 [BRENNTAG], PARAMOVE® 35 AND 50 [SOLVAY], PEROX-AID® 35% [WESTERN CHEMICAL], OR EQUIVALENT)

Medical grade hydrogen peroxide is available over-the-counter as a 3% solution (= 30 mg H_2O_2/ml = 30,000 ppm). However, for any large scale aquaculture use, a highly concentrated solution of 35–50% (= 350–500 mg H_2O_2/ml = 350,000–500,000 ppm) is used; this solution is highly corrosive and the handler must avoid all contact when preparing the solution. Wear rubber gloves and eye protection.

A skirt must be used when treating a cage. The effect of H_2O_2 on protozoa is probably intracellular oxidation. The mechanism of action against other parasites is uncertain. The toxicity of H_2O_2 is greatest at high temperature (probably due to accelerated degradation in the fish) and with smaller fish. Overdosing causes gill damage; fish may take up to 24 hours to die. There are no residue concerns in either the fish or the environment. Transport hazards can present difficulties, especially for island-based farms where hydrogen peroxide tanks cannot be shipped on ferries (Alderman 2002).

Bioassay of a small number of eggs or fish is recommended before treating the entire group. Specific indications for its uses in the United States are provided in Table III-2.

Hydrogen peroxide should never be stored near flammable substances, since spilling it onto such a substance can cause an immediate fire. High-strength peroxide (also called high-test peroxide, or HTP) must be stored in a suitable, vented container to prevent the buildup of oxygen gas, which would otherwise lead to the eventual rupture of the container.

Sodium percarbonate has also been used to generate hydrogen peroxide. Sodium percarbonate is sodium carbonate with hydrogen peroxide bound to the molecule. When dissolved in water, it releases H_2O_2 and sodium carbonate. It allows a slower release and prolonged action of hydrogen peroxide in the bath and thus may be less toxic than pure hydrogen peroxide (Buchmann and Kristensson 2003).

Use No. 1: Treatment of acute environmental hypoxia

This use is predicated upon the fact that when hydrogen peroxide is added to water, it releases oxygen as it decomposes. The use of hydrogen peroxide in treating acute hypoxia is not well studied, and proper application probably requires adding a sufficient amount to supply the required oxygen to the fish while at the same time avoiding toxicity from overdosing. After a certain period of time, additional hydrogen peroxide would need to be added as it decays and the oxygen is consumed by the fish. It would be advisable to monitor this procedure with an oxygen meter.

Water-borne formulations:

1. Prolonged immersion
   a. Add 0.25 ml of 3% H_2O_2 solution/l of water to be treated (= 1.0 ml/gallon = 7.5 ppm; Sterba 1983).
   b. Add 0.10 ml of 3% H_2O_2 solution/l (= 0.40 ml/gallon = 3 ppm) to yield 1.0 mg oxygen/l (Maranthe et al. 1975).

Use No. 2: Parasiticide for protozoan or metazoan ectoparasites. Many fish do not tolerate this treatment.

Water-borne formulations:

1. Bath
   a. Add 10 ml of 3% H_2O_2 solution/l (= 38 ml/gallon = 300 ppm), and treat for 10–15 minutes for protozoan ectoparasites of tropical fish (Sterba 1983).
   b. Add 19 ml of 3% H_2O_2 solution/l (= 70 ml/gallon = 570 ppm) and treat for 4 minutes for protozoan ectoparasites of tropical fish. Use only once (Lewbart 1991).
   c. Add 0.21 ml of 35% H_2O_2 solution/l (= 75 ppm), and treat for 30 minutes for amyloodiniosis. Retreat after 6 days. Fish must be moved to an uncontaminated system immediately after last treatment might cure (Montgomery-Brock et al. 2001).
   d. Add 1.25 ml of 50% H_2O_2 solution/l (= 4.75 ml/gallon = 1,250 ppm), and treat for a maximum of 30 minutes for sea lice (Thomassen 1993). Hydrogen peroxide decays to oxygen so aeration is not needed during treatment. Do not use at >10°C. Margin of safety is good at 6°C but becomes increasingly narrow at higher temperature, making this drug of limited use during summer, the high risk season for sea lice. Treated lice detach from the fish but may recover motility in ~1 hour (Hodneland et al. 1993); *L. salmonis* probably cannot reinfect a fish but *Caligus* spp. might be able to do so.
   e. Add 80 mg of sodium percarbonate/l (= 304 mg/gallon) and treat for 18 hours for monogeneans. This dose eradicates *Gyrodactylus derjavini* on rainbow trout (Buchmann and Kristensson 2003).

Use No. 3: Oomyceticide for water mold infections of eggs

Water-borne formulations:

1. Constant Flow
   a. Add 1.42–2.11 to 2.8 ml of 35% hydrogen peroxide/l (= 5.4–8.1 to 10.8 ml/gallon = 500–750 to 1,000 ppm; 35% hydrogen peroxide = 350,000 ppm H_2O_2), and treat for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch (Dawson et al. 1994). Use 500–1000 mg/liter for coldwater and coolwater fish; use 750–1000 mg/liter for warmwater fish.

Use No. 4: Treating skin and gill flavobacterium infections

Water-borne formulations:

1. Bath
   a. Add 0.14–0.21 to 0.28 ml of 35% hydrogen peroxide/l (= 0.54–0.81 to 1.08 ml/gallon = 50–75 to 100 ppm; 35% hydrogen peroxide = 350,000 ppm H_2O_2). Use once per day on alternate days for 3 treatments.

   For bacterial gill disease on freshwater salmonids: Use either 100 mg/liter for 30 minutes or 50–100 mg/liter for 60 minutes.

   For external columnaris on fingerling and adult freshwater, coolwater fish (except northern pike and paddlefish), and channel catfish: Use 50–75 mg/liter for 60 minutes.

   For external columnaris on freshwater fry (except northern pike, pallid sturgeon, and paddlefish): Use 50 mg/liter for 60 minutes.

   Use with caution on walleye.

HYPOSALINITY

Use No. 1: Treatment of *Cryptocaryon irritans*

Water-borne formulations:

1. Prolonged immersion
   a. Reduce the salinity of the affected tank by ~5–10 ppt/day, using fresh dechlorinated water until

the salinity is <16 ppt (Cheung et al. 1979). Remove all invertebrates before beginning the treatment. Return the tank to normal salinity after 3 weeks. Note that not all marine reef fish may tolerate this salinity. Some fish also tend to become hyperactive. A salinity of 14 ppt (specific gravity 1.010) is reported by some to be well tolerated by many reef fish for at least 3 weeks (Goodlett and Ichinotsubo 1997), allowing time for either ectoparasite treatment or preventive quarantine; however, most if not all elasmobranchs probably do not tolerate this treatment.

b. Reduce the salinity as quickly as possible to 25% of the original salinity for 1–3 hours. (For example, if salinity is 40 ppt, reduce to 10 ppt.) Repeat every 3 days for a total of 4 treatments (Colorni 1985).

Use No. 2: Treatment of marine capsalid monogeneans

Water-borne formulations:

1. Bath

After treating sharks with freshwater for 5 minutes or teleosts for 3–15 minutes, monogeneans become opaque (Fig. III-7), but not all die (Whitaker 2001).

Use No. 3: Treatment of other marine ectoparasites

Water-borne formulations:

1. Prolonged immersion

a. Reduce the salinity of the affected system by ~5–10 ppt/day, using fresh dechlorinated water until it becomes freshwater. This treatment is effective but can only be used on euryhaline species (i.e., can tolerate freshwater).

HYPOTHERMIA

Use: Preventing stress during transport. Lowering the temperature reduces fish metabolic rate and thus oxygen consumption. It also greatly reduces stress hormone release. The specific rate and amount of temperature reduction that can be used varies widely among fish species, but temperate freshwater fish tend to be more tolerant than tropical freshwater fish, which tend to be more tolerant than tropical marine fish. In general, the temperature should not be reduced faster than ~1°C every 15 minutes.

Temperate species can be cooled to as low as 4°C (39°F) depending upon the species and their prior acclimation conditions. This has been done to transport Atlantic salmon broodfish. Hypothermia is not a true anesthetic and does not affect pain receptors. However, it is often used in conjunction with anesthetics to decrease the amount of drug needed.

Packing live fish on ice (for shipping to market) is considered inhumane (ECPAKFP 2006).

Water-borne formulations:

1. Add ~0.5 lb of ice per gallon (~60 grams per liter) to reduce the water temperature by ~12°C (~10°F) (e.g., to reduce the temperature from 70°F to 60°F). Do not use ice made from chlorinated drinking water (Jensen 1990).

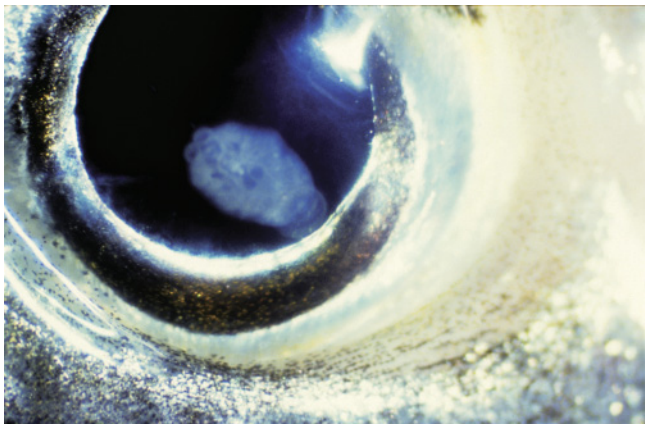


Fig. III-7. A capsalid monogenean on the eye that has become opaque after hyposalinity treatment. (Photograph courtesy of A. Colorni.)

IMMUNOSTIMULANTS

Immunostimulants (Table III-12) are intended to primarily stimulate the nonspecific immune response so that the overall resistance of a fish to infection is increased.

Table III-12. Commercially available immunostimulant products for finfish aquaculture.

Company	Product(s)
Aqua-In-Tech	Immunostimulants (β-glucans, peptidoglycans, nucleotides) and probiotics
Cenzone Tech	Immunostimulant (Aqua Gold) and probiotic (Aqua-Start)
Citura	Immunostimulant derived from chicory root (Bonuline)
Dinatec	Immunostimulant containing β-glucans (Dinamune)
EWOS	Nucleic acid feed immunostimulant (EWOS boost)
ImmuDyne	β-glucan (AquaStim®)
Intervet International/Schering Plough Animal Health	Algine-based immunostimulant (AquaVac Ergosan)
Inve Aquaculture	Proprietary immunostimulants (Sanoguard®)
Levapan SA	Yeast feed additives
Park Tonks	Immunostimulant composed of plant extracts and vitamins (Aquamune XL)
Pharmaq AS	β-glucan (Vetregard)
Zeigler Bros	Proprietary immunostimulant (Vpak™ [Vitality pak])

Immunostimulants can be delivered orally, via injection, or via water. Oral preparations are most commonly used commercially. For more details on immunostimulants, see "**Health Promotion and Maintenance**" (**p. 73**).

IVERMECTIN (IVOMEC® [MERIAL])

Ivermectin is an avermectin parasiticidal agent that has been widely used as a treatment against sea lice infections of farmed fish (salmonids and nonsalmonids) (Roth 2000) without any official license for use in aquaculture. It has also been used to treat gill maggots. Ivermectin has a relatively low margin of safety for fish, presumably due to the reduced blood-brain barrier in fish compared to mammals, resulting in greater potential for neurotoxicity. Ivermectin also has been associated with respiratory distress (Toovey et al. 1999). Ivermectin is photoinactivated (Boxall et al. 2004).

Use No. 1: Treating ectoparasitic copepods

Oral formulations:

1. Feed for treating caligid sea lice in salmon: see general concerns regarding treatment of sea lice under "**Organophosphates**."
   a. Feed 50 µg ivermectin/kg body weight (= 23 µg/lb body weight)/day once weekly × 2 weeks (Pike and Wadsworth 1999).
2. Oral intubation for gill maggots (*Salmincola californiensis*) in rainbow trout
   a. Orally intubate 0.2 µg ivermectin/kg body weight (= 0.09 µg/lb body weight) once. Repeat treatment after 14 days (Roberts et al. 2004).

KETAMINE (KETAMINE HCL, KETALAR [BEDFORD LABORATORIES])

Ketamine acts primarily as an NMDA receptor antagonist and induces what is known as "dissociative anesthesia." It can be used to immobilize fish for short procedures. Complete recovery may require over one hour.

Use: Sedation/anesthesia

Injectable formulations:

1. Inject 66–88 mg of ketamine/kg IM (= 30–40 mg/lb) (Mashima and Lewbart 2000).

KETOPROFEN (FORT DODGE ANIMAL HEALTH)

Use: Alleviation of inflammation (and possibly pain) just after major surgery

Injectable formulations:

1. Inject 2 mg of ketoprofen/kg (= 0.9 mg/lb) of body weight IM as a single dose just before recovery from surgery (Harms 2005). There are no clinical studies to confirm if this is efficacious (it depends upon whether fish can perceive pain).

LEVAMISOLE HYDROCHLORIDE (LEVASOL [PITTMAN-MOORE], TRAMISOL [OVER-THE-COUNTER], OR EQUIVALENT)

Use: Treatment of *Anguillicola* and other susceptible nonencysted nematodes

Levamisole is the levo-isomer of DL-tetramisol. Levamisole has a greater safety margin than the racemic mixture. Levamisole is not effective in treating capillarids in golden shiners (Hoffman 1982) and might cause sterility in zebrafish (Kent and Fournie 2007).

Water-borne formulations:

1. Prolonged immersion
   a. Add 10 mg levamisole HCl/l (= 38 mg/gallon) (Butcher 1993).

Oral formulations:

1. Feed 2.5–10 mg levamisole HCl/kg (= 1.1–4.5 mg/lb) of body weight/day for 7 days (Post 1987). This is equivalent to a feed having 0.025–0.100% levamisole and fed at a rate of 1% of body weight/day. Feeding 8 mg levamisole HCl/kg (= 3.6 mg/lb) of body weight is a proven treatment for *Anguillicola* (Blanc et al. 1992).

Injectable formulations:

1. Inject 8 mg levamisole HCl/kg (= 3.6 mg/lb) of body weight intracardiac (Blanc et al. 1992).

LIDOCAINE (XYLOCAINE® [ASTRA PHARMA])

Lidocaine is a topical anesthetic that is used less often as a fish anesthetic but is commonly available in veterinary clinics. It must be buffered with 1 g/l of sodium bicarbonate or there will be large variability in the effective dose (Carrasco et al. 1984). There is a reasonable safety margin between anesthetic and lethal doses. While not tested, proportionately lower doses would probably be satisfactory for sedation. Lidocaine's activity may vary considerably with water quality, fish species, fish size, and fish density. Dosages given should be used as general guidelines. The clinical response of the fish should also be used to ascertain the proper dosage (see **p. 20**).

Use: Sedation/anesthesia

Water-borne formulations:

1. Bath
   a. Add ~350 mg of lidocaine/l (= ~1330 mg/gallon) (Carrasco et al. 1984).

MAGNESIUM SULFATE ($MgSO_4$, EPSOM SALTS)

Use: Treatment of hexamitosis

Oral formulations:

1. Feed 3% magnesium sulfate in the feed for 2–3 days. This has been used to successfully treat salmonids (Warren 1981).

MALACHITE GREEN (MALACHITE GREEN [MARINE ENTERPRISES], OR EQUIVALENT)

Malachite green is in the triphenyl methane class of dyes and has traditionally been the most effective agent known for treating water mold infections of fish and eggs. It is also effective against protozoan ectoparasites and some myxozoan parasites. Unfortunately, it is also a respiratory poison, teratogen, and suspected carcinogen (Meyer and Jorgensen 1983). It is highly toxic to mammalian cells in culture due to the generation of free radicals; it also causes malignant transformation (Bose et al. 2004). It should be handled with appropriate caution. It is illegal to use on food fish in virtually all countries, although there is some evidence that it may still be used illegally in some countries. With the advent of safer and effective treatments for water molds (e.g., bronopol and hydrogen peroxide), there is much less justification for its use (Sudova et al. 2007). Malachite green persists in tissues for long periods; half-life can be in excess of 2000 degree-days (Alderman and Clifton-Hadley 1993). Repeated treatments cause increased accumulation (Alderman 1988). Malachite green is toxic to gill and liver (Gerundo et al. 1991). It is still extensively used in the pet fish industry and is sold in aquarium stores for use in both freshwater and marine aquaria in several countries. However, safer therapies, especially for marine fish, are available.

In water, malachite green exists in equilibrium between the colored, ionic form and the colorless, nonionic pseudobase (carbinol form). The relative proportion of these two forms is dependent upon pH: At pH 4, malachite green exists in almost entirely ionized (colored) form; while at pH 10, it is totally in the carbinol (colorless) form. Only the colored ionic form has antimicrobial activity, but the carbinol form is lipid-soluble, allowing it to pass across membranes (i.e., across the gill) (Alderman 1991). The toxicity of malachite green is highly temperature dependent, increasing with higher temperature (Alderman 1985). It is more toxic at low pH and low hardness. It is also phytotoxic. It is inactivated (oxidized) by light; aquarium lights should be turned off during treatment.

Malachite green is toxic to young fry and eggs that are near hatching. It should not be used prophylactically but only on eggs that have water mold infection. It is also reported to be toxic to tetras, catfish, and loaches (halving the suggested concentration may be tolerated, but there are little data on individual species responses). Some species of scaleless aquarium fish, such as knife fish and pimelodid catfish, will not tolerate this drug (G. Lewbart, unpublished data) and it may also be toxic to small marine fish. Centrarchids seem especially sensitive to malachite green. The 96-hour LC_{50} for bluegill is 0.035 mg/liter (Bills et al. 1977). It is toxic to largemouth bass eggs and fry and should not be used on this species (Wright 1976). Toxicity in fish usually presents as respiratory distress, since it is a metabolic respiratory poison. Treated fish may become anorexic (Post 1987). Toxicity is cumulative so unnecessary repeated treatments should be avoided.

Malachite green will stain all objects, especially plastics. Malachite green is manufactured as a zinc-free oxalate salt (green crystals with a metallic sheen) or as a zinc chloride salt (yellow crystals). The latter is toxic (mainly due to impurities, not due to the zinc) and should not be used. Dye lots vary considerably in potency (Alderman 1985), although this is usually not considered when calculating doses.

When prolonged immersion is used, remove residual drug with activated carbon 2 days after the last treatment. When malachite green is used in flow-through systems (e.g., treating salmonid eggs), release of drug into the environment should be prevented by treating the effluent with activated carbon (Marking et al. 1990).

It is usually best to prepare a fresh stock solution (1.4 g malachite green in 380 ml of water = 3.7 mg/ml). Old solutions of malachite green rapidly lose their ionizing activity after being dissolved in water (Chinabut 1993) as the oxalate salt tends to come out of solution over time.

Any users should wear protective, waterproof clothing (gloves, apron, boots) when handling malachite green solutions and should wear a breathing protection when handling the powder (it is a respiratory poison).

Use No. 1: Treating water mold infections and protozoan ectoparasites of freshwater fish

Water-borne formulations:

1. Bath
   a. Add 50–60 mg malachite green/l (= 50–60 ml of malachite green stock solution/gallon = 13–16 ml/liter) and treat for 10–30 seconds (Debuf 1991).
   b. Add 1.0 mg malachite green/l (= 1 ml of malachite green stock solution/gallon = 0.26 ml/liter), and treat for 30–60 minutes (Warren 1981; Debuf 1991). Use 2.0 mg malachite green/l if the pH is high. For salmonids, treatment can be repeated up to 4 times each week if the temperature is <14°C (58°F) (Warren 1981).
2. Prolonged immersion
   a. Add 0.10 mg malachite green/l (= 0.10 ml of malachite green stock solution/gallon = 0.026 ml/liter). Treat three times at 3-day intervals. Remove residual chemical after the last treatment with activated carbon. *Epistylis* is successfully treated with 0.10 mg/liter malachite green for 12–24 hours (G. Lewbart, personal communication).
   b. Add zinc-free 0.75% malachite green (Marine Enterprises). Use as directed.

3. Swab
   a. Swab a 100 mg malachite green/l solution onto skin lesions. The colored areas on the skin help to monitor healing (Warren 1981).

Use No. 2: Treating water mold infections of freshwater fish eggs

Water-borne formulations:

1. Bath
   a. Add 15 mg malachite green/l (= 0.15 ml of malachite green stock solution/gallon = 0.04 ml/liter) and treat for 10 seconds for channel catfish, African catfish and carp eggs (Treves-Brown 2000).
   b. Add 10 mg malachite green/l (= 10 ml of malachite green stock solution/gallon = 2.6 ml/liter) for 10–30 minutes (Hoffman and Meyer 1974).
   c. Add 0.50 mg malachite green/l (= 0.50 ml of malachite green stock solution/gallon = 0.13 ml/liter) for 1 hour (Debuf 1991).
2. Flush
   a. Add 42.5 g malachite green to 1 gallon of water (= 11.2 g/liter). Add 88 ml of this stock to the inflow adjusted to a flow rate of 6 gallons/minute (= 23 liters/min). Return the flow to normal after treating for 1 hour (Warren 1981).
3. Constant flow
   a. Add 2.2 mg malachite green/l, and treat for 1 hour (Warren 1981).

Use No. 3: Treatment of proliferative kidney disease

Water-borne formulations:

1. Flush
   a. Add 1 mg malachite green/l, and treat for 1 hour. Treat weekly for 3 weeks (Clifton-Hadley and Alderman 1987; Debuf 1991).

MEBENDAZOLE (TELMINTIC® [PITTMAN-MOORE], OR EQUIVALENT)

Use: Treatment of monogeneans

There is considerable species variation in response. For example, *Pseudodactylogyrus* is effectively treated with 1 mg/liter prolonged immersion (Székely and Molnár 1987), while *Gyrodactylus elegans* is reportedly susceptible to 0.10 mg/liter and *Dactylogyrus vastator* is resistant to even 2 mg/liter prolonged immersion (Scott 1993).

Water-borne formulations:

1. Bath
   a. Add 100 mg mebendazole/l (= 380 mg/gallon) and treat for 10 minutes (Székely and Molnár 1987).
2. Prolonged immersion
   a. Add 1 mg mebendazole/l (= 3.8 mg/gallon) and treat for 24 hours (Székely and Molnár 1987).

METHYLENE BLUE (METHYLENE BLUE [AQUATROL], OR EQUIVALENT)

Some evidence exists that methylene blue reduces incidence of bacterial and water mold infection of the eggs of freshwater aquarium fish (Herwig 1979). Methylene blue has also been advocated in the aquarium literature for treating ectoparasites and nitrite toxicity by prolonged immersion; however, other chemicals have stronger evidence of efficacy. Also, recent evidence indicates that high doses might inhibit normal swim bladder inflation in developing fry (Sanabria et al. 2009). It is illegal to use on food fish in many countries.

Prolonged immersion use of this agent is not recommended in systems with biological filtration because it is toxic to nitrifying bacteria (Collins et al. 1975). Note that many over-the-counter aquarium pharmaceuticals contain this ingredient. Experimental oral administration can produce hemolytic anemia in rodents. Methylene blue stains many objects, especially plastics. It is also phytotoxic (van Duijn 1973). It is best to prepare a stock solution by adding 1.4 g to 380 ml water (= 3.7 mg/ml).

Use No. 1: Preventing infections of freshwater fish eggs

Water-borne formulations:

1. Prolonged immersion
   a. Add 2 mg methylene blue/l (= 2 ml of methylene blue stock solution/gallon = 0.53 ml/liter). Repeat on alternate days for up to a total of three times.

Use No. 2: Treating ectoparasites of freshwater fish

Water-borne formulations:

1. Prolonged immersion
   a. Add 1–3 mg methylene blue/l (= 1–3 ml of methylene blue stock solution/gallon = 0.26–0.79 ml/liter) (Allison 1966).

METHYLTESTOSTERONE (17α-METHYLTESTOSTERONE, 17MT [RANGEN])

Changing the sex of immature tilapia to produce all male populations (Barry et al. 2007).

Oral formulations:

1. Feed 60 mg methyltestosterone/kg (= 27 mg/lb) of feed to larval tilapia during the first 21 days of feeding to produce a predominantly male population (= 9 mg 17MT/kg of body weight per day). Feed should be stored frozen or refrigerated. Only one month at room temperature can reduce potency by 15%.

METOMIDATE (MARINIL®, METHOXYNOL® [SANKYO], AQUACALM® [AQUATIC LIFE SCIENCES], OR EQUIVALENT)

Metomidate hydrochloride is a water-soluble anesthetic that has been used as a hypnotic in humans. It rapidly induces anesthesia, but recovery time is prolonged. It has a relatively large safety margin except for larval fish, in which it can be ineffective and lethal (Massee et al. 1995).

Involuntary muscle movements (twitching, etc.) may not be blocked by anesthesia, making it less desirable to use during bleeding or surgery. Metomidate's activity may vary considerably with water quality, fish species, fish size, and fish density. Dosages given should be used as general guidelines. The clinical response of the fish should also be used to ascertain the proper dosage (see **p. 20**).

Use No. 1: Sedation

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add ~0.1–0.2 mg metomidate/l (~0.4–0.8 mg/gallon).

Use No. 2: Anesthesia

Water-borne formulations:

1. Bath
   a. Add ~1–10 mg metomidate/l (~4–40 mg/gallon). A proper dosage will usually cause anesthesia within 3 minutes.

METRONIDAZOLE (FLAGYL® IV [SEARLE, RHONE MÉRIEUX], FISH-ZOLE [THOMAS], OR EQUIVALENT)

Metronidazole, a nitroimidazole, has traditionally been used for treating intestinal flagellate and anaerobe bacterial infections in humans. It is relatively insoluble in water (maximum solubility ~1 g/100 ml). Make sure that it is totally dissolved before adding to water or mixing in feed. An aquarium brand (Fish-Zole) is also available. Dimetridazole is a more water soluble nitroimidazole that has been used to treat protozoal infections in poultry but is being phased out in food animals because it is mutagenic. Dimetridazole is probably efficacious at a similar concentration.

Use No. 1: Treatment of hexamitosis and spironucleosis

Water-borne formulations:

1. Bath
   a. Add 5 mg metronidazole/l (= 19 mg/gallon) and treat for 3 hours. Repeat every other day for 3 treatments (Gratzek 1988).
2. Prolonged immersion
   a. Add 6.6 mg metronidazole/l (= 25 mg/gallon), and treat once daily for a total of three times (Gratzek and Blasiola 1992).
   b. Add 25 mg metronidazole/l (= 95 mg/gallon), and treat every other day for 3 days (Langdon 1992a).

Oral formulations:

1. There is evidence that a single oral treatment with metronidazole may be as effective as 3 water-borne treatments (Whaley and Francis-Floyd 1991).
   a. Feed 25 mg metronidazole/kg (= 11 mg/lb) of body weight/day for 5–10 days. Then reassess clinical condition, and retreat if needed (Gratzek and Blasiola 1992). This is equivalent to a feed that has 0.25% metronidazole and is fed at a rate of 1% of body weight/day.
   b. Feed 100 mg metronidazole/kg (= 45 mg/lb) of body weight for 3 days (Langdon 1992a). This is equivalent to a feed that has 1% metronidazole and is fed at a rate of 1% of body weight/day.
   c. Soak brine shrimp in a 1% metronidazole solution in a refrigerator for 3 hours. Feed once (Langdon 1992a).
   d. Feed 5 g metronidazole/kg (= 2.3 g/lb) of *feed* for at least 2 days. Experimentally cures rainbow trout of *Hexamita salmonis* in 2 days (Tojo and Santamarina 1998a), but the drug is expensive.

Use No. 2: Treatment of ichthyobodosis

Oral formulations:

1. Feed 40 g metronidazole/kg (= 18 g/lb) of *feed* at 2% of body weight per day for 10 days. Experimentally cures rainbow trout (Tojo and Santamarina 1998b), but the drug is expensive.

MONENSIN SODIUM (COBAN® 60 [ELANCO], RUMENSIN® 60 [ELANCO])

Use: Treatment of coccidiosis

Oral formulations:

1. Feed 100 mg monensin/kg (= 45 mg/lb) of body weight/day. This treatment is experimentally effective against *Calyptospora*.

NITRIFYING BACTERIA

Use: Seeding of filters to improve or speed up development of microbiological filtration to detoxify ammonia and nitrite

Note that these preparations consist of live bacteria. Products should not have been exposed to extreme temperatures and should be used before the expiration date. Commercial preparations of nitrifying bacteria often fail because they are sold well beyond the expected shelf life. Freeze-dried preparations have never been shown to be effective; nitrifiers do not appear to survive freeze-drying (Bower and Turner 1981). One of the best methods of seeding an aquarium with viable bacteria is to use filter

material from a healthy aquarium with an active biological filter.

Water-borne formulations:

1. Prolonged immersion
   a. Add filter material (e.g., floss, gravel) from an aquarium with an active biological filter and healthy fish to a filter to be used in the new aquarium.
   b. Add Fritz-Zyme TurboStart #700 (Fritz) to freshwater aquaria as directed.
   c. Add Fritz-Zyme TurboStart #900 (Fritz) to marine aquaria as directed.
   d. Add Cycle Bacteriological Biological Filter Supplement and Organic Sludge Remover (Rolf Hagen Corporation) to freshwater or marine aquaria, as directed.

ORGANOPHOSPHATE (DICHLORVOS [NUVAN®, 500EC®, AQUAGARD®, VAPONA®, APAVAP®, DDVP, 2,2, DICHLOROETHENYL DIMETHYLPHOSPHATE], TRICHLORFON [NEGUVON®, DIPTEREX®, TUGON®, DYLOX®, METRIPHONATE®, 2,2, TRICHLORO-1-HYDROXYETHYLPHOSPHONATE], AZAMETHIPHOS [ALFACRON®, SALMOSAN®])

Use: Treatment of sea lice, other crustacean ectoparasites (copepods, branchiurans, isopods), monogeneans and leeches

Organophosphates (OPs) are effective treatments for many metazoan ectoparasites, although resistance can be a problem (Goven et al. 1980; Roth et al. 1993; Tully and McFadden 2000; Fallang et al. 2004), and there have been significant environmental concerns, especially when treating caged fish. There are many types of OPs. Some are legal to use for treating fish in some countries; most are not.

The OPs that have been commonly used in aquaculture are dichlorvos and trichlorfon. The commercial formulation of dichlorvos is Aquagard® (Ciba-Geigy), which is 50% dichlorvos, 42% dibutylphthalate and 8% emulsifying agent. Trichlorfon is available in several formulations, including Neguvon® (Bayer), Dipterex® (Bayer), and Dylox® (Bayer) (Roth et al. 1993). Note that OP commercial preparations vary in percentage of active ingredient (e.g., Neguvon® is an 80% trichlorfon formulation).

When it is added to water, trichlorfon degrades to the much more toxic and more lipid-soluble dichlorvos. Dichlorvos degrades more slowly and more predictably to less toxic by-products. These chemical reactions are influenced by several factors: light, high temperature, aeration, and high pH all speed the chemical reactions (Samuelsen 1987). For example, in salmon-rearing areas, the half-life of dichlorvos is typically 5–8 days in seawater at 5°C (41°F) (Samuelson 1987). The half-life of trichlorfon may be over 3 weeks in acid water. In contrast, the half-life of trichlorfon in a typical warm water pond in summer, having a pH of 9.0, is less than 1 day. In such ponds, OPs must be applied in early morning to maintain an effective dose for a long enough time.

Aeration should be provided during bath treatments since oxygen depletion can exacerbate toxicity. Clinical signs of toxicity include dyspnea, rolling on the side, muscle rigidity and congregating at the bottom of the cage. Clinical signs develop more rapidly in smaller fish. OPs inhibit acetylcholinesterase (AChE), the enzyme that catalyzes the breakdown of acetylcholine, a neurotransmitter. This inhibition is greater in arthropods and other invertebrates than it is in vertebrates. However, some inhibition of brain AChE does occur when fish are treated with OPs and inhibition can persist for weeks. This can lead to overdosing if the same fish are treated even weeks apart. This presents a problem with sea lice control, since multiple treatments are often needed to permanently reduce parasite loads. A 75–80% inhibition of brain AChE is lethal or near-lethal to fish (Hoy et al. 1992). Trichlorfon is especially toxic to some larval fish (Flores-Nava and Vizcarra-Quiroz 1988) and potentially toxic to elasmobranchs and characins.

Azamethiphos (Salmosan® [Novartis]) is the most recent OP to be developed for treating sea lice. It has only one-tenth the mammalian toxicity of dichlorvos and is commercially available as a wettable powder, making it available in portion-controlled packets. It is also more active than dichlorvos against sea lice, thus requiring less drug (Roth et al. 1996). It also does not cause cumulative AchE depression from repeated exposure and is better tolerated by fish.

Another OP, fenthion (Spotton®, 20% solution; or Tiguvon®, 3% solution [Bayer]) has been used successfully to treat anchor worms (*Lernaea*) in aquarium fish (J.B. Gratzek, personal communication) and may be useful for other parasites. The dosage of active ingredient to use (i.e., mg/liter fenthion) is the same as for trichlorfon.

Crustaceans (including lobsters) appear to be the nontarget organisms that are most susceptible to OPs. Mollusks such as mussels are relatively resistant. OPs can be inactivated prior to release by raising the pH of the treatment solution (see #1c below). In some cases, toxicity to nontarget marine life might be due more to the carrier than to the OP. For example, Aquagard®, which is 50% dichlorvos, is more toxic than pure dichlorvos. The hydrolysis of the carrier dibutylphthalate is relatively slow and a significant amount is insoluble and can be deposited in the sediment after treatment (Treves-Brown 2000).

OPs must be handled with extreme care because they can also induce neurotoxic poisoning in humans. There is serious risk of exposure during drug preparation and treatment. Intoxication can occur via inhalation, ingestion or through the skin; protective gear, including rubber gloves, overalls, and face shields, should always be worn. Compliance is sometimes problematic since treatments must usually be done during warmer times of the year and the gear is uncomfortable. Trichlorfon is also a possible teratogen. OP residues are rapidly cleared from fish tissues.

Water-borne formulations:

1. Bath for sea lice
   Dichlorvos, trichlorfon and most recently azamethiphos are used for treating salmon with *Lepeophtheirus* and *Caligus* before the stage at which serious skin damage is evident. Dilute 1:16 before adding to the cage.
   Closely monitor for reinfestation at 10–20 days after treatment. One may need to retreat up to twice more at 2- to 3-week intervals, since only preadults and adults are killed (need to let the chalimus larvae mature) (Pike 1989; Debuf 1991).
   a. Add 15–300 mg trichlorfon/l (= 57–1,140 mg/gallon), and treat for 15–60 minutes at 3–18°C (37–64°F) (Horsberg et al. 1987). Use the lower dose at the higher temperatures. The lower dose at higher temperature is used to account for the more rapid conversion of trichlorphon to dichlorvos; using the lower dose reduces potential toxicity as trichlorphon degrades spontaneously.
   b. Add 0.5–2.0 mg dichlorvos/l (= 1.9–7.6 mg/gallon), and treat for 30–60 minutes. Use the lower dose at the higher temperatures in the range of 3–17°C (27–63°F) (Pike 1989). Higher doses are needed for skirted cages versus an enclosed cage because there is more rapid diffusion of chemicals outside of the cage. Withdrawal time is up to 500 degree days (Scott 1993).
   c. Add 15 mg dichlorvos/l (= 53 mg/gallon = 3 g dichlorvos in 200 l), and treat for 1 minute. Net 10 fish at a time, dip into the treatment tank for 1 minute, and then place in another tank; this method can treat a total of 680 fish in less than 2 hours. The residual OP can then be completely degraded by adjusting the pH to 10 with sodium hydroxide. After 18 hours, neutralize to seawater pH with HCl, allowing release into the environment (Messager and Esnault 1991).
   d. Add 0.01 mg azamethiphos/l (= 0.04 mg/gallon), and treat for not more than 30 minutes above 10°C (50°F) and not more than 60 minutes below 10°C (Treves-Brown 2000).
2. Bath for marine capsalid monogeneans
   a. Add 2–5 mg trichlorfon/l (=7.6–19 mg/gallon), and treat for 60 minutes (Langdon 1992a).
3. Bath for isopods
   a. Add 2 mg trichlorfon/l (= 7.6 mg/gallon), and treat for 60 minutes (Langdon 1992a).
4. Prolonged immersion for ectoparasites on pond or aquarium fish
   a. Add 0.25 mg trichlorfon/l (= 0.94 mg/gallon = 0.012 ml Neguvon®/gallon) for freshwater aquaria. Use 0.50 mg trichlorfon/l if temperature is over 80°F (27°C) (Piper et al. 1982). Trichlorfon may not be effective above 80°F (Jensen and Durborow 1984). Use 0.50–1.0 mg trichlorfon/l (=1.9–3.8 mg/gallon) for marine fish. For *Dactylogyrus* and other oviparous monogeneans, give 2 treatments at 3-day intervals. For marine turbellarians, use 1.0 mg/liter every other day for 3 treatments (Blasiola 1976). For anchor worms, treat every 7 days for 28 days. OP are only effective against *Lernaea* at 50–80°F (10–27°C; temperature range that larvae are produced). For other copepods (except sea lice), other monogeneans, *Argulus*, and leeches, one treatment will usually suffice (Jensen and Durborow 1984).

OZONE (O_3)

Use: Disinfection of water supplies

Ozone is a 3-atom modification of oxygen that has very strong oxidizing properties. Ozone is formed from atmospheric oxygen by electrical discharges at a high-tension electrode. Ozonizers can be switched into the air stream of a pump, enriching the air with the desired level of ozone. Rubber and some synthetic materials are quickly destroyed by ozone, and ozone must not be directly introduced into a culture system since even trace amounts are toxic to all aquatic organisms (plants and animals). Thus, ozonation occurs in an external filter or in an ozone reactor. For safety, the output water should be run through activated carbon to remove all residual ozone.

A chief benefit of ozone is greatly increasing the redox potential, so that optimal oxygen saturation is possible. All reducing compounds, especially proteins and their breakdown products, are oxidized without any toxic intermediates. However, the end product of this reaction is ammonia. Thus, there must be sufficient nitrifying bacteria (see PROBLEMS 4, 5) to detoxify the ammonia to nitrate. After feeding, too strong ozonation will lead to a sudden increase in ammonia that will not be converted quickly enough to nitrate.

Because of its strong oxidizing effect, ozone can kill pathogenic viruses, bacteria, water molds, and parasites (Sterba 1983). For example, ozone at 8 mg O_3/hr in a 15 gallon aquarium prevented *Cryptocaryon* infection in

Table III-13. Comparison of methods for disinfecting water supplies used for fish culture.

	Chlorine	Ozone	Ultraviolet radiation
Microbicidal effectiveness	Effective at high concentration against all pathogens	Effective in highly polluted water. Most rapid killing	Effective only in clear water
Equipment cost	Low	High	Moderate
Operating cost	High	Low	Moderate
	Chemical costs	Lower electricity cost than UV	Replace bulbs frequently
Disadvantages	Need to inactivate any residual chlorine before use	Need to be sure residual ozone is not exposed to fish (toxic)	Activity blocked by particles (shading)
	Suspected carcinogens may be produced	Ozone is highly toxic and dangerous to humans if generated in an unventilated area	Lamp must be cleaned frequently

opaleye held in an infected aquarium (Wilkie and Gordin 1969). For rapid disinfection of water supplies to eliminate most pathogens, ozone can be used at 8 mg/l/minute for 3 minutes; this corresponds to a redox potential of 600–750 mV. This level can also be used to treat effluent. It is best to filter the water before ozonation (Liltved et al. 1995).

For a comparison of water disinfection methods, see Table III-13, Piper et al. (1982), and Spotte (1992).

PEAT

Use: Softening and acidifying freshwater

Peat is an anaerobic breakdown product of plant material that consists of a complex mixture of organic acids, resins, waxes, plant hormones, salts, and other compounds. Peat reduces water hardness by releasing organic acids (e.g., tannins, humic acids). It also lowers and stabilizes the pH in the slightly acidic range, making the water more suitable for acidophilic fish. The lower pH is also bacteriostatic and fungistatic. Peat should be crumbly, since strands of peat have considerable undegraded plant remains that prevent good plant growth (Sterba 1983).

Water-borne formulations:

1. Prolonged immersion
   a. Add well-pulverized peat to the filter to effect.

2-PHENOXYETHANOL (PHENOXYETHANOL, ETHYLENE GLYCOL MONOPHENYL ETHER)

Use: Anesthesia/euthanization

2-phenoxyethanol has the advantage of being inexpensive when it is compared with other anesthetics. However, it has disadvantages, including a narrow safety margin and adverse side effects (long induction time; erratic rapid swimming ["motorboating"] with exposure; hyperactivity during recovery). There may be less than a twofold difference between the anesthetic dose and the lethal dose. 2-phenoxyethanol is an irritant (avoid contact with the solution) and may cause liver and kidney damage.

An advantage over tricaine or benzocaine is that it does not accumulate in the fish after induction of anesthesia, so it can be used for relatively long periods. However, involuntary muscle movements (twitching, etc.) may not be blocked by anesthesia, making it less desirable to use during bleeding or surgery. Note that activity may vary considerably with water quality, fish species, fish size, and fish density. Dosages given should be used as general guidelines, with the clinical response of the fish being used to gauge the proper dosage (see **p. 20**).

Water-borne formulations:

1. Bath for sedation/anesthesia/euthanasia
   a. Add ~100–400 µl phenoxyethanol/l (~25–100 µl/gallon) for a 2- to 4-minute induction of anesthesia. For euthanization, use about the same dosage and keep fish in anesthetic for at least 10 minutes after breathing stops.

PIPERAZINE SULFATE (PIPERAZINE 17% [AGRILABS], PIPERAZINE 34% [AGRILABS], PIPFUGE [BUTLER], PIPERAZINE [THOMAS LABS], OR EQUIVALENT)

Use: Treatment of nonencysted nematodes in the gastrointestinal tract. Piperazine is a phenothiazine anthelmintic.

Oral formulations:

1. Feed 10 mg piperazine sulfate/kg (= 4.5 mg/lb) of body weight/day for 3 days. This is equivalent to a feed that has 0.10% piperazine sulfate fed at a rate of 1% of body weight/day (Post 1983).

POTASSIUM PERMANGANATE ($KMnO_4$, CAROX® [CARUS CHEMICAL], OR EQUIVALENT)

Potassium permanganate reduces biological oxygen demand by oxidizing organic matter. It has also been advocated to increase dissolved oxygen levels in ponds; however, there is no evidence for it increasing oxygen at permanganate levels that are nontoxic to fish (Tucker and Boyd 1977) and can depress oxygen levels since it is algacidal. Potassium permanganate is an effective external parasiticide and bactericide. It has also been used to treat water molds, but this use has not yet been proven. It is currently under an INAD by the FDA (United States).

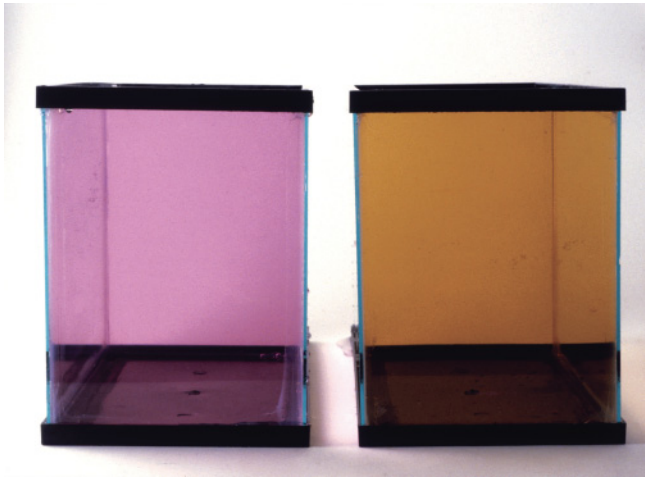


Fig. III-8. Appearance of reduced, active (left) and oxidized, inactive (right) potassium permanganate.

Potassium permanganate kills skin and gill pathogens via its strong oxidizing properties (Duncan 1974). Effective treatment requires 2 mg/liter of active chemical: the permanganate ion (MnO_4^-) imparts a light pink tinge to the water. Permanganate ion is reduced to manganese dioxide (MnO_2), which is relatively nontoxic and colorless; thus, the water will revert to being colorless or light tan when the permanganate becomes inactive. This can be tested by placing a sample of the water in a clear glass container (Fig. III-8). The pink permanganate color can also be seen if the pond water is splashed with an oar or plank to make a wave. Since permanganate reacts with organic matter, the amount needed for effective treatment is higher in organically rich ponds (Tucker and Boyd 1977). If the light pink tinge begins to decay before 8–12 hours has elapsed, more potassium permanganate should be added immediately in 2 mg/liter increments, until the light pink color is restored (Jensen and Durborow 1984). Not more than 6–8 mg/liter total potassium permanganate should be added to a pond. Readjustment to the proper permanganate concentration should be done all at one time to avoid overdosing the fish. Levels of potassium permanganate that exceed approximately 2 mg/liter of active ingredient are not considered safe for fish (Plumb 1979).

A more accurate method of determining the treatment dose is to add 0, 1, 2, 3, 4, 6, 8, 10, and 12 mg/liter of potassium permanganate to separate containers that have 1 liter of water each (small aquarium bags are useful). The lowest concentration in which the pink hue remains after 15 minutes is considered the endpoint (Boyd 1979). The endpoint obtained in this test is multiplied by 2.5 to give a reliable treatment rate for bacterial diseases (Tucker 1989). For example, if the lowest concentration that retains a pink hue after 15 minutes was 2 mg/l, the total amount of potassium permanganate needed for treatment would be $2 \times 2.5 = 5.0$ mg/l. Chemically calculating the permanganate demand of the water to be treated (Tucker 1984) is the most accurate measure of the required permanganate dosage.

Potassium permanganate is toxic in water with high pH, since manganese dioxide may precipitate onto the gills. Thus, it should not be used with caution in seawater. Potassium permanganate should not be mixed with formalin.

Although it is less expensive than formalin, potassium permanganate is still costly to use in large ponds or in those with a high organic content. A source of potassium permanganate may be difficult to locate in some areas, but it may be available from water softening companies if not from farm supply sources. It is considered 100% active.

Use No. 1: Treatment of ectoparasites and skin/gill bacterial infections in freshwater

Water-borne formulations:

1. Bath
   a. Add 1000 mg potassium permanganate/l (= 3,800 mg/gallon) and treat for 10–40 seconds (Debuf 1991).
   b. Add 100 mg potassium permanganate/l (= 380 mg/gallon) and treat for 5–10 minutes for fish lice (Kabata 1985).
   c. Add 5 mg potassium permanganate/l (= 19 mg/gallon) and treat for 30–60 minutes (Aldridge and Shireman 1991; Debuf 1991).
   d. Add 25 g potassium permanganate/m^3 (= 95 g/1,000 gallons) and treat for 30 minutes once for anchor worm (Faisal et al. 1988). Note: This must be done in conjunction with removing adult parasites (see PROBLEM 14).
2. Prolonged immersion in ponds
   a. Add enough potassium permanganate to produce a final concentration of 2 mg/liter (= 7.6 mg/gallon) of active (unreduced) potassium permanganate.
3. Flush
   a. Add 2 mg potassium permanganate/l (= 7.6 mg/gallon) for treating cold water bacterial gill disease (Schachte 1983).

Use No. 2: Oxidation/detoxification of hydrogen sulfide

1. Prolonged immersion
   a. Add enough potassium permanganate to produce a final concentration of 2 mg/liter (= 7.6 mg/gallon) of active (unreduced) potassium permanganate.

POVIDONE IODINE (PVP-IODINE, OVADINE [WESTERN CHEMICAL], IODOPHORE, WESCODYNE® [CIBA-GEIGY], BETADINE® [PURDUE FREDERICK], ARGENTYNE® [ARGENT], OR EQUIVALENT)

Povidone-iodine is a water-soluble complex of iodine with polyvinylpyrrolidone (PVP), with from 9.0% to

12.0% available iodine, calculated on a dry weight basis. It is a stabilized form of iodine. Different iodine formulations vary in concentration, so the amount of drug added is based on the brand that is used. Some potentiated iodine brands are combined with detergents (e.g., Betadine® Scrub); these should not be used.

Use No. 1: Antisepsis ("disinfection") of eggs to kill *Aeromonas salmonicida*, infectious hematopoietic necrosis virus, viral hemorrhagic septicemia virus, and other surface-dwelling pathogens

Povidone iodine can only kill pathogens on the surface of eggs (not inside the egg). Rinse treated ova thoroughly in clean water. Do not treat close to hatching, since this may cause premature hatching and increased mortality (Piper et al. 1982). Povidone iodine is also toxic to unfertilized ova and newly hatched fish. A final concentration of about 100 ppm active ingredient (iodine) is usually recommended. In poorly buffered water (<50 mg/liter total alkalinity), add 1 g of sodium bicarbonate/liter (=3.8 g/gallon) to unbuffered povidone iodine solutions; otherwise, they will lower the pH and kill eggs. A precipitate may form from bicarbonate treatment, but this will not harm the eggs. Fresh povidone iodine solution is brown-to-amber. When batches of eggs are treated, the solution should be discarded when it fades to yellow (Warren 1981). Povidone iodine is much more effective than acriflavine or merthiolate (Piper et al. 1982).

Water-borne formulations:

1. Bath (all formulations described below are prebuffered; therefore, bicarbonate addition is not needed).
   a. Add 3 ml of Wescodyne® ([Ciba-Geigy] 1.6% available iodine solution)/l (= 11 ml/gallon), and treat for 10 minutes (Debuf 1991).
   b. Add 5 ml of Ovadine® ([Western Chemical] 1.0% available iodine solution)/l (= 19 ml/gallon = 50 ppm), and treat for 30 minutes before egg hardening.
   c. Add 10 ml of Ovadine® ([Western Chemical] 1.0% available iodine solution)/l (= 38 ml/gallon = 100 ppm), and treat for 10 minutes after egg hardening.
   d. Add 10 ml of Argentyne® (Argent)/l (= 3.8 ml/gallon), and treat for 10 minutes or use as directed.
   e. Add 20 ml of 0.5% Betadine® solution/l (= 2.6 fluid ounces/gallon). Test before using.

Use No. 2: Antisepsis of wounds

Water-borne formulations:

1. Swab a Betadine® solution (= 1% potentiated iodine) on the wound. Immediately rinse fish in clean water, and then place it in a recovery tank.

Use No. 3: Disinfectant

Water-borne formulations:

1. Bath/spray
   a. Prepare a solution having 10,000 ppm available iodine (= 30–50 mg free iodine). Dip or spray equipment, allowing a contact time of at least 10 minutes before rinsing.

PRAZIQUANTEL (DRONCIT® INJECTION [BAYER], FISH TAPES [THOMAS LABS])

Praziquantel is believed to impair the neuromuscular system, inhibiting attachment of cestodes and possibly other parasites, such as monogeneans. It may also impair permeability of the parasite integument, causing osmotic and nutritional imbalance. Adding praziquantel to feed as a top dressing can reduce palatability, causing rejection of the diet (Williams et al. 2008). This does not appear to be as serious a problem when the drug is thoroughly mixed with the feed.

Use: Treatment of adult cestodes, monogeneans, and possibly larval digeneans

Water-borne formulations:

Immersion is more efficacious when praziquantel is dissolved in dimethyl sulfoxide (DMSO) rather than ethanol (Treves-Brown 2000).

1. Bath for adult cestodes
   a. Add 2 mg praziquantel/l (= 7.6 mg/gallon) for 1–3 hours (Hoffman 1983; Moser et al. 1986; Lewbart and Gratzek 1990; Gratzek and Blasiola 1992). This procedure can be repeated after 1 week if needed.
2. Bath for marine monogeneans
   a. Add 20 mg praziquantel/l (= 76 mg/gallon) for 1.5 hours. Juvenile fish and clupeids are sensitive to this dose, but even higher doses have been used for some other fish species (Schmahl and Tarashewski 1987; Thoney 1989).
   b. Add 10 mg praziquantel/l (= 38 mg/gallon) for 3 hours. This is better tolerated than #2a by some fish (Thoney and Hargis 1991).
   c. Add 100 mg praziquantel/l (= 380 mg/gallon) for 4 minutes to effectively treat the polyopisthocotylean *Microcotyle sebastis* (Kim and Cho 2000).
3. Prolonged immersion in aquaria for monogeneans
   a. Add 2 mg praziquantel/l (= 7.6 mg/gallon).
4. Bath for digenean metacercaria
   a. Add 10 mg praziquantel/l (= 38 mg/gallon) for 1 hour. This dose eliminates >90% of *Diplostomum spathaceum* in carp (Székely and Molnár 1991).
5. Prolonged immersion for digenean metacercaria
   a. Add 1 mg praziquantel/l (= 3.8 mg/gallon) for at least 90 hours. This dose eliminates 100% of *Diplostomum spathaceum* in carp (Székely and Molnár 1991).
   b. Add 2–10 mg praziquantel/l (= 7.6–38 mg/gallon) for 24 hours (Krum et al. 1992).

Oral formulations:

1. Oral for adult cestodes
   a. Feed 50 mg praziquantel/kg (= 23 mg/lb) of body weight/day. One day's treatment is usually sufficient. This is equivalent to a feed having 0.50% praziquantel fed at a rate of 1% of body weight/day (Langdon 1992a).
   b. Intubate 50 mg praziquantel/kg (= 23 mg/lb) of body weight once (Langdon 1992a). This dose has eliminated *Bothriocephalus acheilognathi* from grass carp (Scott 1993).
2. Oral for digenean metacercaria
   a. Feed 50 mg praziquantel/kg (= 23 mg/lb) of body weight (Langdon 1992a). This dose reduces the number of *Diplostomum spathaceum* metacercariae in trout and sculpins (Bylund and Sumari 1981).
   b. Feed 330 mg praziquantel/kg (= 150 mg/lb) of body weight once. This dose eliminates 100% of *Diplostomum spathaceum* in carp (Székely and Molnár 1991).
3. Oral for polyopisthocotylean monogeneans
   a. Feed 20 g of praziquantel/kg of *feed* at 1% of body weight every other day for 3 times. This dose effectively treats *Microcotyle sebastis* (Kim and Cho 2000).
4. Oral for capsalid and polyopisthocotylean monogeneans
   a. Administer 100 mg praziquantel/kg body weight daily via intubation, split into four doses every day. Repeat every day for 3 days. This significantly reduces the burden of the capsalid *Benedenia seriolae* and provides a 100% cure for the polyopisthocotylean *Zeuxapta seriolae* (Williams et al. 2008).

Injectable formulations:

1. Injection for digenean metacercaria
   a. Inject 25 mg praziquantel/kg (= 12 mg/lb) of body weight IM once (Lorio 1989).

PYRETHROID (CYPERMETHRIN, EXCIS® [NOVARTIS], BETAMAX [NOVARTIS], DELTAMETHRIN [ALPHA MAX VET®], PY-SAL®)

Pyrethroids are synthetic analogues of pyrethrins, a group of natural insecticides isolated from plants of the genus *Pyrethrum*. Excis® is a 1% cypermethrin solution dissolved in alcohol and having a biodegradable surfactant; the stock solution should be stored safely to protect against fire. Pyrethroids are neurotoxins, disrupting sodium channels. Excis® has a very short withdrawal time (e.g., 24 hours in Scotland, 3 days in Norway) since little drug is taken up by the fish. Pyrethroids are very persistent in the environment. A skirt must be used around a cage during treatment.

Water-borne formulations:

1. Bath for sea lice: see general concerns regarding treatment of sea lice and handling precautions under "**Organophosphates**."
   Cypermethrin is used for treating salmon with *Lepeophtheirus* and *Caligus* before the stage at which serious skin damage is evident. Adults, subadults and chalimus larvae are killed. Its half-life in water is relatively short (~5 days; Boxall et al. 2004). Resistance to pyrethroids has developed in some populations (Sevatdal et al. 2005).
   a. Add 5 µg cypermethrin/l (= 19 µg/gallon), and treat for 60 minutes (Hart et al. 1997).
   b. Add 2–3 µg deltamethrin/l (= 7.6–11 µg/gallon), and treat for 40 minutes.

QUATERNARY AMMONIUM COMPOUNDS (QAC, ROCCAL® [UPJOHN], HYAMINE 1622, HYAMINE 3500)

Quaternary ammonium compounds are cationic surfactant disinfectants that have also been used as antiseptics to treat skin and gill infections, such as bacterial gill disease. The QACs used to treat fish diseases include benzalkonium chlorides and benzethonium chlorides. QACs are more toxic at high temperature and in soft water. Quaternary ammonium solutions act as surfactants, removing excess mucus that contains parasites and bacteria. There are several different formulas of QAC, including powders and liquids. Roccal is a 10% solution of alkyl-dimethyl-benzyl-ammonium chlorides. Some batches of Roccal® are toxic to trout. Hyamine 3500®, a mixture of dodecyl and tetradecyl homologues of alkyl-dimethyl-benzyl-ammonium chlorides, and Hyamine 1622® are both less toxic at therapeutic doses.

When the powder is used to prepare a solution, a respirator should be worn or mixing should be done under a fume hood to avoid inhaling the dust. The powder should be added directly to water (adding water to the powder produces a sticky mass) (Warren 1981).

Use No. 1: Disinfectant

Water-borne formulations:

1. Prolonged immersion
   a. Add 5 ml of Roccal®/l (= 19 ml/gallon) as a net dip.

Use No. 2: Treatment of external bacterial infections

Salmon and lake trout appear to be sensitive to Hyamine 3500® and are best treated with Hyamine 1622® (Warren 1981). Different lots of Roccal® vary in efficacy and ichthyotoxicity; thus, a bioassay should be run before a new lot is used (Piper et al. 1982).

QACs are more toxic in soft water (cut the dose in half). QACs have a low therapeutic index, so the lower dose should be used when in doubt. The homing ability of salmonids may be affected, so QACs should not be

used on salmonids that are intended for release into the wild (Scott 1993).

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add one of the following (Scott 1993):
      10 mg of active QAC/l (= 38 mg/gallon) and treat for 5–10 minutes
      5 mg of active QAC/l (= 19 mg/gallon) and treat for 30 minutes
      2 mg of active QAC/l (= 7.6 mg/gallon) and treat for 60 minutes
      1 mg of active QAC/l (= 3.8 mg/gallon) and treat for several hours
      0.1–0.5 mg of active QAC/l (= 0.38–1.90 mg/gallon) and treat for 24 hours

      Then place fish in clean, untreated water immediately after treatment. It is usually best to retreat 2 or 3 times.

      The 60-minute exposure can also be performed as a constant flow treatment.

QUINALDINE SULFATE (QUINATE® [KNOLL])

This agent is the most widely used anesthetic for collecting tropical marine fish for the hobbyist trade and research. It appears to have a slightly better therapeutic index than tricaine, making it safer to use. Fish under quinaldine anesthesia do not usually stop breathing and thus are not as susceptible to asphyxiation. Thus, quinate can be used for longer procedures than tricaine or benzocaine. It is more expensive than tricaine. In some fish, toxicity increases with higher temperature, pH, and hardness. Quinaldine is a suspected carcinogen and caution should be exercised in its use. Fish retain a strong reflex response even after total loss of equilibrium which may be unsuitable during certain biopsy or surgical procedures. Some fish (e.g., tilapia) need extremely high doses; largemouth bass are very sensitive and it should not be used in that species. Prolonged exposure of fish to quinaldine is toxic so it should only be used as a short term anesthetic.

Stock solutions are stable but should be stored in a tightly capped, brown bottle. The parent compound (quinaldine) is more cumbersome to use, since it must be dissolved in an organic solvent before adding to water. Like tricaine, quinaldine sulfate acidifies the water (Summerfelt and Smith 1990) and in freshwater should be buffered (at least 1 part sodium bicarbonate buffer:2 parts quinaldine sulfate). It is irritating to the gills, and corneal damage has been observed (Bowser 2001). Buffering is needed not only to reduce irritation but to shift the chemical equilibrium to the unionized free base to facilitate uptake across the gills (it is ineffective below pH 5.0). Quinate is more potent in hard water.

Involuntary muscle movements (twitching, etc.) may not be blocked by anesthesia, making it less desirable to use during bleeding or surgery. Note that activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines, with the clinical response of the fish being used to gauge the proper dosage (see **p. 20**).

Use No. 1: Sedation

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add ~1–50 mg quinaldine sulfate/l (~4–200 mg/gallon).

Use No. 2: Anesthesia

Water-borne formulations:

1. Bath
   a. Add ~2.5 to >100 mg quinaldine sulfate/l (~10 to >400 mg/gallon). A proper dosage will usually cause anesthesia within 60 seconds.

Use No. 3: Euthanasia

Water-borne formulations:

1. Bath
   a. This is similar to the anesthetic dose. Keep fish in the solution for at least 10 minutes after breathing stops.

SALT

Many forms of salt can be effectively used for reducing stress and preventing or treating ectoparasites. Pure sodium chloride is available in coarse (meat-curing salt or rock salt) and fine (table salt) forms. For small volumes of water, table salt can be used. Noniodized table salt should be used for prolonged immersion. For prolonged immersion, it is best to use a balanced salt mixture, since other important minerals (e.g., Ca, Mg) are then added. One of the most reliable sources of balanced salts is the dry, artificial seawater (e.g., Kent Marine, Instant Ocean®) sold in aquarium stores. However, this can be expensive if large volumes of water are to be treated. An alternative balanced salt is dried seawater, or solar salt. Solar salt is available from water-softening companies. Avoid solar salt preparations with anticaking agents, such as sodium ferrocyanide (yellow prussiate of soda). Exposure of sodium ferrocyanide to sunlight generates hydrogen cyanide and is highly toxic to fish. Waste ferrocyanides in streams should not exceed 2 ppm to avoid fish kills. For using salt to treat nitrite toxicity, see "**Chloride**."

Use No. 1: Treatment of *Ichthyophthirius*

Water-borne formulations:

1. Prolonged immersion in aquaria
   a. Add 2 g salt/l (= 2 ppt = 7.6 g/gallon) to the aquarium. Some freshwater fish, such as many catfish, are sensitive to even low concentrations of salt, so this treatment should be used with caution

in those species. This salt level may be toxic to some plants. It has also been used successfully at 5 ppt NaCl to treat ich in warmwater fish in Australia (Selosse and Rowland 1990)

Use No. 2: Treatment of freshwater ectoparasites, columnaris, and bacterial gill disease

Water-borne formulations:

1. Bath
   a. Add 10–30 g salt/l (= 10–30 ppt = 38–114 g/gallon), and treat for up to 30 minutes. The higher doses may only be tolerated for a few minutes. Fish may become excitable when they are first exposed to high salt concentrations. If fish are weak or if they are a salt-sensitive species, use the lower dosage and repeat the next day. Small salmonids (<5 g) should not be exposed to >10 ppt salt, while salmonids <100 g should not be exposed to >20 ppt salt (Scott 1993).
   b. A salt bath can remove excess mucus and debris associated with ectoparasite infestations, columnaris, and bacterial gill disease, facilitating the effectiveness of other chemicals against these pathogens (Warren 1981). It is especially useful in salmonids.

Use No. 3: Prophylaxis or treatment of freshwater ectoparasites and water mold infections

Water-borne formulations:

1. Prolonged immersion in aquaria
   a. Add 1–5 g salt/l (= 1–5 ppt = 3.8–19 g/gallon) (Taylor and Bailey 1979). Some freshwater fish, such as many catfish, are sensitive to even low concentrations of salt, so the lower dosage should be used with these salt-sensitive species. Virtually all tropical freshwater aquarium fish can be maintained indefinitely in 1 ppt seawater (G. Lewbart, personal communication).
   b. Add ~1 teaspoon salt/5 gallons water to prevent freshwater velvet in killies (R. Goldstein, personal communication)
   c. Add up to 35 ppt salt for euryhaline fish.

Use No. 4: Increase mineral content for Rift lake cichlids

Water-borne formulations:

1. Prolonged immersion
   a. Add mineral mix (Aqua-Cichlids [Aquatronics], or equivalent). Use as directed.

Use No. 5: Increase salinity in brackish or marine aquaria. Note that it is best to allow sea salt mixtures to dissolve overnight before adding to the aquarium, since some salts take time to fully dissolve.

Water-borne formulations:

1. Prolonged immersion
   a. Add artificial seawater, and use as directed.

Use No. 6: Prevention of stress-induced mortality in freshwater fish

Water-borne formulations:

1. Prolonged immersion
   a. Add 3–5 ppt solar salt or artificial seawater. A mixture of divalent cations plus sodium chloride is superior to sodium chloride alone in reducing stress-induced mortality (Grizzle et al. 1990).

SECNIDAZOLE (RHONE MÉRIEUX)

This is a nitroimidazole.

Use: Treatment of parasitic flagellates

Oral formulations:

1. Treatment of *Ichthyobodo necator:* Feed 20 g secnidazole/kg (= 9 g/lb) of *feed*/day at 2% of body weight per day for at least 2 days. Experimentally cures rainbow trout in 2 days at this dose (Tojo and Santamarina 1998b), but the drug is expensive.
2. Treatment of *Hexamita salmonis:* Feed 2 g secnidazole/kg (= 0.9 g/lb) of *feed*/day at 2% of body weight per day for 2 days. Experimentally cures rainbow trout in 2 days at this dose (Tojo and Santamarina 1998a), but the drug is expensive.

SEDATIVES; SEE "ANESTHETICS"

SILVER SULFADIAZINE (THERMAZENE [KENDALL], OR EQUIVALENT)

Silver sulfadiazine is a sulfa-derived topical antibacterial. It is used in humans and animals primarily as a topical cream to treat burn wounds. It prevents the growth of bacteria and fungi on damaged skin. Silver sulfadiazine is typically formulated as a 1% solution suspended in a water-soluble base. The chemical itself is poorly soluble, and has only very limited penetration through the skin.

Use: Postoperative treatment of skin wounds

Swab formulations:

1. Using a sterile swab or tongue depressor, gently smear the paste onto the wound. Keep the lesion out of the water for 30–60 seconds following application. Keep gills submerged if possible. This can probably also be used to treat any open wound and repeated as needed until healing is complete (Harms and Wildgoose 2001).

SLAKED LIME (HYDRATED LIME, BUILDER'S LIME, CALCIUM HYDROXIDE, $Ca[OH]_2$)

Slaked lime is caustic and caution should be used in handling the powder. Do not confuse this with agricultural lime (see "**Buffers: Ponds**"), which is most often used for adjusting pH. Slaked lime is a strong alkali and can rapidly raise the pH to over 10, killing all the fish, which is why it is mainly used as a disinfectant. The pond

can be re-stocked with fish once the pH returns to the normal range.

Use No. 1: Disinfecting ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add slaked lime at a rate of 1,784 lb/acre (=2000 kg/ha = 18 g/ft^2). Best results are obtained when lime is disked into the soil of a drained pond. When it is added directly to water, the pH of the water should be allowed to return to <8.5 before adding fish (usually takes about 14 days).

Use No. 2: Adjusting pH/alkalinity of ponds (see "**Buffers: Ponds**")

Use No. 3: Adjusting hardness of ponds (see "**Calcium**")

Use No. 4: Neutralizing free CO_2 in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Add at least 1.7 mg/liter of $Ca(OH)_2$ for every 1.0 mg/liter of CO_2 to be removed (Hansell and Boyd 1980). This dose is about twice the amount that should theoretically be needed for neutralization because slaked lime is poorly soluble in water. This treatment only removes the CO_2 present in the water. The cause of the hypercarbia should also be corrected. Be careful not to rapidly raise pH or cause ammonia poisoning.

Use No. 5: Eradication of snails in ponds

Water-borne formulations:

1. Prolonged immersion
   a. Shoreline treatment: Apply slaked lime in a 3-foot (1-meter) wide swath of 100 lb/100 linear feet (= 45 kg/30 linear meters) or a 6-foot (2-meter) swath of 175 lb/100 linear feet (= 80 kg/30 linear meters) (Mitchell et al. 2007).

SODIUM BICARBONATE (BAKING SODA, Na_2HCO_3)

Use No. 1: Raising acidic pH to normal range in aquaria (see "**Buffers: Freshwater Aquaria**")

Use No. 2: Sedation/anesthesia/euthanasia (also see "**Carbon Dioxide**")

Sodium bicarbonate produces narcosis via the generation of CO_2 from carbonic acid (H_2CO_3). Carbonic acid anesthesia is effective between pH 6.5 and 8.5 (Post 1979). Carbonic acid anesthesia is best used for light sedation. Do not use at levels that cause loss of reflex activity or opercular movement (Post 1979). Some believe that carbonic acid anesthesia should only be used as a last resort because it is easy to overdose and produce a lethal hypercarbia. Concentrated (97–98%) sulfuric acid is most commonly used in conjunction with sodium bicarbonate to generate carbonic acid. Concentrated sulfuric acid should be handled with extreme caution. A 10% (wt/vol) sodium carbonate solution can be used to quickly reverse the anesthesia if desired.

Note that activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines, with the clinical response of the fish being used to gauge the proper dosage (see **p. 20**).

Water-borne formulations:

1. Bath
   a. Mix 6.75% (wt/vol) sodium bicarbonate with 3.95% (wt/vol) concentrated sulfuric acid to obtain the desired CO_2 concentration (Post 1979). The volume of each solution that is needed can be calculated as follows:

$$\frac{\text{mg/liter } H_2CO_3 \text{ concentration} \times \text{Volume of the anesthetic bath in liters}}{50}$$

For example, if one desires to produce a 200 mg/liter carbonic acid concentration in a 40 liter aquarium, one would add the following:

$$\frac{200 \times 40}{50} = 160$$

Therefore, 160 ml of both the sodium bicarbonate and the sulfuric acid solutions would need to be added to the water. Note that acid should always be added to water, not vice versa.

   b. Add 142–642 mg sodium bicarbonate/l (= 538–2430 mg/gallon). Add concentrated sulfuric acid at a wt:wt ratio of 1.7 mg sodium bicarbonate: 1.0 mg sulfuric acid. An appropriate dosage should produce anesthesia in about 5 minutes (Schnick et al. 1989).
   c. Add 1 tablet of Alka-Seltzer®, Bromo-Seltzer®, or equivalent/20 liters (= 2 tablets/10 gallons). This method should only be used as a last resort for anesthesia, since the dosage is difficult to control.
   d. Make a concentrated solution of sodium bicarbonate by adding ~30 g (10 teaspoons or 1/4 cup) of sodium bicarbonate/l (= ~120 g or 40 teaspoons or 1 cup/gallon) of water. Mix well, until virtually all of the powder is dissolved. Add the fish to be euthanized. Leave it in the solution for at least 10 minutes after the fish's breathing has stopped.

SODIUM PENTOBARBITAL

Barbiturate euthanasia has the advantages of being rapid and less expensive than fish anesthetics; however, fish must be restrained for the injection, which may be difficult. Barbiturate stocks must also be closely monitored and kept in a secure place because they are regulated narcotics. In the United States, barbiturate use

requires preregistration with the Drug Enforcement Administration, which usually takes several months.

Use: Euthanization

Injectable formulations:

1. Inject 60 mg sodium pentobarbital/kg (= 27 mg/lb) of body weight intraperitoneally.

SODIUM PHOSPHATE; SEE "BUFFERS: FRESHWATER AQUARIA"

SODIUM SULFITE (Na_2SO_3 [ARGENT], OR EQUIVALENT)

Use: Treating eggs to improve hatchability; also see Table III-3 for other methods to reduce adhesiveness

Water-borne formulations:

1. Bath
   a. Add 15% sodium sulfite to eggs of channel catfish, largemouth bass, or smallmouth bass for 5–8 minutes. Immediately place eggs in clean water after treatment. Sulfites remove oxygen, and thus are toxic with prolonged exposure (APHA 1992).

TEFLUBENZURON (CALCIDE® [NUTRECO], EKTOBANN® [SKRETTING])

Teflubenzuron is a benzyl-urea. See the general discussion under "**Diflubenzuron**."

Teflubenzuron is not effective on adults and must be used before the adult lice appear.

Use: Treating sea lice in salmon

Oral formulations:

1. Feed for treating sea lice: see general concerns regarding treatment of sea lice under "**Organophosphates**."
   a. Feed 10 mg teflubenzuron/kg (= 4.5 mg/lb) of body weight/day for 7 days. This is equivalent to a feed that has 2 kg teflubenzuron per ton and is fed at a rate of 0.5% of body weight/day (Branson et al. 2000; Treves-Brown 2000).

TFM (3-TRIFLUOROMETHYL-4-NITROPHENOL SODIUM SALT, LAMPRECID [H AND S CHEMICAL COMPANY])

Use: Eradication of lamprey larvae

TFM is currently registered by the United States Environmental Protection Agency and is only legal to use by authorized individuals.

TOLTRAZURIL (BAYCOX® [BAYER ANIMAL HEALTH])

Toltrazuril is a symmetrical triazintrione used to treat coccidiosis in poultry, cattle and swine. It has shown experimental efficacy against a number of fish parasites. It is available as 2.5% and 5% water-soluble solutions.

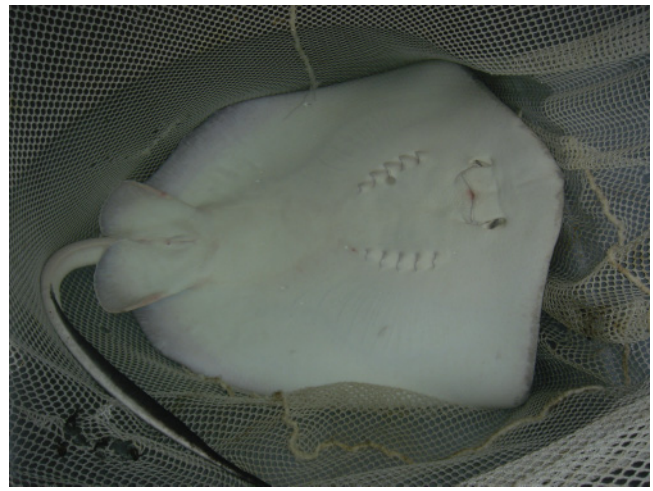


Fig. III-9. "Tonic immobility" induced in a southern stingray. (Photograph courtesy of K. Grant.)

Use No. 1: Treatment of microsporidiosis

Water-borne formulations:

1. Bath
   a. Add 5–20 mg toltrazuril/l (= 19–76 mg/gallon), and treat for 1–4 hours every 2 days for 6 days for *Glugea anomala* infection (Schmahl et al. 1990). Add 2 ml of 2.5% toltrazuril solution to 1 liter (= 7.6 ml to 1 gallon) to prepare a 5 mg/liter solution.

TONIC IMMOBILITY

Use: Restraint of sharks and other elasmobranchs for medical procedures

For many elasmobranchs (sharks, skates, rays), simply placing them in dorsal recumbency induces "tonic immobility" (Fig. III-9), where the elasmobranch becomes very quiescent (Henningsen 1994). Tonic immobility allows for relatively safe handling without the use of chemical restraint. Since many elasmobranch species react adversely to chemical anesthetics, this can be advantageous. Immediately after capture, the individual is placed in dorsal recumbency on a support (e.g., net, stretcher) (Walker and Whitaker 2001).

TRICAINE (TRICAINE METHANESULFONATE, TRICAINE MESILATE, METACAINE, TRICAINE-S [WESTERN CHEMICAL], MS-222, FINQUEL® [ARGENT])

Tricaine is one of the most commonly used sedatives and anesthetics in fish. Finquel® is the only tricaine label approved for use in food fish in the United States. Some

fish need a higher exposure at lower temperatures for the same effect (Schoettger and Julin 1967), but tricaine is safer to use at low temperatures. A higher dosage is also needed in hard water (Schoettger and Julin 1967). Crowding fish also increases the required dosage, with up to 10 times the dosage needed because of absorption by the fish (Dupree and Huner 1984). It is best not to have a fish density greater than ~80 g/L (~300 g/gallon). Tricaine has a narrower safety margin than quinaldine sulfate and is more expensive.

In low-alkalinity water (<50 mg/liter as $CaCO_3$), sodium bicarbonate should be used to buffer the solution. Otherwise, the pH may drop to less than 5. Unbuffered tricaine has been shown experimentally to cause metabolic acidosis (Houston 1990) and severe skin and eye damage (Davis et al. 2008). A suggested stock solution is 100 mg/ml. Stock solutions should not be buffered because this causes chemical dissociation of the sulfonate group. Sodium bicarbonate should be added to the working solution at a ratio of about 2 parts sodium bicarbonate:1 part tricaine (wt:wt).

Tricaine solutions are unstable in light, changing to yellow or brown. Stock solutions should be replaced monthly or stored frozen.

Tricaine causes significant vasoconstriction in the gills. Thus, although initial drug uptake is rapid, the rate soon declines and rapid achievement of a deep plane of anesthesia requires the use of a higher concentration of drug in the water than is in the blood at that stage. Thus, after deep anesthesia has been reached, absorption is slow. This is a disadvantage to tricaine, imposing strict limits on the duration of anesthesia in order to avoid overdosing (Treves-Brown 2000).

Risk of human intoxication appears very low, but there has been a single case report of prolonged exposure being associated with reversible retinal damage in one human (Bernstein et al. 1997). Gloves should always be worn when handling tricaine solutions.

Note that activity may vary considerably with water quality, fish species, fish size, and fish density. Given dosages should be used as general guidelines, with the clinical response of the fish being used to gauge the proper dosage (see **p. 20**). Overdosing is indicated by a recovery time greater than 10 minutes. Induction and recovery is faster at higher temperatures. Tricaine is rapidly cleared by fish and usually no residues are detectable after 24 hours (Houston 1990).

Use No. 1: Sedation for transporting fish

Water-borne formulations:

1. Bath/prolonged immersion
   a. Add ~10–40 mg of tricaine/l (~38–150 mg/gallon). This concentration will reduce oxygen uptake and metabolic rate without causing severe depression. Crowded fish may require higher doses. In general, do not use >100 mg/liter for salmonids or >250 mg/liter for warm water fish, unless the fish are crowded.

      A level 1/4 teaspoon of tricaine (an ~0.8 ml scoop) is ~650 mg. Thus, if added to 4 liters (~1 gallon), it produces a concentration of ~160 mg/l.

Use No. 2: Anesthesia

Water-borne formulations:

1. Bath
   a. Add ~50–250 mg of tricaine/l (= ~190–950 mg/gallon). An optimal concentration will usually cause anesthesia within 60 seconds.
   b. For large fish a 1 g/liter solution of tricaine can be sprayed onto the gills, using an aerosol pump sprayer. This can be reapplied if needed during a procedure.

Use No. 3: Euthanization

Water-borne formulations:

1. Bath
   a. This is similar to the anesthetic dose. Keep fish in the solution for at least 10 minutes after breathing stops to ensure that they are dead.

TRICLABENDAZOLE (CIBA-GEIGY)

This is a noncarbamate benzimidazole.

Use: Treatment of parasitic flagellates

Oral formulations:

1. Feed 40 g triclabendazole/kg (= 18 g/lb) of *feed*/day at 2% of body weight per day for 5 days for treatment of *Ichthyobodo necator* (Tojo and Santamarina 1998b) and 10 days for treatment of *Hexamita salmonis* (Tojo and Santamarina 1998a). Experimentally cures rainbow trout of costia and nearly 100% effective against hexamitosis, but the drug is expensive.

ULTRAVIOLET LIGHT (ULTRAVIOLET "STERILIZATION")

Ultraviolet light is probably the most common method used to disinfect water supplies used for fish culture. It is effective against a number of pathogens but killing power varies greatly among pathogens. Typically, the dose (exposure time) needed for killing increases with the size of the pathogen. Thus parasites are more resistant than bacteria, but even some large pathogens can be effectively controlled (Gratzek et al. 1983). Ultraviolet light is also rapidly attenuated in water, so only very shallow thicknesses of water can be treated. Typically, ultraviolet irradiance of 120–300 mJ/cm^2/second is an appropriate dose for treating water intake supplies (Liltved et al. 1995; Frerichs et al. 2000). Effectiveness is also reduced by turbidity, which can "shade" pathogens from the light.

For a comparison of water disinfection methods, see Table III-13, Piper et al. (1982), and Spotte (1992).

UNSLAKED LIME (QUICK LIME, BURNT LIME, CALCIUM OXIDE, CaO)

Unslaked lime is caustic and caution should be used in handling the powder. Do not confuse this with agricultural lime (see "**Buffers: Ponds**"), which is most often used for adjusting pH. Unslaked lime is an alkali and can rapidly raise the pH to over 10, killing all the fish, which is why it is mainly used as a disinfectant.

Use No. 1: Disinfecting ponds

Water-borne formulations:

1. Prolonged immersion for general disinfection
   a. Add unslaked lime at a rate of ~1500 kg/ha (= 1,338 lb/acre = 14 g/ft^2). Best results are obtained when lime is disked into the soil of a drained pond. When it is added directly to water, the pH of the water should be allowed to return to <8.5 before fish are added (usually takes about 14 days).
2. Prolonged immersion for eradicating *Myxobolus cerebralis* (Hoffman and Hoffman 1972)
   a. Add 2,500 mg unslaked lime/l, and treat for 6 days.

Use No. 2: Adjusting pH/alkalinity of ponds (see "**Buffers: Ponds**")

Use No. 3: Adjusting hardness of ponds (see "**Calcium**")

VACCINES

Vaccines are available for treating many important diseases of fish, especially bacterial infections, but also some viral diseases. No vaccines against parasites or water molds are commercially available. Most vaccines are killed, but some are modified live preparations. Oral, injectable, and bath preparations are available. Bath preparations are commonly used and usually give good protection. Injectable preparations, although more labor intensive, give superior protection; these are mostly administered as oil-adjuvated vaccines. Oral vaccines are least effective, being used mainly as booster vaccines.

The great majority of vaccines are intended for use in salmonids, but some vaccines are available for use in channel catfish, European seabass, gilthead seabream, yellowtail, amberjack, Atlantic cod and tilapia. Three companies supply the majority of fish vaccines worldwide: Intervet (Schering-Plough Animal Health), Novartis Animal Health, and Pharmaq. In Japan, vaccines are mostly produced and distributed by Japanese companies. Some limited use, locally developed vaccines are also available in some countries (e.g., China, Russia, Spain, Germany, Israel; Sommerset et al. 2006). Autogenous vaccines are also used to a much lesser extent. See "**Health Promotion and Maintenance**" (**p. 73**) for details on proper vaccine use.

VIRKON® AQUATIC (DUPONT)

Virkon® Aquatic is an oxidizing disinfectant that is effective against many viruses, as well as bacteria, fungi and water molds. It is a mixture of peroxygen compounds, surfactant, organic acids and inorganic salts, maintaining a low pH. It is approved for use by the U.S. Environmental Protection Agency and Health Canada, and is also approved in Europe. Virasure Aquatic (www.fishvet.co.uk) has a similar composition.

The oxidizing agent in Virkon® Aquatic is potassium monopersulphate (21%), which works best at low pH. Malic and sulphamic acid produce a low pH, acting as catalysts. An inorganic buffer (sodium hexametaphosphate) stabilizes the acidic conditions. A surfactant (sodium alkyl benzene sulphonate) aids cleaning.

After adding water to the Virkon® Aquatic powder, sodium chloride is oxidized by the potassium monopersulfate. Instead of the resulting chlorine being given off as a gas, it interacts with the sulphamic acid (acting as a chlorine acceptor) to form an intermediary complex. This complex is hydrolyzed to release hypochlorous acid, another biocide. The reaction is cyclic—the chloride released from the sulphamic acid produces more sodium chloride, refueling the cyclic system.

Virkon® Aquatic is supplied as a dry powder that is highly corrosive; skin, eye, and respiratory protection should be used when handling the power.

Use: Disinfection of equipment. For other applications (fogging, etc.), see the Dupont product insert. Test strips (Virkon® Test Strips [Dupont]) are available to monitor the potency of foot baths and other long-standing disinfectant solutions.

Water-borne formulations:

1. Bath
   a. Add 50–100 g of Virkon® Aquatic powder to 10 liter of water to produce a 0.5–1% working solution. The solution prepared at room temperature must remain in contact with the surface to be disinfected for at least 10 minutes (do not exceed 30 minutes for metal objects).

WATER CHANGE

Use: Diluting of toxins in closed systems

For aquaria, changing about 10–25% of the water every month (or 3–5% per week) is usually recommended (Axelrod et al. 1980; Moe 1992a). Systems with high fish densities may require larger changes. If rapid dilution is needed, do 50% or more, but be cautious about environmental shock (see PROBLEM 97).

WOUND SEALANT (ORAHESIVE® OR ORABASE® [ConvaTec], OR EQUIVALENT)

These preparations consist of equal quantities of gelatin, pectin and methylcellulose. They are used for treatment of oral ulcers in humans.

Use: Waterproof sealing of open wounds after surgical debridement of skin ulcers

Swab formulations:

1. After applying antiseptic to the wound, smear a thin layer of wound sealant on the entire wound using a sterile swab or tongue depressor. Swab the sealant past the periphery of the wound. Then return fish to its recovery tank (Harms and Wildgoose 2001).

ZEOLITE (CLINOPTILITE)

Use: Removal of ammonia from water

Zeolites are tectosilicate minerals that act as ion-exchange resins, exchanging ammonium ions for sodium ions. Clinoptilite is a very active form of zeolite (Marking and Bills 1982). Under optimal conditions (low hardness, neutral pH, freshwater, 20 × 30 mesh particle size), 1.0 g of zeolite can remove 9 mg of ammonia. More realistic removal rates are around 2 mg ammonia/g zeolite in freshwater (1 lb of zeolite in a 100-gallon tank [or approximately 1 kg in 840 liters] will totally remove 3 mg/liter of ammonia). Temperature is not important under aquaculture conditions. High hardness reduces removal by about 50% because of the binding of calcium and magnesium to the resin. Large particle sizes are less efficient, while smaller particles are easily clogged.

At 36 ppt salinity, there is a 95% reduction in zeolite's ability to remove ammonia, but it still can remove dyes and organic matter at the same rate. Zeolite is less effective than activated carbon in removing dyes and organics. It is better than low-grade carbon. Zeolite can also reduce ammonia build-up while shipping fish.

When zeolite becomes saturated with ammonia, it can be reused by placing it in a strong, alkaline, NaCl solution (~1 lb salt/3 gallons water [~1 kg/25 liters] at pH 11–12) overnight or by treating it with a 200 ppt salt solution for 30 minutes. The resin should be rinsed before reuse. Resins have been regenerated up to 500 times. The brine solution can also be reused (Marking and Bills 1982).

Water-borne formulations:

1. Prolonged immersion to reduce or prevent ammonia toxicity

   When fish are at a density of about 20–40 g of fish/l, adding about 20 g of clinoptilite/l of water reduces the total ammonia nitrogen that accumulates after 24 hours by about 75–85% (Bower and Turner 1982a).

   a. Zeolite Ammonia Remover (Mars Fishcare). Use as directed.
   b. Ammonex® (Argent) bags or loose pieces containing clinoptilite. Use as directed.

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APPENDIX I

Fish Disease Diagnosis Form

Date: _____ Case No.: _____

Name: _____ Phone: _____

Address: _____

HISTORY

Freshwater: _____ Marine: _____ System size: _____ gal (l)/ac (ha)

Species affected: _____

Species in system: _____

No. fish in system: _____ No. fish and % affected: _____(%)

Average fish size: _____ in (cm)/oz (g)

Age(s) of affected fish: _____

When morbidity started: _____ When mortality started: _____

When morbidity ended: _____ When mortality ended: _____

How long has system been set up? _____ Temperature: _____

Types of life support present: _____

Any new introductions? Y __N __If yes, when and what? _____

_____

Water source: _____ Plumbing: metal _____ plastic _____

Water appearance (cloudy, colored?): _____

History of routine maintenance, including water changes and water quality checks:

_____

Behavioral changes? Y __ N __ Describe: _____

Respiratory rates: (normal____ faster____ slower____)_____

Appearance of fish: _____

Appetite (normal ___ less ___ more ___): _____

Other clinical signs: _____

WATER QUALITY

DO: _____ mg/l Temp: _____ pH: _____

Ammonia: TAN _____ mg/l UIA _____ mg/l

Nitrite: _____ mg/l Chloride: _____ mg/l Nitrate: _____ mg/l

Hardness: _____ mg/l Alkalinity: _____ mg/l Salinity: _____ ppt

Water samples preserved for further analysis: _____

PHYSICAL EXAM

Behavior: _____ Respiration (depth and rate): _____

Skin: _____

Gills: _____

BIOPSIES AND CULTURE

Skin biopsy: _____

Gill biopsy: _____

Blood smears taken? Y ____ N ____ Results: _____

Bacterial cultures taken? Y ____ N ____

Organs cultured: Kidney _____ Other _____

Results of cultures: _____

NECROPSY

Peritoneal cavity/visceral fat: _____

Gonads: _____

Liver/gall bladder: _____

Stomach/intestine: _____

Spleen: _____

Swim bladder: _____

Kidney: _____

Heart: _____

Brain: _____

Other: _____

Tissues preserved for histology or other further analysis: _____

Problem(s) Identified	Recommended Treatment(s)
1. _____	1. _____
2. _____	2. _____
3. _____	3. _____

Results of treatment: _____

APPENDIX II

Suppliers

The following includes contact information for companies whose products were mentioned in the text. Note that many of the addresses and telephone numbers only refer to a single regional office. Many of these listings are multinational companies with additional offices in other geographic locations. To find the closest location and telephone number to you, check the website.

Note that this is only a partial listing of suppliers. A listing of additional aquaculture and fishery suppliers is available at the following websites:
www.aquafind.com, www.thefishsite.com

3M Corporate Headquarters
3M Center
St. Paul, MN 55144-1000
888-364-3577
www.mmm.com

Abbott Laboratories
100 Abbott Park Rd.
Abbott Park, North Chicago, IL 60064-3500
847-937-6100
www.abbott.us

Abraxis LLC
54 Steamwhistle Dr.
Warminster, PA 18974
www.abraxiskits.com

Agri Laboratories, Ltd.
6221 North K Highway
P.O. Box 3101
St. Joseph, MO 64503
816-233-9533
www.agrilabs.com

Agri-Pro Enterprises
Box 27
Iowa Falls, IA 50126
641-648-4696
www.agri-pro.com

Alpharma
Animal Health Division
440 Route 22 East
Bridgewater, NJ 08807
908-566-3800
www.alpharma.com

American Marine, Inc.
54 Danbury Rd.
Suite 172
Ridgefield, CT 06877
800-925-4689
www.americanmarineusa.com

Apothekernes Laboratorium
Harbitzalléen 3 Postboks 158 Skoyen
0212 Oslo
Norway
47-22529000
http://www.randburg.com/no/alpharma.htm

Applied Biochemists, Inc.
6120 West Douglas Ave.
Milwaukee, WI 53218
www.appliedbiochemists.com

Aqua Health, Ltd.
37 McCarville St.
Charlottetown, Prince Edward Island
C1E 2A7, Canada
902-566-4966

Aqua-In-Tech Inc.
425-787-5218
www.aqua-in-tech.com

Aquacenter
166 Seven Oaks Rd.
Leland, MS 38756
800-748-8921
www.aquacenterinc.com

Aquaculture Vaccines, Ltd. (AVL)
24-26 Gold St.
Saffron Walden
Essex, CB10 1EJ
United Kingdom
01799-528167

Aqua Logic Inc.
8268 Clairemont Mesa Blvd., Suite 302
San Diego, CA 92111
858-292-4773
www.aquanetics.com
www.aqualogicinc.com

Aquarium Pharmaceuticals, Inc.
Mars Fishcare, Inc.
P.O. Box 218
Chalfont, PA 18914-0218
215-822-2181
http://aquariumpharm.com

Aquarium Systems, Inc.
8141 Tyler Boulevard
Mentor, OH 44060
800-822-1100
www.unitedpetgroup.com

Aquatic Diagnostics, Ltd.
Institute of Aquaculture
University of Stirling
Stirling FK9 4LA
Scotland, UK
0044 1786 466568
aquaticdiagnostics@stir.ac.uk
http://www.aquaticdiagnostics.com

Aquatic Eco-Systems, Inc.
23295 Apopka Boulevard
Apopka, FL 32703
877-347-4788
www.aquaticeco.com

Aquatic Health Resources
Box 175
17410 Minnetonka Blvd.
Minnetonka, MN 55345
877-280-2856
info@aquatichealthresources.com
www.aquatichealthresources.com

Aquatic Life Sciences, Inc.
parent company for Syndel Laboratories (CAN)
and Western Chemical (USA)
http://www.aquaticlifesciences.com

AQUI-S New Zealand Ltd.
PO Box 44-269
Lower Hutt, New Zealand
64-4-587-0389
sales@aqui-s.com
http://www.aqui-s.com

Argent Chemical Laboratories
8702 152nd Ave. NE
Redmond, WA 98052
425-885-3777
www.argent-labs.com

Astra Pharmaceutical Products
50 Otis St.
Westboro, MA 01581;
U.S. subsidiary of Astra AB, now AstraZeneca
http://www.astrazeneca-us.com

Aurum Aquaculture, Ltd.
RR1, Box 1-H
Leland, MS 38756
800-817-5808

Axcentive SARL
Chemin de Champouse
Quartier Violesi
13320 Bouc Bel Air
France
+33 442 694 090
info@axcentive.com
www.halamid.com

Baxter Worldwide
One Baxter Parkway
Deerfield, IL 60015-4625
847-928-2000
www.baxter.com

Bayer Animal Health
Bayer HealthCare LLC
Animal Health Division
PO Box 390
Shawnee Mission, KS 66201
800-633-3796
www.bayer-ah.com

Bedford Laboratories
300 Northfield Rd.
Bedford, OH 44146
440-232-3320
800-521-5169
http://www.bedfordlabs.com

Becton-Dickinson (BD)
1 Becton Dr.
Franklin Lakes, NJ 07417
201-847-6800
www.bd.com

Biomark, Inc.
7615 West Riverside Dr.
Boise, Idaho 83714
208-275-0011
www.biomark.com

Bionor Laboratories AS
PO Box 2870
NO-3702 Skien
Norway
+47-35908530
http://www.bionor.no

B.L. Mitchell, Inc.
103 US Highway 82 East
Leland, MS 38756-9392
800-817-5808
http://www.blmitchell.com

Brenntag
http://www.brenntag.com

Butler Animal Health Supply
5600 Blazer Parkway
Dublin, OH 43017
800-848-5983
www.accessbutler.com

Carolina Biological Supply Company
2700 York Rd.
Burlington, NC 27215-3398
800-334-5551
www.carolina.com

Carus Chemical Co.
315 5th St.
Peru, IL 61354
800-435-6856
www.caruscorporation.com

Cenzone Tech
2110 Low Chaparral Dr.
San Marcos, CA 92069
888-825-2585
www.cenzone.com

CEVA, Inc.
2033 Gateway Place, Suite 150
San Jose , CA 95110-3710
408-514-2900
info@ceva-dsp.com
http://www.ceva-dsp.com

Charm Sciences
659 Andover St.
Lawrence, MA
978-687-9200
http://www.charm.com

Chemetrics, Inc.
4295 Catalett Rd.
Calverton, VA 20138
800-356-3072
www.chemetrics.com

Citura B.V.
PO Box 5063
3008 AB
The Netherlands
+31-10-423-96-00
www.citura.com

Cole-Parmer Instrument Company
625 East Bunker Ct.
Vernon Hills, IL 60061
800-323-4340
www.coleparmer.com

ConvaTec
PO Box 5254
Princeton, NJ 08543-5254
800-422-8811
www.convatec.com

Dinatec
Diversified Nutri-Agri Technologies Inc.
3292 Thompson Bridge Rd.
Gainesville, GA 30506
888-346-2832
www.dinatec.com

DuPont Animal Health Solutions
Windham Rd.
Chilton Industrial Estate
Sudbury, Suffolk, CO10 2XD
United Kingdom
44 (0)1787 377305
http://www2.dupont.com

Eco Enterprises
1240 NE 175th St., Suite B
Shoreline, WA 98155
800-426-6937
206-523-9300
www.ecogrow.com

Eka Chemicals Inc.
1775 West Oak Commons Ct.
Marietta, GA 30062
770-321-5849
www.eka.com

Elanco Animal Health
2001 W. Main St.
PO Box 708
Greenfield, IN 46140
800-428-4441
www.elanco.us

EWOS
7721-132nd St.
Surrey, British Columbia
Canada V3W 4M8
800-663-0476
http://www.ewos.com/ca

Fisher Scientific
2000 Park Lane Dr.
Pittsburgh, PA 15275
800-766-7000
www.fishersci.com

Fishman Chemical, LLC
215 Ojibway Ave.
Tavernier, Florida 33070
305-852-6121
sales@fishchemical.com
http://www.fishchemical.com

Floy Tag Inc.
4616 Union Bay Place NE
Seattle, WA 98105
800-843-1172
www.floytag.com

Fort Dodge Animal Health
9225 Indian Creek Pkwy #400
Overland Park, KS 66210
913-664-7000
www.wyeth.com/animalhealth

Fritz Pet
230 Sam Houston Rd.
Mesquite, TX 75149
800-955-1323
www.fritzpet.com

Ginger, Inc.
PO Box 381
Toledo, OH 43697-0381
800-537-4075
www.gingerinc.com

GlaxoSmithKline
U.K.: 44 (0)20 8047 5000
U.S.: 888-825-5249
www.gsk.com

H & S Chemical Company, Inc.
1025 Mary Laidley Dr.
Covington, KY 41017
859-356-5000
www.hschem.com

Hach Company
P.O. Box 389
Loveland, CO 80539
800-227-4224
www.hach.com

Rolf C. Hagen, Inc.
50 Hampden Rd.
Mansfield, MA 02048
www.hagen.com

Hoechst-Roussel Vet—Intervet, Inc.
PO Box 2500
Route 202-206
Sommerville, NJ 08876-1258
800-247-4838

Hoffman-LaRoche
Roche Animal Health and Nutrition
Hoffman-LaRoche, Inc.
340 Kingsland St.
Nutley, NJ 07110
973-235-5000
www.rocheusa.com

ImmuDyne, Inc.
7453 Empire Dr., Suite 300
Florence, KY 41042
888-246-6839
www.immudyne.com

Intervet International/Schering-Plough Animal Health
P.O. Box 31
5830 AA Boxmeer
The Netherlands
31 485 587600
www.intervet.com

INVE Aquaculture, Inc.
3528 W 500 S
Salt Lake City, UT 84104
801-956-0203
www.inve.com

Jellett Biotek
4654 Chester Basin
Highway 3, Nova Scotia
Canada B0J 1K0
902-275-5104
http://www.jellett.ca

Kendall
Subsidiary of Covidien
15 Hampshire St.
Mansfield, MA 02048
800-962-9888
customersupport@covidien.com
www.kendallhealthcare.com

Kent Marine
9675 South 60th St.
Franklin, WI 53132
800-255-4527
www.kentmarine.com

Kirkegaard and Perry Laboratories, Inc.
KPL, Inc.
910 Clopper Rd.
Gaithersburg, MD 20878
800-638-3167
www.kpl.com

Knoll Chemische Fabriken AG
BASF Future Business GmbH
Bau Z 025
4. Gartenweg
67063 Ludwigshafen
621 60-76811
info_fb@basf.com
www.basf-fb.de

Kordon LLC
2242 Davis Ct.
Hayward, CA 94545-1114
800-877-7387
www.novalek.com/kordon

LaMotte Company
P.O. Box 329
802 Washington Ave.
Chestertown, MD 21620
800-344-3100
www.lamotte.com

Levapan S.A.
Calle 153 #101-26
Bogotá, Colombia
+57-1-681-5606
www.levapan.com

Lustar Products Co.
101 Victory Rd.
Springfield, NJ 07081
973-379-4435

Mardel Laboratories, Inc.
1958 Brandon Ct.
Glendale Heights, IL 60139
800-323-3557
630-351-0606

Marine Enterprises International, Inc.
8800 A Kelso Dr.
Baltimore, MD 21221-3125
800-200-7258
www.meisalt.com

Mazuri/Purina Mills, Inc.
St. Louis, MO
800-227-8941
www.mazuri.com

Mercury Science Inc.
4802 Glendarion Dr.
Durham, NC 27713
866-861-5836
info@mercuryscience.com
www.mercuryscience.com

Merial
3239 Satellite Blvd.
Building 500
Duluth, GA 30096
678-638-3000
http://us.merial.com

Microbial ID
125 Sandy Dr.
Newark, DE 19713
800-276-8068
servicelab@microbialid.co
www.microbialid.com

Microtek International Inc.
6761 Kirkpatrick Crescent
Saanichton, British Columbia
Canada V8M 1Z8
250-652-4482
800-667-5062
www.microtek-intl.com

Mid-Continent Agrimarketing
1150 W 151st St., Suite D
Olathe, KS 66061
913-768-8967
www.mid-conagri.com

Miles, Inc.
Bayer Animal Health Division
Agricultural Division
Animal Health Products
Box 390
12707 W 63rd St.
Shawnee, KS 66201
www.bayer-ah.com
www.animalhealth.bayerhealthcare.com

Nachez Animal Supply Company
201 John R. Junkin Dr.
Nachez, MS 39120
601-445-0997

Neogen Corporation
620 Lesher Place
Lansing, MI 48912
517-372-9200
http://www.neogen.com

Novartis Animal Health, Inc.
Schwarzwaldallee 215
CH-4058 Basel
Switzerland
800-637-0281
www.ah.novartis.com

NT Laboratories, Ltd.
Unit B Manor Farm
Wateringbury
Kent ME18 5PP
United Kingdom
+44 (0)1622 817 692
www.ntlabs.co.uk

Nutreco Holding N.V.
Prins Frederiklaan 4
3818 KC Amersfoort
P.O. Box 299
3800 AG Amersfoort
The Netherlands
+31 (0)33 422 6100
http://www.nutreco.com

Olin Corporation
190 Carodelet Plaza
Suite 1530
Clayton, MO 63105-3443
www.olin.com

Ortho-McNeil-Janssen Pharmaceuticals, Inc.
1125 Trenton-Harbourton Rd.
P.O. Box 200
Titusville, NJ 08560-200
800-526-7736
www.ortho-mcneil.com

Oxyrase Inc.
P.O. Box 1345
Mansfield, OH 44901
419-589-8800
info@oxyrase.com
http://www.oxyrase.com

Park Tonks
48 North Rd.
Great Abington
Cambridge CB21 6AS
England
44(0)-1223-891-721
www.parktonks.co.uk

Pfizer Animal Health
www.pfizerah.com

Pfizer Company
235 East 42nd St.
New York, NY 10017
212-733-2323
http://pfizer.com

Pharmacal Research Labs, Inc.
562 Captain Neville Dr.
Waterbury, CT 06705
Pharmaq AS
P.O. Box 267
Skøyen N-0213
Oslo, Norway
+47 23 29 85 00
http://www.pharmaq.no

Phelps Dodge Refining Corp.
897 Hawkins Blvd.
El Paso, TX 79915-1217
915-778-9881
www.pdec.com

Phibro Animal Health Corporation
65 Challenger Rd., Third Floor
Ridgefield Park, NJ 07660
201-329-7300
http://www.pahc.com

Phoenix Scientific
1790-104 La Costa Meadows Dr.
San Marcos, CA 92069
760-471-5396
http://www.phnx-sci.com

Polysciences, Inc.
400 Valley Rd.
Warrington, PA 18976
800-523-2575
http://www.polysciences.com

Prominent Environmental Ltd.
Room 611, Hong Leong Plaza
33 Lok Yip Rd.
Fanling N.T. Hong Kong
852-26762545
pie@prominent.com.hk
http://www.prominent.com.hk

Purdue Frederick
One Stamford Forum
201 Tresser Blvd.
Stamford, CT 06901-3431
203-588-8000
www.pharma.com

Rangen Inc.
115 13th Ave. S
Buhl, ID 83316
800-657-6446
http://www.rangen.com

Red Sea Fish pHarm
www.redseafish.com

Research Associates Laboratory
14556 Midway Rd.
Dallas, Texas 75244
972-960-2221
http://www.vetdna.com

Rhone Mérieux
Subsidiary of Synbiotics Corporation
12200 NW Ambassador Dr., Suite 101
Kansas City, MO 64163 USA
800-228-4305
www.synbiotics.com

Salifert Worldwide B.V.
Dijkgraaf 13 6921
RL Duiven
The Netherlands
www.salifert.com

Sandpoint Aquarium Products
1365B Interior St.
Eugene, OR 97402
503-683-0600

Sankyo, Ltd.
Two Hilton Ct.
Parsippany, NJ 07054
877-726-5961
http://www.sankyopharma.com

Save My Bait, Inc.
www.savemybait.com

Schering-Plough
2000 Galloping Hill Rd.
Kenilworth, NJ 07033-0530
908-298-4000
http://www.schering-plough.com

Schering-Plough Animal Health Corp.
IntervetSera North America, Inc.
158 Keystone Dr.
Montgomeryville, PA 18936
800-659-1970
www.sera-usa.com
http://www.sera.de

Sigma-Aldrich Corp.
St. Louis, MO
314-771-5765
800-325-5832
http://www.sigmaaldrich.com

Skretting A/S
1140 Industrial Way
Longview WA 98632
800-962-2001
info@skretting.com
www.skretting.com

Solvay Chemicals
3333 Richmond Ave.
Houston, TX 77098
800-443-2785
http://www.solvaychemicals.us

SpectraPure
2167 E 5th St.
Tempe, AZ 85281
800-685-2783
www.spectrapure.com

Spectrum Chemical
14422 S San Pedro St.
Gardena, CA 90248-2027
800-813-1514
http://www.spectrumchemical.com

StatSpin, Inc.
60 Glacier Dr.
Westwood, MA 02090-1825
800-782-8774
www.statspin.com

Swift Optical Instruments
999 W Taylor St., Suite C
San Jose, CA 95126
877-967-9438
http://www.swiftoptics.com

Syndel Laboratories Ltd.
958 Chatsworth Rd.
Qualicum Beach
British Columbia
Canada V9K 1V5
800-663-2282
info@syndel.com
www.syndel.com

Syngenta Crop Protection
PO Box 18300
Greensboro, NC 27419
336-632-6000
http://www.syngentacropprotection.com

Syva Laboratories SA
Avda Parroco Pablo Diez 49-57
Leon, 24010 Spain
34-987-800800
expotacion@syva.es
www.syva.es

Takeda Chemical Industries, Ltd.
1-1, Doshomachi 4-chome,
Chuo-ku, Osaka 540-8645
Japan
81 6 6204-2111
www.takeda.com

Tetra Sales USA
3001 Commerce St.
Blacksburg, VA 24060
800-423-6458
www.tetra-fish.com

Thomas Labs
9165 W Van Buren
Tolleson, AZ 85353
800-359-8387
tlabsorders@hotmail.com
www.thomaslabs.com

Union Carbide Company
Old Ridgebury Rd.
Danbury, CT 06817
www.dow.com

Vetrepharm Ltd.
Unit 15, Industrial Estate
Sandleheath, Fordingbridge,
Hants 1PA
United Kingdom
01425 656081

Wardley Products
The Hartz Mountain Corporation
400 Plaza Dr.
Secaucus , NJ 07094
800-275-1414
www.hartz.com

Western Chemical, Inc.
1269 Lattimore Rd.
Ferndale, WA 98248
800-283-5292
360-384-5898
www.wchemical.com

Wisconsin Pharmacal Company
1 Pharmacal Way
Jackson, WI 53037
262-677-4121
800-558-6614
www.pharmacalway.com

Wyeth
5 Giralda Farms
Madison, NJ 07940
800-533-8536.
www.wyeth.com

Yellow Springs Instruments, Inc.
YSI, Inc.
1700/1725 Brannum Lane
Yellow Springs, OH 45387-1107
937-767-7241
800-765-4974
www.ysi.com

Zeigler Bros, Inc.
PO Box 95
Gardners, PA 17324
800-841-6800
www.zeiglerfeed.com

Ziggity Systems, Inc.
101 Industrial Parkway
P.O. Box 1169
Middlebury, IN 46540
574-825-5849
219-825-5849

APPENDIX III

Scientific Names of Fish Mentioned in the Text

Common name	Scientific name
Adjutant	*Lethrinus haematopterus*
Albacore	*Thunnus thynnus*
Amberjack	*Seriola dumerili*
Amberjack, goldstriped	*Seriola aureovittata*
Anabantids (family)	Anabantidae
Anchovy, European	*Engraulis encrasicolus*
Anemonefish, clown	*Amphiprion ocellaris*
Anemonefish, sebae	*Amphiprion sebae*
Anemonefish (family)	*Amphiprionidae*
Angelfish, deep	*Pterophyllum altum*
Angelfish, French	*Pomacathus paru*
Angelfish, freshwater	*Pterophyllum scalare*
Angelfish, Koran	See semicirculatus angelfish
Angelfish, marine (family)	Pomacanthidae
Angelfish, semicirculatus	*Pomacanthus semicirculatus*
Archerfish	*Toxotes jaculator*
Argentine, lesser	*Argentina sphyriaena*
Atherinid, boyeri	*Atherina boyeri*
Ayu	*Plecoglossus altivelis*
Barb, dashtail	*Barbus poechii*
Barb, lineatus	*Barbus lineatus* (= *Puntius lineatus*)
Barb, pool	*Puntius sophore*
Barb, rosy	*Barbus conchonius* (= *Puntius conchonius*)
Barb, striped	See lineatus barb
Barb, tiger	*Barbus tetrazona* (= *Capoeta tetrazona*)
Barbel	*Barbus barbus*
Barbs	*Barbodes, Capoeta, Puntius, Barbus*
Barbs (family)	Cyprinidae
Barramundi	*Lates calcarifer*
Bass, Australian	*Macquaria novemaculatea*
Bass, Australian sea	See barramundi
Bass, European sea	See Mediterranean sea bass
Bass, hybrid striped	*Morone saxatilis* × *Morone chrysops*
Bass, Japanese sea	*Lateolabrax japonicus*
Bass, largemouth	*Micropterus salmoides*
Bass, Latolabrax sea	*Lateolabrax* sp.
Bass, Mediterranean sea	*Dicentrarchus labrax*
Bass, percichthyid (family)	Percichthyidae
Bass, rock	*Amblopites rupestris*
Bass, smallmouth	*Micropterus dolomieui*
Bass, striped	*Morone saxatilis*
Bass, white	*Morone chrysops*
Bass, white sea	*Atractoscion nobilis*
Blackfish, largescale	*Girella punctata*
Bleak	*Alburnus alborella*

Common name	Scientific name
Bluefish	*Pomatomus saltatrix*
Bluegill	*Lepomis machrochirus*
Bream	*Abramis brama*
Bream, rock	*Oplegnathus fasciatus*
Bream, white	*Blicca bjoerkna*
Bream, yellowfin	*Acanthopagrus australis*
Brill, New Zealand	*Colistium guntheri*
Bullhead, black	*Ictalurus melas*
Bullhead, brown	*Amieurus nebulosus*
Bullhead, yellow	*Ictalurus natalis*
Buri-hira	*Seriola quinqueradiata* (m) × *S. aureovittata* (f)
Burbot	*Lota lota*
Butterflyfish, freshwater	*Pantodon buchholzi*
Butterflyfish, marine (family)	Chaetodontidae
Carangids (family)	Carangidae
Carp, bighead	*Hypophthalmichthys molitrix*
Carp, black	*Mylopharyngodon piceus*
Carp, common	*Cyprinus carpio carpio*
Carp, Crucian	*Carassius carassius*
Carp, grass	*Ctenopharyngodon idella*
Carp, Israel	*Cyprinus carpio nudus*
Carp, silver	*Hypophthalmichthys nobilis*
Carps (family)	Cyprinidae
Carpione	*Salmo carpio*
Catfish, African	*Clarias gariepinus*
Catfish, ancistrid	*Ancistrus* spp.
Catfish, Asian stinging	*Heteropneustes fossilis*
Catfish, blue	*Ictalurus furcatus*
Catfish, bristlenose	*Ancistrus cirrhosus*
Catfish, bullhead	See bullhead
Catfish, bullhead (family)	Ictaluridae
Catfish, channel	*Ictalurus punctatus*
Catfish, corydoras	*Corydoras* spp.
Catfish, electric	*Malapterurus electricus*
Catfish, European	See sheatfish
Catfish, golden nugget loricarid	*Baryancistrus* sp.
Catfish, hardhead sea	*Arius felis*
Catfish, labyrinth (family)	Clariidae
Catfish, pimelodella	*Pimelodella* spp.
Catfish, plecostomus	*Plecostomus* spp.
Catfish, saltwater	*Plotosus anguillaris*
Catfish, striped	*Pangasius hypophthalmus*
Catfish, suckermouth (family)	Loricaridae
Catfish, tapah	*Wallago attu*
Catfish, walking	*Clarias batrachus*
Catfish, white	*Ictalurus catus*
Cavallas (family)	Carangidae
Char, arctic	*Salvelinus alpinus*
Characins (family)	Characidae
Chebachek	*Pseudorasbora parva*
Chub	*Coregonus zenithicus*
Churchill	*Petrocephalus catostoma*
Cichlid, chromide	*Etroplus maculatus*
Cichlid, convict	*Cichlasoma nigrofasciatum*
Cichlid, firemouth	*Herichthys meeki* (= *Cichlasoma meeki*)
Cichlid, jewel	*Hemichromis bimaculatus*
Cichlid, Ramirez's dwarf	*Apistogramma ramirezi*

Common name	Scientific name
Cichlid, Rio Grande	*Cichlasoma cyanoguttatum*
Cichlid, severum	*Cichlasoma severum*
Cichlid, zilli	*Tilapia zilli*
Cichlids	Cichlidae
Cichlids, African Rift Lake	*Cynotilapia*, *Callochromis*, *Cyphotilapia*, *Eretmodus*, *Haplochromis*, *Iodotropheus*, *Julidochromis*, *Lamprologus*, *Labeotropheus*, *Melanochromis*, *Petrotilapia*, *Pseudotropheus*, *Tropheus* spp.
Cisco	*Coregonus artedii*
Clariids (family)	Walking catfish
Clownfish, Clark's	*Amphiprion clarkii*
Clownfish, false percula	*Amphiprion ocellaris*
Clownfish, tomato	*Amphiprion frenatus*
Clupeids (family)	Herring, sardines, menhaden
Coalfish	See sablefish
Cobia	*Rachycentron canadum*
Cod, Atlantic	*Gadus morhua*
Cod, black	*Anoplopoma fimbria*
Cod, Malabar rock	*Epinephelus malabaricus*
Cod, Murray	*Maccullochella peeli peeli*
Cod, Pacific	*Gadus macrocephalus*
Cod, poor	*Trisopterus minutus*
Cod, sleepy	*Oxyeleotris lineolatus*
Cods (family)	Gadidae
Crappie, black	*Pomoxis nigromaculatus*
Crevalle, jack	*Caranx hippos*
Croaker, Atlantic	*Micropogonias undulatus*
Croaker, yellow	*Pseudosciaena crocea*
Ctenopoma	*Ctenopoma* spp.
Cunner	*Tautoglabrus adspersus*
Cyclopterids (family)	Lumpfish, snailfish
Cyprinids (family)	Carps, barbs
Dab	*Limanda limanda*
Damselfish, bicolor	*Pomacentrus partitus*
Damselfish, blacksmith	*Chromis punctipinnis*
Damselfish, blue	*Chromis* sp.
Damselfish, domino	*Dascyllus tricmaculatus*
Damselfish, humbug	*Dascyllus aruanus*
Damselfish, staghorn	*Amblyglyphidodon curacao*
Damselfish (family)	Pomacentridae
Danio, devario	*Danio devario*
Danio, zebra	*Danio rerio*
Danios	*Brachydanio* spp., *Danio* spp.
Dart, snubnose	*Trachinotus blochii*
Discus	*Symphysodon discus*
Dogfish, smooth	*Mustelus canis*
Dolphin	*Coryphaena hippurus*
Drum, freshwater	*Aplodinotus grunniens*
Drum, red	*Sciaenops ocellatus*
Drum, shi	*Umbrina cirrosa*
Drums (family)	Sciaenidae
Eel, American	*Anguilla rostrata*
Eel, electric	*Electrophorus electricus*
Eel, European	*Anguilla anguilla*
Eel, Japanese	*Anguilla japonica*
Eel, moray (family)	Muraenidae
Eel, Pacific sand	*Ammodytes personatus*

Common name	Scientific name
Eel, sand	*Ammodytes* sp.
Eel, short finned	*Anguilla australis*
Eel, swamp	*Fluta alba*
Eel, tire track	*Mastacembelus armatus*
Eels, anguillid (family)	Anguillidae
Eels, true (family)	Anguillidae
Emperor, spangled	*Lethrinus nebulosus*
Eulachon	*Thaleichthys pacificus*
Filefish, threadsail	*Stephanolepis cirrhifer*
Fish, mandarin	See Chinese perch
Flatfishes (order)	Pleuronectiformes
Flathead, barfin	*Platycephalus* sp.
Flounder, barfin	*Verasper moseri*
Flounder, European	See flesus flounder
Flounder, flesus	*Platichthys flesus*
Flounder, Japanese	*Paralichthys olivaceus*
Flounder, olive	See Japanese flounder
Flounder, southern	*Paralichthys lethostigma*
Flounder, winter	*Pseudopleuronectes americanus*
Flounders, lefteye (family)	Bothidae
Four-eyes	*Anableps anableps*
Fox, flying	*Epalzeorhynchos kalopterus*
Fundulids	*Fundulus* spp. killifish
Galaxias, mountain	*Galaxias olidus*
Gasterosteids (family)	Sticklebacks
Gibel	*Carassius auratus gibelio*
Gobies (family)	Gobiidae
Goby, neon	*Elacatinus* (= *Gobiosoma*) *oceanops*
Goby, round	*Neogobius melanostomus*
Goby, sand	*Pomatoschistus minutus*
Goby, yellowfin	*Acanthogobius flavimanus*
Goldfish	*Carassius auratus auratus*
Gourami, colisa	*Colisa* sp.
Gourami, dwarf	*Colisa lalia*
Gourami, three-spot	*Trichogaster trichopterus*
Gouramies (family)	Belontiidae
Gouramies, kissing (family)	Helostomatidae
Grayling	*Thymallus thymallus*
Graylings (family)	Thymallidae
Greenling, fat	*Hexagrammos otaki*
Greenling, spotbelly	*Hexagrammos agrammus*
Grouper, banded	*Epinephelus awoara*
Grouper, blackspotted	*Epinephelus fuscogutatus*
Grouper, brownspotted	*Epinephelus malabaricus*
Grouper, chlorostigma brownspotted	*Epinephelus chlorostigma*
Grouper, coioides	*Epinephelus coioides*
Grouper, dusky	*Epinephelus marginatus*
Grouper, greasy	*Epinephelus tauvina*
Grouper, humpback	*Cromileptes altivelis*
Grouper, kelp	*Epinephelus moara*
Grouper, leopard coral	*Plectropomus leopardus*
Grouper, malabaricus	See brownspotted grouper
Grouper, melanostigma	*Epinephelus melanostigma*
Grouper, orangespotted	*Epinephelus coicoides*
Grouper, red	See redspotted grouper
Grouper, redspotted	*Epinephelus akaara*
Grouper, sevenband	*Epinephelus septemfasciatus*

Common name	Scientific name
Grouper, tauvina	*Epinephelus tauvina*
Grouper, yellow	*Epinephelus awoara*
Groupers (family)	Serranidae
Grunt, threeline	*Parapristipoma trilineatum*
Grunters (family)	*Teraponidae*
Gudgeon	*Pseudorasbora parva*
Gudgeon, Asian topmouth	*Pseudorasbora parva*
Guppy	*Poecilia reticulata*
Haddock	*Melanogrammus aeglefinus*
Hake, Pacific	*Merluccius productus*
Halibut, Atlantic	*Hippoglossus hippoglossus*
Halibut, Greenland	*Reinhardtius hippoglossoides*
Halibut, shotted	*Eopsetta grigorjewi*
Halibut, spotted	*Verasper variegatus*
Halibuts, bastard (family)	Paralichthyidae
Herring, Atlantic	*Clupea harengus harengus*
Herring, Pacific	*Clupea harengus pallasi*
Herring (family)	Clupeidae
Hogfish, Spanish	*Bodianus rufus*
Ictalurids (family)	Bullhead catfish
Ide, golden	*Leuciscus idus*
Jack, striped	*Pseudocaranx dentex*
Jacks (family)	Carangidae
Jacopever	*Sebastes schlegeli*
Jacopever, fox	*Sebastes vulpes*
Jawfish (family)	Opistognathidae
Jurupari	*Geophagus jurupari*
Killifish, gulf	*Fundulis grandis*
Killifish (family)	Cyprinodontidae
Kingfish, yellowtail	*Seriola lalandi*
Knifefish (order)	Gymnotiformes
Knifefish, green	*Eigenmannia virescens*
Koi	*Cyprinus carpio koi*
Lamprey, American sea	*Petromyzon marinus dorsatus*
Lampreys (family)	Petromyzontidae
Lionfish	*Pterois* spp., *Dendrobates* spp.
Loach, clown	*Botia macracanthus*
Loach, kuhlii	*Acanthophthalmus kuhlii*
Loaches (family)	Cobitidae
Lookdown	*Selene vomer*
Luderick	*Girella tricuspidata*
Mackerel, Atlantic	*Scomber scombrus*
Mackerel, chub	*Scomber japonicus*
Mackerel, jack	*Trachurus japanicus*
Mackerel, Japanese horse	*Trachurus japonicus*
Mackerel, Japanese Spanish	*Scomberomorus niphonius*
Mackerel, Pacific	*Scomber japonicus*
Mackerels (family)	Scombridae
Matrinxa	*Brycon cephalus*
Medaka	*Oryzias latipes*
Menhaden, Atlantic	*Brevoortia tyrannus*
Menhaden, gulf	*Brevoortia patronus*
Milkfish	*Chanos chanos*
Minnow, blue	*Fundulus grandis*
Minnow, bluntnose	*Pimephales notatus*
Minnow, fathead	*Pimephales promelas*
Minnow, sheepshead	*Cyprinodon variegatus*

Common name	Scientific name
Mollies (family)	Poeciliidae
Molly, black	*Poecilia sphenops*
Mono	*Monodactylus sebae*
Mosquitofish	*Gambusia affinis*
Mouthbrooder, Egyptian	*Pseudocrenilabrus* multicolor
Mudskipper	*Periophthalmus* sp.
Mullet, grey	*Mugil capito*
Mullet, large scale	*Liza macrolepis*
Mullet, striped	*Mugil cephalus*
Mullets (family)	Mugilidae
Mummichog	*Fundulus heteroclitus*
Muskellunge	*Esox masquinongy*
Opaleye	*Girella nigricans*
Opistognathids (family)	Opistoganthidae
Oscar	*Astronotus ocellatus*
Oval fish	*Navodan modestus*
Pacu	*Piaractus mesopotamicus*
Pacu, red	*Piaractus brachypomus*
Paddlefish, American	*Polyodon spathula*
Paddlefish (family)	Polyodontidae
Paradisefish	*Macropodus opercularis*
Paradisefishes (family)	Belontiidae
Parrotfish, Japanese	*Oplegnathus faciatus*
Parrotfish, spotted	*Oplegnathus punctatus*
Parrotfishes (family)	Scaridae
Perch, Australian silver	*Bidyanus bidyanus*
Perch, Chinese	*Siniperca chuatsi*
Perch, climbing	*Anabas testudineus*
Perch, Eurasian	*Perca fluviatilis*
Perch, European	*Perca fluviatilis*
Perch, golden	*Macquaria ambigua*
Perch, Macquarie	*Macquaria australasica*
Perch, redfin	*Perca fluviatilis*
Perch, shiner	*Cymatogaster aggregata*
Perch, silver	*Bairdiella chrysura*
Perch, white	*Morone americana*
Perch, yellow	*Perca flavescens*
Perches, freshwater (family)	Percidae
Plecostomus, blue-eyed	*Panaque suttoni*
Pickerel, chain	*Esox niger*
Pike, northern	*Esox lucius*
Pike-perch	*Stizostedion lucioperca*
Pikes (family)	Esocidae
Pilchard	*Sardinops sagax*
Pilchard, Australasian	*Sardinops sagax neopilchardus*
Pinfish	*Lagodon rhomboides*
Piranha	*Serrasalmus* spp.
Piranha, spotted	*Serrasalmus rhombeus*
Pirarucu	*Arapaima gigas*
Plaice	*Pleuronectes* (= *Platessa*) *platessa*
Platyfish	*Xiphophorus maculatus*
Platys	*Xiphophorus* sp.
Platys (family)	Xiphiidae
Pleuronectids (family)	Pleuronectid flatfishes
Poecilids (family)	Poeciliidae; Mollies, platies, swordtails
Pollock	*Pollachirus virens*
Pollock, walleye	*Theragra chalcogramma*

Common name	Scientific name
Pomfret, silver	*Pampus argenteus*
Pompano	*Trachinotus carolinus*
Pompano, ovate	*Trachinotus ovatus*
Pompano, snubnose	*Trachinotus blochii*
Pompano (family)	Carangidae
Porgy	*Stenotomus versicolor*
Porgy, rock	*Oplegnathus punctatus*
Porgy, silvery black	*Acanthopagrus cuvieri*
Pout, Norway	*Trisopterus esmarki*
Puffer, fine-patterned	*Takifugu poecilonotus*
Puffer, grass	*Takifugu niphobles*
Puffer, panther	*Takifugu pardalis*
Puffer, tiger	*Takifugu rubripes*
Pumpkinseed	*Lepomis gibbosus*
Rabbitfish	*Siganus cahaliculatus*
Rabbitfish, rivulatus	*Siganus rivulatus*
Rabbitfish, white-spotted	*Siganus canaliculatus*
Rainbowfish, Australian	*Melanotaenia* spp.
Rainbowfish, Madagascar	*Bedotia geayi*
Rainbowfish (family)	Melanotaenidae
Ratfish	*Hydrolagus colliei*
Ray, cownose	*Rhinoptera bonasus*
Ray, southern eagle	*Myliobatis australis*
Redfish	See red drum
Roach	*Rutilus rutilus*
Rockfish	*Sebastes* sp.
Rockfish, black	*Sebastes inermis*
Rockfish, gopher	*Sebastes carnatus*
Rockfish, Schlegel's black	*Sebastes schlegeli*
Rockling, four-beard	*Enchelyopus cimbrius*
Rohu	*Labeo rohita*
Rudd	*Scardinius erythrophthalmus*
Sablefish	*Anoplopoma fimbria*
Sailfish	*Istiophorus platypterus*
Salmon, amago	*Oncorhynchus rhodurus*
Salmon, Atlantic	*Salmo salar*
Salmon, chinook	*Oncorhynchus tshawytscha*
Salmon, coho	*Oncorhynchus kisutch*
Salmon, kokanee	*Oncorhynchus nerka*
Salmon, masou	*Oncorhynchus masou*
Salmon, Pacific	*Oncorhynchus gorbuscha*, *O. keta*, *O. kisutch*, *O. nerka*, and *O. tshawytscha*
Salmon, pink	*Oncorhycnchus gorbuscha*
Salmon, silver	See coho salmon
Salmon, sockeye	See kokanee salmon
Salmon, yamame	See masou salmon
Salmonids (family)	Salmon and trouts; Salmonidae
Sandlance, Pacific	*Ammodytes hexapterus*
Sardine, Atlantic	*Sardina pilchardus*
Sardines (family)	Clupeidae
Scat	*Scatophagus* sp.
Sculpin, sunrise	*Pseudoblennius cottoides*
Sea bream, black	*Mylio macrocephalus*
Sea bream, cantharus black	*Spondyliosoma cantharus*
Sea bream, crimson	*Evynnis japonicus*
Sea bream, gilthead	*Sparus aurata*
Sea bream, pagrus	*Pagrus major*

Common name	Scientific name
Sea bream, red	*Chrysophrys major*
Sea bream, red-banded	*Pagrus auriga*
Sea bream, schlegeli black	*Acanthopagrus schlegeli*
Sea bream, sharp-snout	*Diplodus puntazzo*
Sea bream, silver	*Sparus sarba*
Sea dragon, leafy	*Phycodurus eques*
Sea dragon, weedy	*Phyllopteryx taeniolatus*
Seahorse	*Hippocampus hudsonius*
Seahorse, lined	*Hippocampus erectus*
Sea trout	*Cynoscion regalis*
Sea trout, silver	*Cynoscion nothus*
Shad, gizzard	*Dorosoma cepedianum*
Shark, bonnethead	*Sphyrna tiburo*
Shark, brown	See sandbar shark
Shark, lemon	*Negraprion brevirostris*
Shark, leopard	*Triakis semifasciata*
Shark, sandbar	*Carcharinus plumbeus*
Shark, scalloped hammerhead	*Sphyrna lewini*
Shark, smooth dogfish	*Mustelus canis*
Shark, spiny dogfish	*Squalus acanthias*
Sharks, dogfish (order)	Squaliformes
Sheatfish	*Siluris glanis*
Shiner, emerald	*Notemigonus atherinoides*
Shiner, golden	*Notemigonus crysoleucas*
Shiner, spottail	*Notropis hudsonius*
Shiners	*Notropis* spp.
Siamese fighting fish	*Betta splendens*
Silver dollar	*Metynnis argenteus*
Silverside	*Menidia* sp.
Silversides (family)	Atherinidae
Smelt, rainbow	*Osmerus mordax*
Smelt, rainbow	*Osmerus mordax*
Smelt, surf	*Hypomesus pretiosus*
Smelts	*Osmerus* spp.
Smelts (family)	Osmeridae
Snakehead, bullseye	*Channa marulius*
Snakehead, Formosa	*Ophicephalus maculatus*
Snakehead, green	*Channa punctatus*
Snakehead, striped	*Ophicephalus striatus*
Snakeheads	*Ophicephalus* spp.
Snapper, gray	*Lutjanus griseus*
Snook	*Centropomus unidecimalis*
Snook, common	See snook
Sole, Dover	*Solea vulgaris/Solea solea*
Sole, English	*Parophrys vetulus*
Sole, Senegalese	*Solea senegalensis*
Soles (family)	Soleidae
Spadefish, Atlantic	*Chaetodipterus faber*
Spinefoot, dusky	*Siganus fuscenscens*
Spot	*Leiostomus xanthurus*
Sprat	*Sprattus sprattus*
Stickleback, brook	*Culaea inconstans*
Stickleback, ninespine	*Pungitius pungitius*
Stickleback, ten-spined	*Pungitius pungitius*
Stickleback, three-spined	*Gasterosteus aculeatus*
Sticklebacks (family)	Gasterosteidae
Stingray	*Dasyatis* sp.

Common name	Scientific name
Stingray, southern	*Dasyatis americana*
Stingrays (family)	Dasyatidae
Stonefish	*Synanceia* sp.
Sturgeon, baeri	*Acipenser baeri*
Sturgeon, lake	*Acipenser fluvescens*
Sturgeon, pallid	*Scaphirhynchus albus*
Sturgeon, Russian	*Acipenser guldenstadi*
Sturgeon, Siberian	*Acipenser baeri*
Sturgeon, spiny	*Acipenser nudiventris*
Sturgeon, white	*Acipenser transmontanus*
Sturgeon (family)	Acipenseridae
Sucker, shorthead redhorse	*Moxostoma macrolepidotum*
Sucker, silver redhorse	*Moxostoma anisurum*
Sucker, white	*Catostomus commersoni*
Suckers (family)	Catostomidae
Sunbleak	*Leucaspius delineatus*
Sunfish, green	*Lepomis cyanellus*
Sunfish, marine	*Mola mola*
Sunfishes, freshwater (family)	Centrarchidae
Sweetlips, threeband	*Plectorhynchus cinctus*
Swordtails (family)	Xiphiidae
Tang, naso	*Naso lituratus*
Tang, palette	See powder blue tang
Tang, powder blue	*Paracanthurus hepatus*
Tang, yellow	*Zebrasoma flavescens*
Tangs (family)	Acanthuridae
Tautog	*Tatutoga onitis*
Tench	*Tinca tinca*
Tengra	*Mystus tengra*
Tetra, neon	*Hyphessobrycon innesi*
Tetras	*Cheirodon*, *Crenuchus*, *Hemigrammus*, *Hyphessobrycon*, *Megalamphodus*, *Moenkhausia*, *Paracheirodon* spp.
Tetras (family)	Characidae
Tigerfish, three-striped	*Terapon jarbua*
Tilapia, blue	*Tilapia aurea*
Tilapia, Mozambique	*Tilapia mossambica*
Tilapia, Nile	*Tilapia nilotica*
Tilapia, nilotica	See Nile tilapia
Tilapias	*Oreochromis* spp., *Tilapia* spp., *Sarotherodon* spp.
Tomcod, Atlantic	*Microgadus tomcod*
Tomcod, Pacific	*Microgadus proximus*
Topminnows	*Fundulus* sp., *Cyprinodon* sp.
Triggerfish, sargassum	*Xanthichthys ringens*
Triggerfish, vidua	*Melichthys vidua*
Trout, brook	*Salvelinus fontinalis*
Trout, brown or sea	*Salmo trutta*
Trout, cutthroat	*Oncorhynchus clarki*
Trout, golden	*Oncorhynchus aguabonita*
Trout, Kamloops	*Oncorhynchus mykiss kamloops*
Trout, lake	*Salvelinus namaycush*
Trout, marble	*Salmo trutta marmoratus*
Trout-perch	*Percopsis omiscomaycus*
Trout, rainbow	*Oncorhynchus mykiss*
Trout, steelhead (marine)	*Oncorhynchus mykiss*
Trumpeter, striped	*Latris lineata*
Tube-snout	*Aulorhynchus maximus*
Tuna, Atlantic bluefin	*Thunnus thynnus*

Common name	Scientific name
Tuna, southern bluefin	*Thunnus maccoyii*
Turbot	*Psetta maxima* (= *Scophthalmus maximus*)
Turbot, New Zealand	*Colistium nudipinnis*
Walleye	*Sander vitreus* (= *Stizostedion vitreum*)
Weatherfish, oriental	*Misurgunus anguillicaudatus*
Weatherfishes (family)	Cobitidae
Whitefish, clupeaformis	See lake whitefish
Whitefish, lake	*Coregonus clupeaformis*
Whitefish, muksun	*Coregonus muksun*
Whitefish, peled	*Coregonus peled*
Whitefishes (family)	Coregonidae
Whiting	*Merlangius merlangus*
Whiting, blue	*Micromesistius poutassou*
Whiting, sand	*Silago ciliata*
Wolf fish, common	*Anarhichas lupus*
Wrasse, blue-lined cleaner	*Labroides dimidiatus*
Wrasse, corkwing	See *C. melops* wrasse
Wrasse, C. melops	*Crenilabrus melops*
Wrasse, C. ocellatus	*Crenilabrus ocellatus*
Wrasse, goldsinny	*Ctenolabrus rupestris*
Wrasse, Red Sea	*Coris aygula*
Wrasse, rockcook	*Ctenolabrus exoletus*
Wrasses (family)	Labridae
Yellowtail	*Seriola quinqueradiata*
Zebrafish	See zebra danio

APPENDIX IV

Definitions of Terms

Acute: Having severe clinical signs or a short course

Agonal: Pertaining to the death struggle; occurring at the time of or just before death

Algicidal: Lethal to algae

Alkalinity: The ability of a solution to neutralize acids expressed as carbonate or bicarbonate equivalents

Amplified fragment length polymorphism (AFLP): A highly sensitive genetic method for detecting polymorphisms in DNA

Anorexic/anorexia: Lack or loss of appetite for food

Anoxia: Total lack of oxygen

Ante-mortem: Before death

Antiseptic: A substance that prevents the growth or development of a microorganism on living tissue

Asepsis: Freedom from infection; aseptic (adj.)

Autolysis: Spontaneous disintegration of cells or tissues by the body's own enzymes, as occurs after death; autolyze (verb)

Bacteremia: Bacterial infection of the blood

Bilateral: Affecting both sides

Biofiltration: Process by which specific bacteria detoxify nitrogenous wastes (ammonia, nitrite) using oxygen; biofilter (noun)

Biosecurity: Preventing the introduction or spread of infectious disease by placing barriers to transmission

Brackish water: Water that is saline but less salty than full-strength seawater

Branchial: Pertaining to the gill

Cachexia: General ill health and malnutrition

Cathartic: An agent that causes evacuation of intestinal contents

Chlorinity: The mass of chlorine, measured as the total mass of halogen, contained in water (usually seawater)

Chondrodysplasia: The abnormal formation of cartilage

Chronic: Persists for a long time

Clinical hypoxia: Clinical signs associated with hypoxia, such as labored breathing and piping

Clinical signs: Any evidence of disease observed by the clinician (e.g., reddening of the body, abnormal swimming)

Community tank: An aquarium that has peaceful, compatible, easily maintained species of fish

Conditioned: Refers to an aquaculture system that has a stable and functioning biofilter

Congestion: Abnormal accumulation of blood in a body part

Conspecific: Individual that is in the same species

Cyst: (1) A developmental stage in some protozoan parasites; (2) Any closed epithelium-lined cavity

Dematiaceous: A family of imperfect fungi having hyphae and/or conidia that are brownish or black colored

Depression: A lowering or decrease in activity; depressed (adj.)

Diagnosis: Determination of the nature of a case of a disease; diagnostic (adj.)

Differential diagnosis: The determination of which one of several diseases may be producing the clinical signs

Dyspnea: Labored or difficult breathing; dyspneic (adj.)

Ecchymosis: A hemorrhagic spot, larger than a petechia, in the skin or mucous membrane

-emia: An affliction of the blood (e.g., bacteremia is a bacterial infection in the blood)

Enophthalmos: Recession of the eyeball within the orbit (eye socket)

Epithelium: The cellular covering of external and internal body surfaces

Erosion: A shallow or superficial loss of epithelium; shallower than an ulcer

Etiology: The science that deals with causes of disease; etiologic, etiological (adj.)

Euryhaline: Capable of tolerating a wide range of salinity

Eutrophic: An ecosystem that has a large input of nutrients

Exophthalmos: Abnormal protrusion of the eye; exophthalmic (adj.)

Facultative: Not obligatory; able to adopt an alternative mode of living

Fallow: Removing all cultured fish from a culture system or site for a period of time

FAT (fluorescent antibody test): A diagnostic test, usually used to detect a pathogen in tissue, that uses a specific antibody with a fluorescing molecule (fluorescein) attached to the antibody. When the antibody binds to the pathogen, its binding can be detected using ultraviolet light under a microscope

Fistula: An abnormal passage from an organ to the body surface

Fluctuant: Movable and compressible

Focus: (1) The chief center of a morbid process; (2) A discrete area having a morbid process; focal (adj.)

Fomite: An inanimate object or material on which disease-producing agents may be conveyed

Fontanelle: One of the membrane-covered spaces that remain at the junction of the sutures of the incompletely ossified skull in some immature animals

Gangrene: Death of tissue; gangrenous (adj.)

Germicide: An agent (as heat or radiation or a chemical) that destroys microorganisms that might carry disease

GMS: Gomori methenamine silver; silver stain used to stain tissue section for carbohydrate, including fungal cell walls

Hardness: See PROBLEM 9

HE: Hematoxylin and eosin stain; the stain routinely used to stain histological sections for routine examination

Hemorrhage: The escape of blood from vessels; bleeding; hemorrhagic (adj.)

History: The events preceding and associated with a disease outbreak; also known as subjective data

Holotrichous: Cilia distributed evenly over the body; usually refers to protozoa

Horizontal transmission: The transmission of a microbial infection between members of the same species that are not in a parent-offspring relationship

Hyperemia: An excess of blood in a body part

Hyperplasia: Abnormal increase in the number of normal cells in normal arrangement in an organ or tissue, which increases the organ's or the tissue's volume

Hypertrophy: Enlargement of an organ or its part caused by an increase in the size of its cells

Hyphema: Hemorrhage in the anterior chamber of the eye

Hypoxia: Deficiency of oxygen, such as reduction of oxygen in tissues below physiologically required levels

-iasis: A condition or state; e.g., parasitiasis is the state of being parasitized; also see -osis

Iatrogenic: Resulting from the actions of a clinician, usually referring to an adverse effect

Idiopathic: Occurring without known cause

IFAT (indirect fluorescent antibody test): A variation of the fluorescent antibody test (see FAT) in which two antibodies are used in the test—an antibody that specifically binds to the pathogen is first added, followed by a fluorescein-containing antibody that specifically binds to the first antibody. This test is more commonly used than FAT because it tends to be more sensitive

In toto: Entirely; totally

Infection: Invasion and multiplication of organisms in body tissues

Infestation: Subsistence on the surface of the skin or gills, without invasion into these tissues

Inflammation: A protective tissue response to injury, which serves to destroy, dilute, or wall off both the injurious agent and the injured tissues

Intensive culture system: A culture system designed to hold a large amount of fish in a small amount of water; e.g., aquarium, raceway

-itis: Inflammation of a tissue or organ (e.g., splenitis is inflammation of the spleen)

Keratinized: Formation of a horny, outer layer on the skin, typically found in terrestrial vertebrates (mammals, reptiles)

Latent: Dormant or concealed

LC_{50}/LD_{50}: The concentration or dose of a chemical that causes 50% mortality in a specified period of time (e.g., the 96-hour LC_{50} is the concentration of a chemical that will kill 50% of the individuals after 96 hours' exposure to the chemical)

Lesion: Any pathological or traumatic discontinuity of tissue or loss of function of a part

Lethargy: Drowsiness or indifference

Macroalgae: Macrophytes that are members of the algae

Macronucleus: In ciliate protozoa, the larger of two types of nucleus in each cell, which controls nonreproductive functions

Macrophyte: A large macroscopically visible aquatic plant (e.g., hydrilla, cryptocorynes, hair algae, and kelp are macrophytes, while dinoflagellates, diatoms, and other microscopic plants are not)

Mesohaline: Refers to brackish water between ~5 and 18 ppt salinity

Morbidity: (1) The condition of being diseased; (2) The sickness rate; the ratio of sick to well animals in a population

Moribund: In a dying state

Mucous membrane: The tissue lining various canals and cavities of the body; also see epithelium

Necrosis: Death of individual cells or groups of cells, or of localized areas of tissue; necrotic (adj.)

Nocioception: Ability to detect an adverse stimulus; afferent nerve response produced in the peripheral and central nervous system by stimuli that have the potential to damage tissue

Obligate: Characterized by the ability to survive only in a particular environment (e.g., obligate pathogen)

Ocular: Pertaining to the eye

Oligohaline: Refers to slightly brackish water (between ~0.5 and 5 ppt salinity)

Operculum: The bony covering of the gill

Opportunistic: Capable of adapting to the tissue or host other than the normal one, or capable of taking advantage of an immunocompromised host; said of microrganisms and parasites

-osis: Disease, morbid state; e.g., parasitosis is being sick from parasite infection or infestation; also see -iasis

Pain: A sensory experience that is the unpleasant awareness of a noxious stimulus or bodily harm; also see nocioception

Parasitemia: Parasite infection of the blood

Paratenic host: A host that is not absolutely required for completion of a parasite's life cycle (i.e., is not an obligate host); transport host (syn.)

Parenchyma: The essential or functional elements of an organ, as distinguished from its stroma or framework

Paresis: Slight or incomplete paralysis

Pathognomonic: Specifically distinctive or characteristic of a disease or pathologic condition

Pathology: The branch of medicine that studies the changes in body tissues and organs that are caused by disease

PCR: See polymerase chain reaction

Peracute: Very acute

Pericardium: The sac enclosing the heart

Peritoneum: The membrane that lines the wall of the abdominal cavity and covers the viscera

Peritonitis: Inflammation of the peritoneum

Petechia: A minute red spot caused by escape of a small amount of blood; petechial (adj.)

pH: The negative logarithm of the hydrogen ion concentration, expressed on a scale of 0–14, values <7 being increasingly acidic (more hydrogen ion), values >7 being increasingly basic (less hydrogen ion), and 7 being neutral

Pharmacokinetics: The study of quantifying how an administered drug becomes distributed throughout various tissues and excreted from the body

Phytoplankton: Microscopic plants found in the water column (e.g., microscopic algae, such as diatoms, dinoflagellates, and green algae)

Piping: The act of fish gulping air at the surface of the water

Poikilothermy: The state of having a body temperature that varies with the temperature of the environment; poikilothermic (adj.)

Polyhaline: Refers to brackish water approaching full-strength seawater (~18–30 ppt salinity)

Polymerase chain reaction: A genetic technique in which a specific strand of DNA is exponentially amplified in a very short period of time using a *polymerase* enzyme via a *chain reaction,* allowing the very sensitive detection and identification of even a miniscule amount of genetic material

Postmortem: After death

ppt (parts per thousand): The approximate grams of solids per liter of water; usually refers to the amount of salt in water

Prepatent: Period before being evident

Primary infection: The infectious agent that is responsible for initiating damage to tissue; also see secondary infection

Probiotic: Live microorganisms that when administered in adequate amounts confer a health benefit on the host

Prodromal: Referring to prodrome, which is the stage of early nonspecific clinical signs indicating the start of a disease before specific clinical signs occur

psu (practical salinity units): The conductivity ratio of a seawater sample to a standard KCl solution; this is an alternative measure of expressing the salinity of water

Quarantine: Imposing isolation or restriction of free movement to prevent the spread of contagious disease

Recrudescence: Recurrence of clinical signs after temporary abatement

Secondary infection: An infectious agent that invades the tissue after another agent has initially damaged the tissue; also see opportunistic, primary infection

Septicemia: Toxin in the blood; often refers to the presence of bacterial toxin

Sequela: A morbid condition following or occurring as a consequence of another condition or event

Serosanguineous: Composed of serum and blood

Sessile: Attached

Sexual dimorphism: Characteristics that distinguish male from female

Shimmies: Swimming in one place in a slow, weaving fashion; usually associated with some skin ectoparasite infestation

Splenomegaly: Enlargement of the spleen

Sporozoite: The infective form of a sporozoan, which undergoes asexual reproduction (schizogony) in the body of the host

Stenohaline: Unable to withstand a wide variation in salinity

Systemic: Pertaining to or affecting the body as a whole (e.g., versus only affecting the skin or gills)

Theront: The free-swimming, nonfeeding, infective stage of certain parasitic protozoa (e.g., *Ichthyophthirius*)

Tissue: A group or layer of similarly specialized cells that together perform certain specialized functions

Tomite: The daughter cells produced by a tomont

Tomont: The "encysted," benthic, dividing stage of certain parasitic protozoa (e.g., *Ichthyophthirius*)

Toxicosis: Any diseased condition caused by poisoning

Trophont: The attached, fish-feeding stage of certain parasitic protozoa (e.g., *Ichthyophthirius*, *Cryptocaryon*, *Amyloodinium*, *Piscinoodinium*)

Trophozoite: General term for the feeding stage of a parasitic protozoan

Ulcer/ulceration: A local defect on the surface of an organ or tissue, usually produced by sloughing of necrotic tissue

Unilateral: Affecting only one side

Vaccine: A biological preparation that is used to establish or improve immunity to a particular disease

Vascular plant: The evolutionarily advanced plants that have a specialized conduction system, which includes xylem and phloem; such plants include almost all of the commonly propagated aquarium plants, as well as many aquatic pond weeds (e.g., hydrilla); the other major group of aquatic plants is the algae

Vascularized: Supplied with blood vessels

Vent: The posterior opening that serves as the only such opening for the intestinal and urinary tracts of fish

Vertical transmission: Transmission of an infection from a parent to its offspring during the period immediately before and after hatching/birth

Viremia: Virus infection of the blood

Viscera: Plural of viscus

Viscus: Any large interior organ in any of the great body cavities (e.g., pericardial and abdominal cavities of fish), especially those in the abdomen

Wet mount: The technique of placing wet tissue on a microscope slide so that it can be examined for infectious agents or pathology

Xenoma: An extremely enlarged host cell filled with spores and developmental stages of microsporidia

Zoonosis: A disease of animals that can be transmitted to man; zoonotic (adj.)

APPENDIX V

Example Form for Shipping Fish to a Clinic or Diagnostic Laboratory

YOU ARE ENCOURAGED TO CONTACT THE DIAGNOSTIC LABORATORY BEFORE SUBMITTING FISH FOR DIAGNOSIS. In order to determine the cause of a fish disease outbreak, it is imperative that properly prepared specimens be submitted to the diagnostic laboratory. Fish *must* be alive when collected, and it is desirable to have fish alive at the time of laboratory examination. However, it is usually not practical to ship live fish unless they are small and appear capable of surviving the time needed for transport. If specimens cannot be maintained alive, the samples should be stored in individual sealed plastic bags and placed on wet ice. If the fish cannot be shipped to the diagnostic lab within 4 hours of death, they should be frozen; they can then be shipped later on dry ice.

While specimens fixed in alcohol or formalin are of limited diagnostic value by themselves, they can be useful when submitted along with fresh or frozen specimens. The formalin should preferably be buffered by adding 10 grams of sodium phosphate per liter (= 35 grams per gallon) of 10% formalin. The body cavity should be exposed to allow for adequate preservation. This can be done by making two incisions, one along the belly running the entire length of the body cavity, the other running at a right angle to the first, just behind the gill cover. This flap of muscle should be removed. The swim bladder should also be slit lengthwise. Fish that are less than 5 cm (2 inches) long can simply be placed in fixative without slitting the body cavity. There should be about ten times as much volume of formalin solution as there is volume of fish tissue for adequate preservation. Seventy percent alcohol can be used if formalin is not available.

At least ten fish displaying signs typical of the disease should be collected. Try to include fish that are only mildly affected as well as those that are extremely ill. At least five apparently normal fish should also be obtained if possible. If more than one area is affected, each area should be treated as a separate disease outbreak and an appropriate number of fish collected.

Many times the information surrounding the outbreak of a disease can be as important as the examination of the affected fish. Water quality is especially important when a toxin is suspected in causing a fish kill. If a toxin is suspected, several gallons of water should be collected. If this cannot be sent immediately along with the sample of fish, it should be frozen for later shipment on dry ice.

Shipment of Specimens:

Important: Check with the intended carrier to be sure that the materials to be sent are in compliance with their regulations.

Live specimens: Place the smallest affected fish in a strong plastic bag. Add only enough water to allow the fish to remain upright and fill the rest of the bag with compressed air or pure oxygen. Tie the bag securely and pack it in a strong cardboard, plastic, or Styrofoam box with shredded paper or Styrofoam chips. During summer, a plastic bag containing crushed ice should be placed next to the fish. Mark the container "Scientific Specimens—Perishable" and ship it by overnight carrier.

Refrigerated specimens: Fish should be placed in individual plastic bags without water, the bags sealed and placed on crushed ice. Shipment should be in a well-insulated container with 10–15 lb (4.5–7 kg) of crushed ice.

Frozen specimens: Live fish collected in the field should be placed immediately in a plastic bag with a small amount of water that is then placed on ice to be immediately frozen. Ship on dry ice as soon as possible using instructions for live specimens. Five pounds (2.2 kg) of dry ice will keep specimens frozen for 24–36 hours if the carton is well insulated.

Fixed specimens: Fish should be placed in a strong sealed plastic bag, which should then be placed within a strong crush-resistant container.

Ship specimens to:

Dr. Edward J. Noga
NCSU College of Veterinary Medicine
4700 Hillsborough Street, Raleigh, NC 27606

FISH DISEASE OUTBREAK DATA SHEET

Biologist or Owner _____ Case No._____

Address _____ Date _____

Email _____ Phone No. _____ FAX No. _____

Name of body of water
(exact location if known) _____

water temp _____ dissolved O_2 (mg/L) _____ pH _____ alkalinity (mg/L) _____ salinity (ppt) _____

unionized ammonia (mg/L) _____ nitrite (mg/L) _____ Cl:NO_2 ratio: _____

Other water quality values tested:

Unusual aquatic or weather conditions:

Feed used:

Date mortality began _____ Date of collection
(including exact time) _____

Suspected cause of mortality: parasites _____ other infectious disease _____

oxygen _____ pollution _____ unknown _____

Species affected and extent of kill:

Species	Estimated number	Percentage of population	Age (size)
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Body condition of fish: good _____ fair _____ poor _____

Brief description of characteristics or mortality:

Behavior

Physical appearance

Additional remarks:

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