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EMERSION AND THERMAL TOLERANCES OF THREE SPECIES OF UNIONID MUSSELS: SURVIVAL AND BEHAVIORAL EFFECTS

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ABSTRACT We evaluated the behavior and survival of unionid mussels after emersion in air temperatures across a range that is likely to be encountered during status surveys or relocations. Five laboratory tests were performed with pocketbook *Lampsilis cardium* Rafinesque (2 tests), pimpleback *Quadrula pustulosa* Lea (1 test), and spike *Elliptio dilatata* Rafinesque (2 tests) mussels, each conducted in a completely randomized, nested experimental design. For each mussel species (except *Q. pustulosa*), treatments tested included two water temperatures (25 and 10 °C), five air temperatures (ranging within ±20 °C of the water temperature), three aerial exposure durations (15, 30, and 60 min), and a no emersion control. All treatments were duplicated, with 10 organisms per emersion time and aerial exposure temperature ($n = 320$ mussels per test). Behavioral response (ability to upright) and mortality were measured daily for 14 d postemersion. Both water and aerial exposure temperature (air shock) were important predictors of times to first uprighting. The intensity function of first uprighting differed among species ($P < 0.01$), and there was a significant interaction between *E. dilatata* versus the other species and water temperature ($P < 0.01$). Over-all mussel survival after emersion was high (93%); however, *E. dilatata* experienced significant treatment related mortality at the 25 °C test water, 45 °C aerial exposure temperature. Because of the high incidence of uprighting and survival of mussels in our study, emersion at moderate temperatures (15 to 35 °C) and durations (15 to 60 min) does not seem harmful to mussels, and, therefore, conducting relocations and status surveys under these conditions should not impair mussel survival and over-all success.

KEY WORDS: Unionid mussel, conservation, emersion, temperature, behavior, mortality

INTRODUCTION

The imperiled status of unionid mussels (Williams et al. 1993) has prompted conservation efforts by public and private natural resource agencies that include status surveys, restocking, and relocation. The effects of collection and handling on mussels in field studies are generally considered benign and inconsequential to mussels relative to most threats (construction, zebra mussel infestation, habitat loss). However, Cope and Waller (1995) reviewed the success of relocation projects and found that mortality of mussels after relocation can be significant (>70% in 30% of projects reviewed). Mortality was highest within 1 year of the event, suggesting that effects of collection, handling, and displacement of mussels may be greater than were previously considered. The environmental conditions that mussels experience during collections and surveys may contribute to low survival, but can also be controlled to some extent. Determination of the emersion and thermal tolerances of unionid mussels would provide guidelines on the conditions in which surveys and relocations should occur to enhance mussel survival and over-all success.

Past studies suggest that mussels can tolerate emersion for hours or even days (Byrne and McMahon 1994, Dietz 1974, Holland 1991, Schanzle and Kruze 1994, Waller et al. 1995). However, survival of mussels is related to such environmental conditions during emersion as relative humidity and air temperature. For example, Waller et al. (1995) emersed *Amblema plicata plicata* Say and *Obliquaria reflexa* Rafinesque for a maximum of 8 h and found that mussels had greater survival when handled during the fall (water temperature ~15 °C; air temperatures ranged from 12 to 25 °C) compared to those handled during the spring (water temperature ~23 °C; air temperature ranged from 18 to 29 °C). In the present laboratory study, we augment these data by evaluating a range of extreme air temperatures and water–air thermal differentials. We selected the minimum and maximum water and air temperature and emersion times based on conditions likely to be found in field collecting situations. In addition to survival, the uprighting behavior of mussels after emersion was selected as a potential indicator of emersion stress; presumably, the ability to upright and burrow into the substratum indicates normal functioning. Waller et al. (1999) found significant species and water temperature related differences in the uprighting and movement intensity of four mussel species after displacement. Thus, displacement, coupled with a thermal and emersion challenge, may also produce significant behavioral changes.

In this study, we evaluated the effects of emersion and temperature on the survival and behavior of three mussel species *Lampsilis cardium* Rafinesque (pocketbook), *Quadrula pustulosa* Lea (pimpleback), and *Elliptio dilatata* Rafinesque (spike), and examined the variation in survival and behavioral response within and among the three species. These mussel species represent two subfamilies (Lampsilinae and Ambleminae) and two contrasting life history strategies (long-term and short-term brooders) within the Unionidae. Additionally, *L. cardium* and *Q. pustulosa* served as surrogates for two U.S. Federally Endangered species, the *L.*

higginsi Lea (Higgins' eye) and *Q. fragosa* Conrad (winged mapleleaf), both found in the Upper Mississippi River basin. *Elliptio dilatata* was chosen as a second surrogate for *Q. fragosa,* because too few *Q. pustulosa* were available for testing at low (10 °C) water temperature.

MATERIALS AND METHODS

Test Organisms

Three species of unionid mussels were collected from the Wolf River at Shawano, Shawano County, Wisconsin. Mussels were transported in holding tanks, containing Wolf River water (25 °C), to the Upper Midwest Environmental Sciences Center, in La Crosse, Wisconsin. Holding tank water temperatures were maintained at 25 ± 3 °C (with addition of nonchlorinated ice as needed), and the dissolved oxygen concentration was maintained at >60% saturation with aeration. Water temperature and dissolved oxygen (Yellow Springs Instrument Model 58 oxygen meter) were measured at 1-h intervals. At the laboratory, mussels were placed into submerged cages held in the Black River (water temperature, 27 °C), near La Crosse, Wisconsin until study initiation. The mussel cages (122-cm length × 122-cm wide × 46-cm height) were constructed of angle and strap iron frame with netting (1.9-cm diam. polyethylene) attached to the iron frame by tie wraps and nylon rope. One species of mussel (111 total; density of 75/m^2) was placed into each cage. During collection, transport, and allocation to cages, mussels were continually immersed in river water.

Experimental Design and Exposure System

Five laboratory tests were performed with *L. cardium* (2 tests), *Q. pustulosa* (1 test), and *E. dilatata* (2 tests), each conducted in a completely randomized design as a nested experiment. For each mussel species tested (except *Q. pustulosa*), there were two water temperature treatments (25 and 10 °C), five air temperatures (ranging within ± 20 °C of the water temperature), three aerial exposure duration treatments (15, 30, and 60 min), and a no emersion control treatment (Fig. 1). Because of limited availability, *Q. pustulosa* was tested only at 25 °C, the treatment we assumed to be more lethal. All treatments were duplicated, with 10 organisms per emersion time and temperature (n = 320 mussels/test), for a total of 32 experimental units. Ten mussels were placed into a flow-through, stainless steel tank (61-cm length × 30-cm wide × 36-cm height) containing sand (13 ± 0.5-cm depth) and 42 ± 0.5 L of overlying well water. Each tank was placed into one of six water baths (305-cm length × 84-cm wide × 46-cm height) maintained at the test temperature (10 or 25 ± 1 °C) with a thermostatically controlled, liquid circulation pump (Remcor Model CFF-501, Remcor Products Co., Franklin Park, IL) connected to the water bath. Tanks were aerated to maintain dissolved oxygen concentrations at >60% saturation. The flow rate of water into each tank was 200 mL/min with a turnover rate of seven times per day. The photoperiod was 16-h light and 8-h dark.

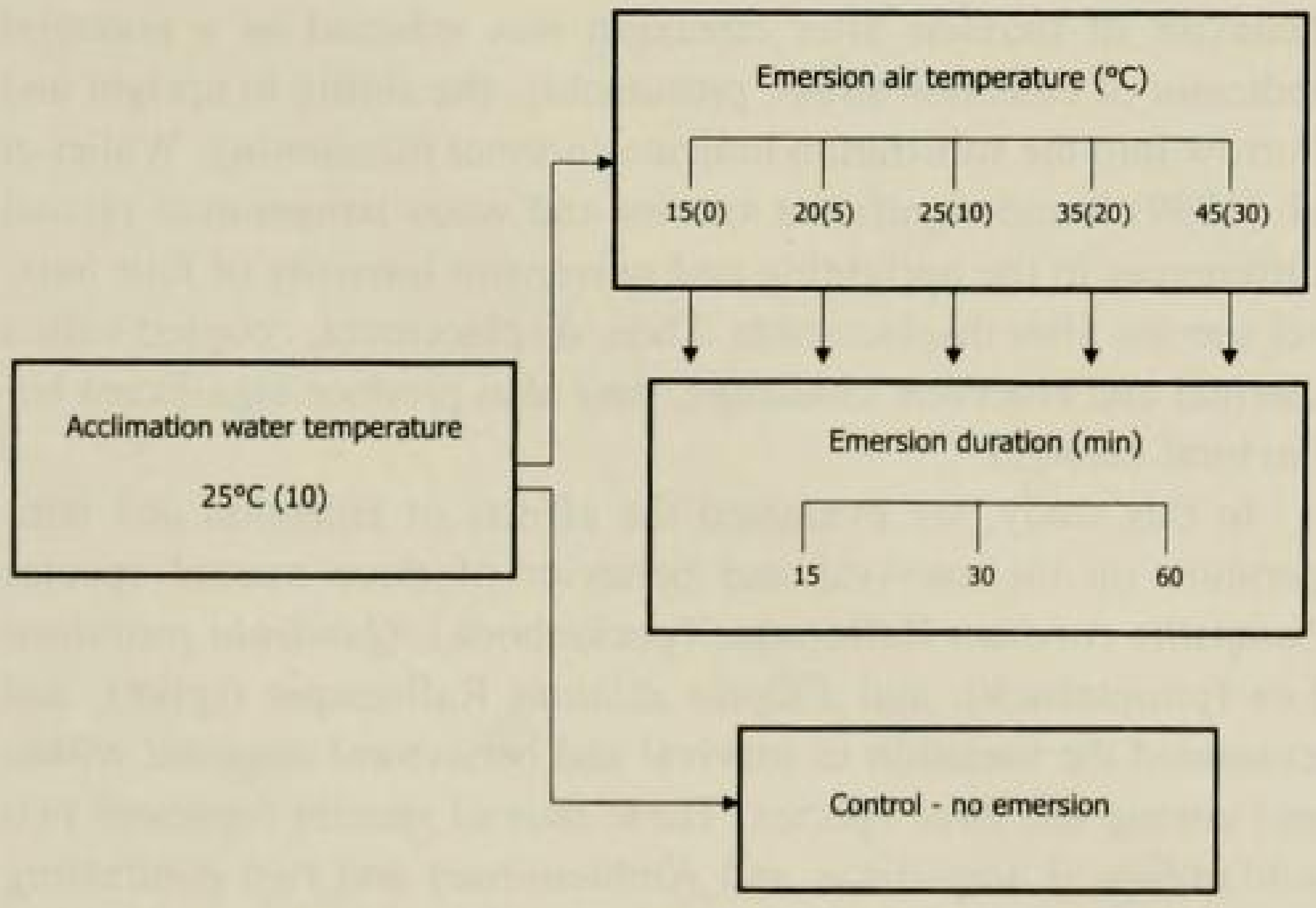

Figure 1. Experimental design for the thermal and emersion exposure tests with three species of unionids. The numbers in parentheses represent the 10 °C test water–aerial exposure regime.

Laboratory tests began when the water temperature of the Black River reached the desired test water temperature (25 °C; July/August and 10 °C; November/December). Each mussel species was tested individually, and a given species was transported in coolers containing Black River water to the laboratory for testing. Ten mussels were randomly selected for each experimental unit. Plastic mesh netting was placed on top of the sand substrate to prevent mussels from burrowing into the substrate before aerial exposure. Mussels were acclimated in their respective tanks at a water temperature of 25 ± 1 °C for 2 d; mussels were not fed during the acclimation period. Mussels within a replicate were numerically marked (1 to 10) on their right valve with a permanent marker. To enable identification of mussels after burrowing into the substrate, each mussel was uniquely tagged with a numbered fishing bobber (3.81-mm dia.) that was attached to a 22.9-cm piece of cotton thread and secured to the umbonal region of the right valve with cyanoacrylate (Krazy Glue® Gel, Borden, Inc., Columbus, OH). Both siphons (incurrent and excurrent) remained immersed while bobbers were being attached to the shells; total handling time was less than 3 min per mussel.

For each treatment, 20 mussels (10 from each replicate) were removed from the test water (25 or 10 °C), transported in water (held at test temperature), and placed into an environmental chamber (Hotpack® Biological Chamber, Hotpack Corp., Philadelphia, PA) at a given air temperature (25 °C water temperature; 15, 20, 25, 35, 45 °C air temperature; 10 °C water temperature; 0, 5, 10, 20, 30 °C air temperature) for a duration of 15, 30, or 60 min. Treatments were conducted in order of increasing air temperature and emersion duration. The target relative humidity in the environmental chambers was 60 ± 5%. This relative humidity was selected based on average ambient air conditions experienced in our geographic region (Steve Thompson, National Oceanic Atmospheric Administration, La Crosse, WI, pers. comm.). Following emersion, mussels were removed from the environmental chamber, transported in well water (held at the test temperature, 25 or 10 °C), and returned to their respective tanks. Each mussel was placed directly on top of the sand substrate, with the right valve (tagged side) facing upward. Test organisms were fed a mixture of C4 algae diet (Coast Seafoods Co., South Bend, WA; 0.2 mL per mussel) and dry *Chorella* (0.013 g dry weight per mussel) daily. Mussels were monitored for mortality and uprighting response for 14 d postemersion. At test termination (14 d postemersion), mussels were recovered from each tank and measured for total length and whole mussel wet weight. Sex of *L. cardium* was determined by shell dimorphism. *Elliptio dilatata* and *Q. pustulosa* are not sexually dimorphic; thus, we examined histological sections of half of the mussels from each replicate in the 25 °C test to determine the sex ratio, and assumed animals tested at the 10 °C water temperature had a similar sex ratio, because all mussels came from the same population and were randomly sampled.

Statistical Analyses

For each mussel species, we examined patterns between two response variables, times to first uprighting and death, as a function of water temperature (°C), duration of aerial exposure, and air shock temperature (°C), which we define as the difference between water temperature and air emersion temperature. We refer to both first uprighting and death as events, and our primary data consist of elapsed times to occurrences of those events for each mussel. Some event durations may have exceeded the study duration (14 d) and, therefore, went unobserved; these events are said to be "right-censored" (Hosmer and Lemeshow 1999). Proper accommodation of censoring is critical to valid interpretation of time-to-event data. For both events (first uprighting and death), we used the Cox proportional hazards regression model (Cox 1972, Newman 1995, Hosmer and Lemeshow 1999) to identify factors that explained the pattern in uprighting and survival. We arbitrarily selected *L. cardium* as the baseline species for our analysis; this choice does not affect the over-all results. The baseline temperature was 0 °C, and other temperatures were coded as deviations from this baseline. Denote E = U and E = D for the events uprighting and survival, respectively. Our full regression models for both first uprighting and survival are

$$\lambda_{Ei}(t) = \lambda_{E0}(t)\exp(S_i + \beta_1 T + \beta_2 A + \beta_3 M + \beta_4 A^2 + \beta_5 M^2 + \beta_{1,i} T + \beta_{2,i} A + \beta_{3,i} M + \beta_6 TA + \beta_7 TM + \beta_8 AM + \beta_9 TA^2 + \beta_{4,i} A^2 + \beta_{6,i} TA + \beta_{7,i} TM + \beta_{8,i} AM + \beta_{11} TAM + \beta_{11,i} TAM), \quad (1)$$

where: $\lambda_{Ei}(t)$ is the hazard function for event type E for the ith species at time t; $\lambda_{E0}(t)$ is the corresponding baseline hazard; S_i, i = 1, 2, are two fixed-effects parameters for identification of the three species (S_1 denotes *E. dilatata*, and S_2 denotes *Q. pustulosa*); T represents water temperature (°C) with coefficient β_1; A represents air shock temperature (°C), which we define as the difference between water and air emersion temperatures with coefficient β_2; M represents air exposure duration (min) with coefficient β_3; A^2 and M^2 are quadratic (∪ or ∩-shaped) effects of air shock and exposure duration, respectively; $\beta_{1,i}T$ represents the species × water temperature interaction; $\beta_{2,i}A$ represents the species × air shock interaction; $\beta_{3,i}M$ represents the species × air-exposure duration interaction; TA, TM, and AM are two-way interactions among T, A and M; TA^2 is the interaction between T, and the quadratic effect of A; $\beta_{4,i}A^2$ is the interaction between species and A; $\beta_{6,i}TA$, $\beta_{7,i}TM$, and $\beta_{8,i}AM$ represent three-way interactions among species, A, T, and M; TAM is the three-way interaction between T, A, and M; and $\beta_{11,i}TAM$ is the four-way interaction among species, T, A, and M. In this model, "interactions" are on the log scale. Although in such terminal events as death, it is customary to refer to $\lambda(t)$ as the hazard function for deleterious events such as death, the term intensity is more appropriate than hazard for events such as first uprighting. Therefore, we refer to $\lambda(t)$ as either the hazard or intensity function, depending on whether we are addressing survival or first uprighting, respectively. We fitted Eq. (1) to the uprighting and survival data by maximizing the partial likelihood (Cox 1972), and constructed likelihood-ratio and Wald chi-square tests for each parameter (Hosmer and Lemeshow 1999) with the SAS PHREG software (SAS Institute 1997). For each event type, we began with our full regression model [Eq. (1)] and, one-by-one, deleted terms for which the corresponding likelihood-ratio chi-square test was not significant at the α = 0.05 level, except we did not delete terms for which a higher-order interaction was statistically significant. This model reduction process identifies the simplest model for each event type that preserves the hierarchical structure of Eq. (1). Our recorded event times were based on observations at fixed times rather than exact measurements of event times, and, therefore, contained ties. We used Efron's method to adjust for tied event times, which has been shown to perform better than alternatives (Hertz-Picciotto and Rockhill 1997). We assumed that events occurred at the observation time rather than, for example, the temporal midpoint between successive observations to produce conservative estimates of the intensity of first uprighting or the hazard of mortality.

TABLE 1.

Physical characteristics of three mussel species after aerial exposure at various water–air temperature differentials.

Water Temperature (°C)	Species	Mean Length (mm)	Wet Weight (g)
25	*Elliptio dilatata*	80.57 (9.7)	53.79 (18.4)
	Quadrula pustulosa	61.82 (13.2)	78.27 (42.0)
	Lampsilis cardium	100.32 (10.7)	185.59 (54.0)
10	*E. dilatata*	78.64 (10.4)	52.21 (19.6)
	L. cardium	99.71 (11.6)	179.74 (33.1)

Numbers in parentheses are the standard deviation of the mean.

A particularly desirable feature of proportional hazard regression models is that the parameters have natural interpretations that provide informative descriptions of the event times. Because these models are still unfamiliar in ecology, interpretation requires some explanation. The hazard function $\lambda(t)$ quantifies the number of events per interval of time at time t. From Eq. (1), the dimensionless hazard ratio (risk ratio) at time t is given by $\mathrm{HR}(t) = \lambda_i(t)/\lambda_0(t) = \exp[S_i + \cdots + \beta_{11,i}TAM]$. For such categorical variables as species S_i in our analysis, the hazard ratio for species i relative to the baseline species is $\exp(S_i)$, and the hazard ratio for species 1 relative to species 2 is $\exp[S_1 - S_2]$ in the absence of higher-order interactions. If, for example, $\exp(S_i) = 0.5$, we say that the *relative* hazard (or intensity) for species i is only 50% of that for the baseline species. For continuous covariates such as water temperature, the statistic $100[\exp(\beta_1) - 1]$ is the estimated percentage change in the hazard (or intensity) ratio for each unit change in temperature. If, for mortality, $\beta_1 = 0.5$, then the hazard ratio increases by approximately 65% for each 1 °C increase in temperature (Allison 1995). These interpretations extend to more com-

TABLE 2.

Proportional hazards (intensity) model fitted to the time to first uprighting for *Elliptio dilatata*, *Quadrula pustulosa*, and *Lampsilis cardium*. *Lampsilis cardium* at 0 °C constitute baseline conditions.

Parameter (Effect)	Estimate (SE)	Wald χ^2	*P*-Value
S_1 (species: *E. dilatata*)	0.3142 (0.1965)	2.6	0.11
S_2 (species: *Q. pustulosa*)	− 0.3031 (0.0821)	13.6	< 0.01
β_1 (water temperature T)	0.1491 (0.0083)	319.0	< 0.01
β_2 (air shock A)	− 0.0677 (0.0146)	31.4	< 0.01
β_4 (A^2)	0.0055 (0.0011)	26.9	< 0.01
$\beta_{1,1}$ ($S_1 \times T$)	− 0.0562 (0.0096)	34.1	< 0.01
β_6 ($T \times A$)	0.0027 (0.0007)	16.2	< 0.01
β_9 ($T \times A^2$)	− 0.0003 (0.0001)	30.9	< 0.01

See text for explanation of the model and parameters.

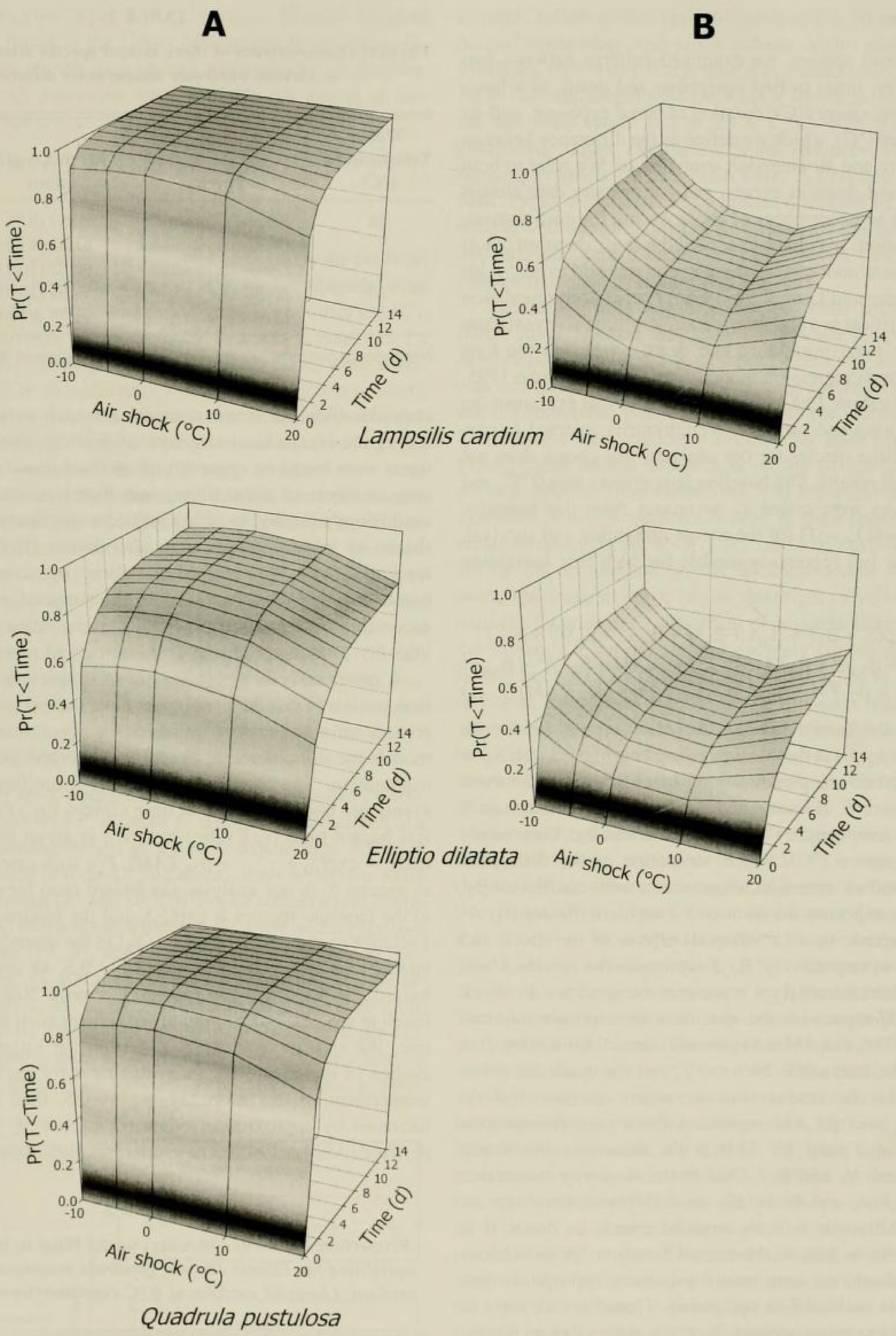

Figure 2. Estimated probabilities of times to first uprighting, T, were less than times on the axis marked Time for *L. cardium*, *E. dilatata*, and *Q. pustulosa* at five air shock temperatures obtained from proportional hazards model (Table 2). Air shock was defined as the difference between water and air emersion temperatures. Letters A and B refer to the 25 and 10 °C water exposure treatments, respectively.

plicated models having significant interactions. For example, from Eq. (1) the hazard ratio for species 1 to species 2 at water temperature T, air shock temperature A, and exposure duration M is given by $\exp[S_1 - S_2 + (\beta_{1,1} - \beta_{1,2})T + (\beta_{2,1} - \beta_{2,2})A + (\beta_{3,1} - \beta_{3,2})M + (\beta_{4,1} - \beta_{4,2})A^2 + (\beta_{6,1} - \beta_{6,2})TA + (\beta_{7,1} - \beta_{7,2})TM + (\beta_{8,1} - \beta_{8,2})AM + (\beta_{11,1} - \beta_{11,2})TAM]$.

Although hazard ratios have natural interpretations that provide the means to assess the relative importance of the explanatory variables, we display model features using graphs of the "survivor" functions (Hosmer and Lemeshow 1999), which are probabilities that times until events exceed some time t. We computed product-limit survival estimates (Kalbfleisch and Prentice 1980) of the survivor function and used those to display model features for both first uprightings and deaths.

RESULTS

Physiochemical Characteristics of Water

Temperature, dissolved oxygen (Yellow Springs Instrument Model 58 oxygen meter), and pH (Beckman Model Φ11 meter) were measured daily in each tank during each test. Averages and standard deviations (SD, in parentheses) for physiochemical characteristics of water in all tanks at each water temperature (25 and 10 °C, respectively) were as follows: temperature 24.5 °C (0.8), 10.1 (0.4); dissolved oxygen 8.2 mg/L (0.4), 11.5 mg/L (0.6); pH 8.10 (0.06), 8.13 (0.09). Un-ionized ammonia concentrations (mg/L) were measured in six randomly selected tanks for each test (range, 0.0013 to 0.0054 mg/L), and were well below the concentrations reported to adversely affect mussel growth (0.036 mg/L at 6 weeks, Sparks and Sandusky 1981) or survival (96 h LC50 = 1.1 mg/L, Arthur et al. 1987). The mean relative humidity over all five tests during aerial exposures was 63.6 (2.19).

Mussel Characteristics

The average length and wet weight of mussels were similar within a species between water temperatures (Table 1). The sex ratios (male:female) of *E. dilatata* and *Q. pustulosa* in the 25 °C treatment were similar (66 male:67 female and 74 male:65 female, respectively); however, the sex ratio for *L. cardium* was approximately 2:1 males to females (209 male:111 female) in the 25 °C water treatment and approximately 3:1 males to females (239 male:80 male) in the 10 °C water treatment.

Uprighting Behavior

The intensity of first uprighting differed among species, water temperatures, and air shock temperatures in a complex way involving multiple interactions (Wald $\chi^2 = 633.2$, $df = 8$, $P < 0.01$). There was a significant interaction between *E. dilatata* versus the other species and water temperature ($P < 0.01$; Table 2). Both water temperature and air shock temperature were important predictors of times to first uprighting. As expected, the intensity of uprighting was greater at the higher water temperature. Moreover, the effects of air shock differed with water temperature and showed a significant quadratic response (∪ or ∩-shaped, Fig. 2). At the 10 °C water temperature, the uprighting response was ∪-shaped; whereas, at the 25 °C water temperature, the response was ∩-shaped. Although air exposure duration had no statistically significant effect, it is important to note that, by definition, any response to air shock temperature requires exposure. In this experiment, the briefest air shock duration was apparently sufficient to affect uprighting intensity, and longer durations showed no additional effect.

In addition to uprighting, we also observed other behavioral responses to emersion. Shell gaping behavior was observed in *L. cardium* during emersion in >25 °C air for 30 min and in *E. dilatata* during emersion in 45 °C air for 15 min. Also, the occurrence of foot extension increased with emersion time in *E. dilatata* at 45 °C (~70% at 15 min duration to ~100% in the 60-min exposure duration). All three species extruded mucus from the siphonal region after emersion in 45 °C air for 60 min.

Survival

Survival of mussels differed among species and with water temperature, air shock, and air exposure time in a complicated way involving both two- and three-way interactions (Wald $\chi^2 = 253.82$, $df = 14$, $P < 0.01$). Survival of *Q. pustulosa* did not differ significantly from *L. cardium*, the baseline species, at any water or air shock temperature, or with air exposure time (Table 3, Fig. 3). *Elliptio dilatata* differed significantly from *L. cardium* up through interactions with the linear and quadratic effect of air shock temperature, and the three-way interaction among species, water temperature, and air shock temperature (Table 3). The parameters (effects) for *E. dilatata* (S_1), air shock (A), squared air shock (A^2), and the *E. dilatata* × air shock ($S_1 \times A$), water temperature × air shock ($T \times A$), air shock × exposure duration ($A \times M$), *E. dilatata* × squared air shock ($S_1 \times A^2$), *E. dilatata* × water temperature × air shock ($S_1 \times T \times A$), and water temperature × air shock × exposure duration interactions ($T \times A \times M$) were significantly different from zero (Table 3). Through the last day of the experiment, survival varied only slightly except for the *E. dilatata* in the 25 °C water temperature treatment that were exposed to large positive air shocks (Fig. 3). For *E. dilatata* in the 25 °C water treatment, survival probabilities decreased significantly in the 60-min air exposure duration treatments (Fig. 4).

DISCUSSION

Over-all mussel survival after emersion was high (93%) and indicated that these mussel species are remarkably resistant to emersion and thermal shock. For example, in the 10 °C water tests, both *L. cardium* and *E. dilatata* survived the air shock treatments despite a 20 °C air–water differential and emersion in subzero air. However, variations in tolerances to water–air treatments were evident among species at the higher water temperature. *Elliptio dilatata* died within 24-h postemersion to the 45 °C air temperature treatment, with 100% mortality at the 60-min aerial exposure duration and 50% mortality at the 30-min duration. Surprisingly, the other two species survived the highest air shock treatment. Several studies have documented that mussel survival, during and after emersion, is directly related to relative humidity (Byrne and Mc-

TABLE 3.

Final fitted proportional hazards model (equation 1) for *Elliptio dilatata*, *Quadrula pustulosa*, and *Lampsilis cardium* survival data. *Lampsilis cardium* at 0 °C constitute baseline conditions.

Parameter (effect)	Estimate (SE)	Wald χ^2	*P*-Value
S_1 (species: *E. dilatata*)	−1.8979 (0.8667)	4.8	0.02
S_2 (species: *Q. pustulosa*)	− 0.0261 (0.4375)	<0.1	0.95
β_1 (water temperature T)	−0.0352 (0.0433)	0.7	0.42
β_2 (air shock A)	0.2702 (0.0731)	13.7	< 0.01
β_3 (exposure duration M)	0.0266 (0.0163)	2.6	0.10
β_4 (A^2)	−0.0034 (0.0018)	3.7	0.05
$\beta_{1,1}$ ($S_1 \times T$)	0.0253 (0.0471)	0.3	0.59
$\beta_{2,1}$ ($S_1 \times A$)	−0.3527 (0.0633)	31.0	<0.01
β_6 ($T \times A$)	−0.0148 (0.0034)	18.4	< 0.01
β_7 ($T \times M$)	− 0.0004 (0.0009)	0.2	0.63
β_8 ($A \times M$)	−0.0042 (0.0014)	9.4	<0.01
$\beta_{4,1}$ ($S_1 \times A^2$)	0.0126 (0.0029)	19.2	<0.01
$\beta_{6,1}$ ($S_1 \times T \times A$)	0.0164 (0.0032)	25.8	<0.01
β_{11} ($T \times A \times M$)	0.0003 (0.0001)	17.1	<0.01

See text for explanation of the model and parameters.

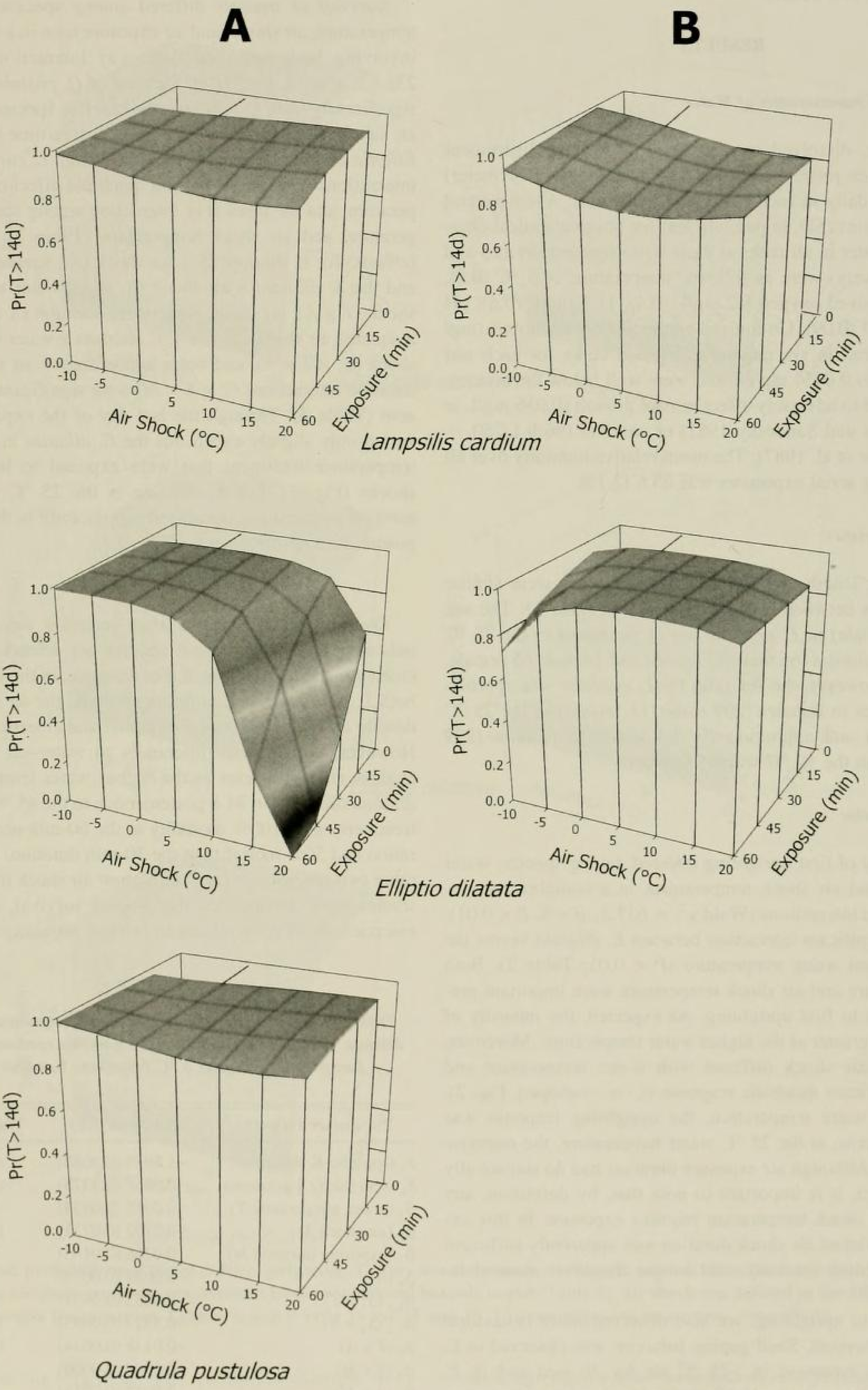

Figure 3. Estimated survival probabilities, T, were greater than times on the axis marked Time for *L. cardium*, *E. dilatata*, and *Q. pustulosa* at five air shock temperatures and three exposure durations obtained from proportional hazards model (Table 3). Air shock was defined as the difference between water and air emersion temperatures. Letters A and B refer to the 25 and 10 °C water exposure treatments, respectively.

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Rafmesque {2 tests), pimpleback Quadrula pusrulosa Lea (1 test), and spike Elliptio dilataia Rafmesque (2 tests) mussels, each

conducted in a completely randomized, nested experimental design. For each mussel species (except Q. pustulosa). treatments tested

included two water temperatures (25 and 10 °C). five air temperatures (ranging within ±20 °C of the water temperature), three aerial exposure durations (15, 30, and 60 min), and a no emersion control. All treatments were duplicated, with 10 organisms per emersion time and aerial exposure temperature ($n = 320$ mussels per test). Behavioral response (ability to upright) and mortality were measured daily for 14 d postemersion. Both water and aerial exposure temperature (air shock) were important predictors of times to first uprighting. The intensity function of first uprighting differed among species (/> < 0.01), and there was a significant interaction between E. dilatara versus the other species and water temperature ($P < 0.01$). Over-all mussel survival after emersion was high (939c); however, E. dilatata experienced significant treatment related monality at the 25 °C test water. 45 °C aerial exposure temperature. Because of the high incidence of uprighting and survival of mussels in our study, emersion at moderate temperatures (15 to 35 °C) and durations (15 to 60 min) does not seem harmful to mussels, and, therefore, conducting relocations and status surveys under these conditions should not impair mussel survival and over-all success.

KEY WORDS: Unionid mussel, conservation, emersion, temperature, behavior, mortality

INTRODUCTION

The imperiled status of unionid mussels (Williams et al. 1993) has prompted conservation efforts by public and private natural resource agencies that include status surveys, restocking, and relocation. The effects of collection and handling on mussels in field studies are generally considered benign and inconsequential to mussels relative to most threats (construction, zebra mussel infestation, habitat loss). However. Cope and Waller (1995) reviewed the success of relocation projects and found that mortality of mussels after relocation can be significant (>70% in 30% of projects reviewed). Mortality was highest within 1 year of the event, suggesting that effects of collection, handling, and displacement of mussels may be greater than were previously considered. The environmental conditions that mussels experience during collections and surveys may contribute to low survival, but can also be controlled to some extent. Determination of the emersion and thermal tolerances of unionid mussels would provide guidelines on the conditions in which surveys and relocations should occur to enhance mussel survival and over-all success.

Past studies suggest that mussels can tolerate emersion for hours or even days (Byrne and McMahon 1994. Dietz 1974, Holland 1991, Schanzle and Kruze 1994, Waller et al. 1995). However, survival of mussels is related to such environmental conditions during emersion as relative humidity and air temperature. For example. Waller et al. (1995) emersed Amblema plicata plicata Say and ObUquaria reflexa Rafmesque for a maximum of 8 h and

found that mussels had greater survival when handled during the fall (water temperature -15 °C: air temperatures ranged from 12 to 25 °C) compared to those handled during the spring (water temperature -23 °C; air temperature ranged from 18 to 29 °C). In the present laboratory study, we augment these data by evaluating a range of extreme air temperatures and water-air thermal differentials. We selected the minimum and maximum water and air temperature and emersion times based on conditions likely to be found in field collecting situations. In addition to survival, the uprighting behavior of mussels after emersion was selected as a potential indicator of emersion stress; presumably, the ability to upright and burrow into the substratum indicates normal functioning. Waller et al. (1999) found significant species and water temperature related differences in the uprighting and movement intensity of four mussel species after displacement. Thus, displacement, coupled with a thermal and emersion challenge, may also produce significant behavioral changes.

In this study, we evaluated the effects of emersion and temperature on the survival and behavior of three mussel species Lampsilis cardium Rafinesque (pocketbook). Quadrula pustulosa Lea (pimpleback), and Elliptio dilatata Rafinesque (spike), and examined the variation in survival and behavioral response within and among the three species. These mussel species represent two subfamilies (Lampsilinae and Ambleminae) and two contrasting life history strategies (long-term and short-term brooders) within the Unionidae. Additionally, L. cardium and Q. pustulosa served as surrogates for two U.S. Federally Endangered species, the L.

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higginsi Lea (Higgins'' eye) and Q. fragosa Conrad (winged mapleleaf). both found in the Upper Mississippi River basin. Elliptio dilatata was chosen as a second surrogate for Q. fragosa. because too few Q. pustulosa were available for testing at low (10 °C) water temperature.

MATERIALS AND METHODS

Test Organisms

Three species of unionid mussels were collected from the Wolf River at Shawano, Shawano County. Wisconsin. Mussels were transported in holding tanks, containing Wolf River water (25 °C), to the Upper Midwest Environmental Sciences Center, in La Crosse, Wisconsin. Holding tank water temperatures were maintained at 25 ± 3 °C (with addition of nonchlorinated ice as needed), and the dissolved oxygen concentration was maintained at >60% saturation with aeration. Water temperature and dissolved oxygen (Yellow Springs Instrument Model 58 oxygen meter) were measured at 1-h intervals. At the laboratory, mussels were placed into submerged cages held in the Black River (water temperature, 27 °C), near La Crosse, Wisconsin until study initiation. The mussel cages (122-em length x 122-cm wide x 46-cm height) were constructed of angle and strap iron frame with netting (1.9-cm diam. polyethylene) attached to the iron frame by tie wraps and nylon rope. One species of mussel (111 total; density of 75/m-) was placed into each cage. During collection, transport, and allocation to cages, mussels were continually immersed in river water.

Experimental Design and Exposure System

Five laboratory tests were performed with L cardiiim (2 tests), Q. pustulosa (1 test), and E. dilatata (2 tests), each conducted in a completely randomized design as a nested experiment. For each mussel species tested (except Q. pustulosa), there were two water temperature treatments (25 and 10 °C), five air temperatures (ranging within ± 20 °C of the water temperature), three aerial exposure duration treatments (15, 30, and 60 min), and a no emersion control treatment (Fig. 1). Because of limited availability, Q. pustulosa was tested only at 25 °C, the treatment we assumed to be more lethal. All treatments were duplicated, with 10 organisms per emersion time and temperature (n = 320 mussels/test), for a total of 32 experimental units. Ten mussels were placed into a flow-

Acclimation water temperature

25°C(10)

Emersion air temperature {"C)

15(0) 20(5) 25(10) 35(20) 45(30)

rr

Emersion duration (min)

Figure \. Experimental design for the thermal and emersion exposure tests with three species of unionids. The numbers in parentheses represent the 10 °C test water-aerial exposure regime.

through, stainless steel tank (61 -cm length x 30-cm wide x 36-cm height) containing sand (13 ± 0.5-cm depth) and 42 ± 0.5 L of overlying well water. Each tank was placed into one of six water baths (305-cm length x 84-cm wide x 46-cm height) maintained at the test temperature (10 or 25 ± 1 °C) with a thermostatically controlled, liquid circulation pump (Remcor Model CFF-501, Remcor Products Co., Franklin Park, IL) connected to the water bath. Tanks were aerated to maintain dissolved oxygen concentrations at >60% saturation. The flow rate of water into each tank was 200 mL/min with a turnover rate of seven times per day. The photoperiod was 16-h light and 8-h dark.

Laboratory tests began when the water temperature of the Black River reached the desired test water temperature (25 °C: July/August and 10 °C; November/December). Each mussel species was tested individually, and a given species was transported in coolers containing Black River water to the laboratory for testing. Ten mussels were randomly selected for each experimental unit. Plastic mesh netting was placed on top of the sand substrate to prevent mussels from burrowing into the substrate before aerial exposure. Mussels were acclimated in their respective tanks at a water temperature of 25 ± 1 °C for 2 d; mussels were not fed during the acclimation period. Mussels within a replicate were numerically marked (1 to 10) on their right valve with a permanent marker. To enable identification of mussels after burrowing into the substrate, each mussel was uniquely tagged with a numbered fishing bobber (3.81-mm dia.) that was attached to a 22.9-cm piece of cotton thread and secured to the umbonal region of the right valve with cyanoacrylate (Krazy Glue® Gel. Borden, Inc., Columbus, OH). Both siphons (incurrent and excurrent) remained immersed while bobbers were being attached to the shells; total handling time was less than 3 min per mussel.

For each treatment, 20 mussels (10 from each replicate) were removed from the test water (25 or 10 °C), transported in water (held at test temperature), and placed into an environmental chamber (Hotpack® Biological Chamber. Hotpack Corp.. Philadelphia, PA) at a given air temperature (25 °C water temperature; 15, 20, 25, 35, 45 °C air temperature; 10 °C water temperature; 0, 5, 10, 20, 30 "C air temperature) for a duration of 15. 30. or 60 min. Treatments were conducted in order of increasing air temperature and emersion duration. The target relative humidity in the environmental chambers was 60 ± 57c. This relative humidity was selected based on average ambient air conditions experienced in our geographic region (Steve Thompson. National Oceanic Atmospheric Administration. La Crosse. WI, pers. comm.). Following emersion, mussels were removed from the environmental chamber, transported in well water (held at the test temperature, 25 or 10 °C), and returned to their respective tanks. Each mussel was placed directly on top of the sand substrate, with the right valve (tagged side) facing upward. Test organisms were fed a mixture of C4 algae diet (Coast Seafoods Co., South Bend, WA; 0.2 niL per mussel) and dry Chorclla (0.013 g dr> weight per mussel) daily. Mussels were monitored for mortality and uprighting response for 14 d postemersion. At test termination (14 d postemersion), mussels were recovered from each tank and measured for total length and whole mussel wet weight. Sex of L. cardiwn was determined by shell dimorphism. Elliplio dilatutu and Q. pustulosa are not sexually dimorphic; thus, we examined histological sections of half of the mussels from each replicate in the 25 °C test to determine the sex ratio, and assumed animals tested at the 10 °C water temperature had a similar sex ratio, because all mussels came from the same population and were randomly sampled.

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Statistical Analyses

For each mussel species, we examined patterns between two response variables, times to first uprighting and death, as a function of water temperature (°C). duration of aerial exposure, and air shock temperature CO. which we define as the difference between w ater temperature and air emersion temperature. We refer to both first uprighting and death as events, and our primary data consist of elapsed times to occurrences of those events for each mussel. Some event durations may have exceeded the study duration (14 d) and, therefore, went unobserved; these events are said to be "right-censored" (Hosmer and Lemeshow 1999). Proper accommodation of censoring is critical to valid interpretation of time-to-event data.

For both events (first uprighting and death), we used the Cox proportional hazards regression model (Cox 1972. Newman 1995. Hosmer and Lemeshow 1999) to identify factors that explained the pattern in uprighting and survival. We arbitrarily selected L cardiiim as the baseline species for our analysis; this choice does not affect the over-all results. The ba.seline temperature was °C. and other temperatures were coded as deviations from this baseline. Denote E = U and E = D for the events uprighting and survival, respectively. Our full regression models for both first uprighting and survival are

\^,(,) = \£(,(r)exp(5, -t- P,r-H P^A + P,M + PjA- + (3,M- + P,,r
+ ^2.A + Ps.,-^ + Pe^A + ^-,TM + PsAM -I- P^rA" + ^^,A'
+ Pft',TA -I- pV.TM + ^sAM + ^uTAM + P, ..JAM). (1)

where; X^/r) is the hazard function for event type E for the /th species at time f; XfoW 's the corresponding baseline hazard; 5,/ = I. 2. are two fixed-effects parameters for identification of the three species (5, denotes E. dilatata. and S, denotes Q. piistiilosa): T represents water temperature (°C) with coefficient P,; A represents air shock temperature (°C). which we define as the difference between water and air emersion temperatures with coefficient p,; M represents air exposure duration (min) with coefficient p,; A' and M- are quadratic (u or n-shaped) effects of air shock and exposure duration, respectively; P|,r represents the species x water temperature interaction; P-,y4 represents the species x air shock interaction; P^.M represents the species x air-exposure duration interaction; TA. TM, and AM are two-way interactions among 7". A and M: TA' is the interaction between T. and the quadratic effect of A; P4.,A- is the interaction between species and A; Pf, ,TA. ^j,TM, and Pg/IM represent three-way interactions among species, A. T, and M: TAM is the three-way interaction between T.A. and M: and p,, ,7AM is the four-way interaction among species. 7". A, and M. In this model, "interactions" are on the log scale. Although in such terminal events as death, it is customary to refer to X(f) as the hazard function for deleterious events such as death, the term intensity is more appropriate than hazard for events such as first uprighting. Therefore, we refer to \(t) as either the hazard or intensity function, depending on whether we are addressing survival or first uprighting. respectively. We fitted Eq. (1) to the uprighting and survival data by maximizing the partial likelihood (Cox 1972). and constructed likelihood-ratio and Wald chi-square tests for each parameter (Hosmer and Lemeshow 1999) with the SAS PHREG software (SAS Institute 1997). For each event type, we began with our full regression model [Eq. (1)] and. one-by-one. deleted terms for which the corresponding likelihood-ratio chi-square test was not significant at the a = 0.05 level, except we did not delete terms for which a higher-order interaction was statistically significant. This model reduction pro-

TABLE 1.

Physical characteristics of three mussel species after aerial exposure at various water-air temperature differentials.

Numbers in parentheses are the standard deviation of the mean.

cess identifies the simplest model for each event type that preserves the hierarchical structure of Eq. (1). Our recorded event times were based on observations at fixed times rather than exact measurements of event times, and, therefore, contained ties. We used Efron's method to adjust for tied event times, which has been shown to perform better than alternatives (Hertz-Picciotto and Rockhill 1997). We assumed that events occurred at the observation time rather than, for example, the temporal midpoint between successive observations to produce conservative estimates of the intensity of first uprighting or the hazard of mortality.

A particularly desirable feature of proportional hazard regression models is that the parameters have natural interpretations that provide informative descriptions of the event times. Because these models are still unfamiliar in ecology, interpretation requires some explanation. The hazard function \{t) quantifies the number of events per interval of time at time /. From Eq. (I), the dimensionless hazard ratio (risk ratio) at time t is given by HR(r) = \,(r)/ \o(?) = exp[5, +   -^ P^jfAA/]. For such categorical variables as species S, in our analysis, the hazard ratio for species ; relative to the baseline species is exp(S,), and the hazard ratio for species 1 relative to species 2 is exp[S, - 5,] in the absence of higher-order interactions. If. for example. exp(S,) = 0.5, we say that the relative hazard (or intensity) for species / is only 50% of that for the baseline species. For continuous covariates such as water temperature, the statistic 100[exp(P|) - 1] is the estimated percentage change in the hazard (or intensity) ratio for each unit change in temperature. If, for mortality, P, = 0.5, then the hazard ratio increases by approximately 65% for each 1 °C increase in temperature (Allison 1995). These interpretations extend to more com-

TABLE 2.

Proportional hazards (intensity) model fitted to the time to first

uprighting for Elliptio dilatata, Quadrula puslulosa. and Lampsilis

cardium. Lampsilis cardium at C constitute baseline conditions.

See text for explanation of the model and parameters.

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B

""°'^n°g

Lampsilis cardium

'^^°c.,o,;

Elliptio dilatata Air s/,^

c<r^og

Air cA '"

Quadrula pustulosa

Figure 2. Estimated probabilities of times to first uprighting, T, were less than times on the axis marked Time for L. cardium, E. dilatata, and

Q. puslulnsa at five air shock temperatures obtained from proportional hazards model (Table 2). Air shock was defined as the difference between

water and air emersion temperatures. Letters A and B refer to the 25 and 10 C" water exposure treatments, respectively.

plicated models having significant interactions. For example, from

Eq. (1) the hazard ratio for species I to species 2 at water tem-

perature T. air shock temperature A, and exposure duration M is

given by expl.V, - S, + (P, , - (3, ,)r + (3,., - (3, ,)/\ + O, , -

P,.,)M + (P, , - p^,)A- + (p^, - 3^,)rA + (P, , - <^,2)TM +

(ps., -P«.,)/IW + (P,, , -P,,,)7-/1M|.

Although hazard ratios have natural interpretations that provide

the means to assess the relative importance of the explanatory

variables, we display model features using graphs of the "survivor"

functions (Hosnicr and Lemcshow 1999). which are probabilities

that times until events exceed some time /. We computed product-

limit survival estimates (Kalbtleisch and Prentice 1980) of the

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survivor function and used those to display model features for both first uprightings and deaths.

RESULTS

Physiochemical Characteristics of Water

Temperature, dissolved oxygen (Yellow Springs Instrument Model 58 oxygen meter), and pH (Beckman Model Oil meter) were measured daily in each tank during each test. Averages and standard deviations (SD, in parentheses) for physiochemical characteristics of water in all tanks at each water temperature (25 and 10 "C. respectively) were as follows: temperature 24.5 °C (0.8), 10.1 (0.4); dissolved oxygen 8.2 mg/L (0.4), 1 1.5 mg/L (0.6); pH 8.10 (0.06), 8.13 (0.09). Un-ionized ammonia concentrations (mg/L) were measured in six randomly selected tanks for each test (range. 0.0013 to 0.0054 mg/L). and were well below the concentrations reported to adversely affect mussel growth (0.036 mg/L at 6 weeks, Sparks and Sandusky 1981) or survival (96 h LC50 = 1.1 mg/L, Arthur et al. 1987). The mean relative humidity over all five tests during aerial exposures was 63.6 (2.19).

Mussel Characteristics

The average length and wet weight of mussels were similar within a species between water temperatures (Table 1). The sex ratios (male:female) of E. dilatata and Q. pustulosa in the 25 °C treatment were similar (66 male:67 female and 74 male:65 female, respectively); however, the sex ratio for L. cardium was approximately 2:1 males to females (209 male: 1 1 1 female) in the 25 °C water treatment and approximately 3:1 males to females (239 male:80 male) in the 10 °C water treatment.

Uprighting Behavior

The intensity of first uprighting differed among species, water temperatures, and air shock temperatures in a complex way involving multiple interactions (Wald x" = 633.2, <//= 8, P < 0.01). There was a significant interaction between E. dilatata versus the other species and water temperature (P < 0.01; Table 2). Both water temperature and air shock temperature were important predictors of times to first uprighting. As expected, the intensity of uprighting was greater at the higher water temperature. Moreover. the effects of air shock differed with water temperature and showed a significant quadratic response (u or n-shaped. Fig. 2). At the 10 °C water temperature, the uprighting response was U-shaped; whereas, at the 25 °C water temperature, the response was n-shaped. Although air exposure duration had no statistically significant effect, it is important to note that, by definition, any response to air shock temperature requires exposure. In this experiment, the briefest air shock duration was apparently sufficient to affect uprighting intensity, and longer durations showed no additional effect.

In addition to uprighting, we also observed other behavioral responses to emersion. Shell gaping behavior was observed in L. cardium during emersion in >25 °C air for 30 min and in E. dilatata during emersion in 45 °C air for 15 min. Also, the occurrence of foot extension increased with emersion time in £. dilatata at 45 °C (-70% at 15 min duration to -100% in the 60-min exposure duration). All three species extruded mucus from the siphonal region after emersion in 45 °C air for 60 min.

Sun'ival

Survival of mussels differed among species and with water temperature, air shock, and air exposure time in a complicated way involving both two- and three-way interactions (Wald x'' = 253.82, df= 14, P < 0.01). Survival of Q. pustulosa did not differ significantly from L. cardium, the baseline species, at any water or air shock temperature, or with air exposure time (Table 3. Fig. 3). Eliiplio dilatata differed significantly from L. cardium up through interactions with the linear and quadratic effect of air shock temperature, and the three-way interaction among species, water temperature, and air shock temperature (Table 3). The parameters (effects) for E. dilatata (S,). air shock (A), squared air shock (A''). and the E. dilatata x air shock (S, x A), water temperature x air shock (T X A), air shock x exposure duration (A x M), E. dilatata X squared air shock (S, x A''), E. dilatata x water temperature x air shock (5, x r X A), and water temperature x air shock x exposure duration interactions (7'' x A x M) were significantly different from zero (Table 3). Through the last day of the experiment, survival varied only slightly except for the £. dilatata in the 25 °C water temperature treatment that were exposed to large positive air shocks (Fig. 3). For £. dilatata in the 25 °C water treatment, survival probabilities decreased significantly in the 60-min air exposure duration treatments (Fig. 4).

DISCUSSION

Over-all mussel survival after emersion was high (93%) and indicated that these mussel species are remarkably resistant to emersion and thermal shock. For example, in the 10 °C water tests, both L. cardium and £. dilatata survived the air shock treatments despite a 20 °C air-water differential and emersion in subzero air. However, variations in tolerances to water-air treatments were evident among species at the higher water temperature. Elliptio dilatata died within 24-h postemersion to the 45 °C air temperature treatment, with 100% mortality at the 60-min aerial exposure duration and 50%' mortality at the 30-min duration. Surprisingly, the other two species survived the highest air shock treatment. Several studies have documented that mussel survival, during and after emersion, is directly related to relative humidity (Byrne and Mc-

TABLE 3.

Final fitted proportional hazards model (equation 1) for Elliptio

dilatata, Quadrula pustulosa, and Lampsilis cardium survival data.

Lampsilis cardium at °C constitute baseline conditions.

See text for explanation of the model and parameters.

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Lampsilis cardium (Q

Elliptio dilatata

'°"^(°C)

Quadrula pustulosa

Figure 3. Kstimatcd survival probiihilitii's. T, were greater than times on the axis marked Time for /,. cardium. K. dilatata. and Q. pustulosa at

five air shocl\ temperatures and three exposure durations obtained from proportional hazards model (Table 3|. Air shock was defined as the difference between water and air emersion temperatures. Letters A and B refer to the 25 and III C water exposure treatments, respectively.