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Tamara J. Pandolfo

W. Gregory Cope

Consuelo Arellano

Robert B. Bringolf

M. Christopher Barnhart
Missouri State University

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Recommended Citation

Pandolfo, Tamara J., W. Gregory Cope, Consuelo Arellano, Robert B. Bringolf, M. Christopher Barnhart, and Edward Hammer. "Upper thermal tolerances of early life stages of freshwater mussels." *Journal of the North American Benthological Society* 29, no. 3 (2010): 959-969.

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Upper thermal tolerances of early life stages of freshwater mussels

Tamara J. Pandolfo^{1,6}, W. Gregory Cope^{1,7}, Consuelo Arellano^{2,8}, Robert B. Bringolf^{3,9}, M. Christopher Barnhart^{4,10}, AND Edward Hammer^{5,11}

¹Department of Environmental and Molecular Toxicology, North Carolina State University, Campus Box 7633, Raleigh, North Carolina 27695 USA

²Department of Statistics, North Carolina State University, Raleigh, North Carolina 27695 USA

³Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602 USA

⁴Department of Biology, Missouri State University, Springfield, Missouri 65897 USA

⁵US Environmental Protection Agency, Chicago, Illinois 60604 USA

Abstract. Freshwater mussels (order Unioniformes) fulfill an essential role in benthic aquatic communities, but also are among the most sensitive and rapidly declining faunal groups in North America. Rising water temperatures, caused by global climate change, industrial discharges, drought, or land development, could further challenge imperiled unionid communities. The aim of our study was to determine the upper thermal tolerances of the larval (glochidia) and juvenile life stages of freshwater mussels. Glochidia of 8 species of mussels were tested: *Lampsilis siliquoidea*, *Potamilus alatus*, *Ligumia recta*, *Ellipsaria lineolata*, *Lasmigona complanata*, *Megaloniais nervosa*, *Alasmidonta varicosa*, and *Villosa delumbis*. Seven of these species also were tested as juveniles. Survival trends were monitored while mussels held at 3 acclimation temperatures (17, 22, and 27°C) were exposed to a range of common and extreme water temperatures (20–42°C) in standard acute laboratory tests. The average median lethal temperature (LT50) among species in 24-h tests with glochidia was 31.6°C and ranged from 21.4 to 42.7°C. The mean LT50 in 96-h juvenile tests was 34.7°C and ranged from 32.5 to 38.8°C. Based on comparisons of LT50s, thermal tolerances differed among species for glochidia, but not for juveniles. Acclimation temperature did not affect thermal tolerance for either life stage. Our results indicate that freshwater mussels already might be living close to their upper thermal tolerances in some systems and, thus, might be at risk from rising environmental temperatures.

Key words: freshwater mussel, Unionidae, glochidia, juvenile, temperature, thermal tolerance, LT50, LT05.

Thermal regimes of freshwater environments can be altered by a variety of anthropogenic impacts, including climate change, landuse change, and thermal effluents from industry (Kinouchi et al. 2007, Encina et al. 2008). The effects of altered thermal regimes on fish have been elucidated (e.g., Eaton and Scheller 1996, Beitinger et al. 2000, Daufresne et al. 2003, Mohseni et al. 2003), but the effects of temperature on bivalves are less studied. The effects of temperature on the development, release, and viability of the larval life stage (glochidia) of fresh-

water mussels of the bivalve order Unioniformes have been investigated (Roberts and Barnhart 1999, Jansen et al. 2001, Zimmerman and Neves 2002, Akiyama and Iwakuma 2007, Cope et al. 2008), but a data gap exists in the determination of acute lethal temperatures for the early life stages (Dimock and Wright 1993). Determination of the upper thermal limits of mussels is vital because changes in extreme temperatures, resulting from higher summer maximum temperatures induced by global climate change, thermal effluent discharges, or droughts, are more likely to be ecologically detrimental than gradual warming (Hastie et al. 2003, Mouthon and Daufresne 2006). Mussel population declines are most strongly associated with atypical conditions that extend beyond their normal tolerances (Golladay et al. 2004), and acute upper thermal limits might provide an indication of the extreme temperatures that mussels can tolerate in such circumstances.

⁶ E-mail addresses: tjpandol@ncsu.edu

⁷ greg_cope@ncsu.edu

⁸ arellano@stat.ncsu.edu

⁹ rbringolf@warnell.uga.edu

¹⁰ chrisbarnhart@missouristate.edu

¹¹ hammer.edward@epa.gov

Mussels are among the most sensitive and rapidly declining faunal groups in North America and elsewhere in the world, and rising ambient temperatures and exposure to extreme thermal events could pose additional risks to threatened mussel species (Hastie et al. 2003). Of the ~300 freshwater mussel species native to North America, nearly 70% are extinct or vulnerable to extinction (Bogan 1993, Williams et al. 1993). This decline has been broadly attributed to pollution, water-quality degradation, and habitat destruction from anthropogenic influences. Specific causes of mussel declines are generally unknown but chronic, low-level stressors are presumed (Strayer et al. 2004, Cope et al. 2008). In addition, the unique life cycle of freshwater mussels makes them particularly susceptible to disruption by environmental stressors, such as temperature. Larval mussels (glochidia) infest the gills or fins of host fish as parasites before transforming into the juvenile life stage and dropping to the sediment to continue their development as benthic-dwelling adults (e.g., Watters 2007).

Climate change might put mussels closer to their thermal limits, and additional heat inputs from thermal discharges, drought, or landuse changes could further alter the thermal environment of these sessile organisms. In the midwestern US, summer water temperatures can exceed 30°C (Otero-Benitez and Davis 2009), and temperatures in the southern US can reach 34 to 40°C (Dyar and Alhadeff 1997, Spooner and Vaughn 2008). To address thermal effects on larval and juvenile mussels, we determined the upper thermal tolerances for glochidia of 8 species and juveniles of 7 species representing 3 tribes of the Unionidae family (Graf and Cummings 2007). Mussels were held at 3 acclimation temperatures and tested over a range of experimental temperatures from 20 to 42°C. These temperatures encompass the upper range of common and extreme temperatures encountered in rivers and streams in the US during the summer.

Methods

Test organisms

Eight species representing 3 tribes (Lampsilini, Anodontini, and Quadrulini) of the Unionidae family were used in our study (Graf and Cummings 2007): *Lampsilis siliquoidea* (Barnes), *Potamilus alatus* (Say), *Ligumia recta* (Lamarck), *Ellipsaria lineolata* (Rafinesque), *Villosa delumbis* (Conrad), *Megaloniais nervosa* (Rafinesque), *Lasmigona complanata* (Barnes), and *Alasmidonta varicosa* (Lamarck). All test organisms were propagated via host-fish infection in facilities at Missouri State University and North Carolina State

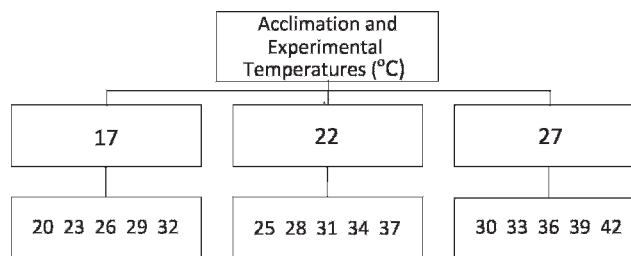


FIG. 1. Experimental design showing acclimation (20, 22, and 27°C) and experimental temperature schemes for freshwater mussel tests. A nonacclimated 20°C control was included with each test.

University with standard propagation and culture methods (Barnhart 2006).

Test conditions

Glochidia and juvenile mussels were acclimated to 3 different temperatures (17°C, 22°C, and 27°C) and tested at 6 experimental temperatures (the acclimation temperature and 5 additional temperatures increased by 3°C increments) (Fig. 1). Unacclimated individuals maintained at 20°C (unacclimated control) were assessed side-by-side with individuals in experimental temperature treatments within each acclimation temperature. Glochidia were <24 h old at the start of each test. Glochidia were acclimated by adjusting their shipping temperature upon arrival by 1°C/h, with a 2-h acclimation period once the target temperature was reached. From January through April, shipping temperatures averaged 17°C ($\pm 3^\circ\text{C}$), and from May through July shipping temperatures averaged 22°C ($\pm 3^\circ\text{C}$). Tests were 24-h nonaerated static experiments done in reconstituted hard water according to the American Society of Testing and Materials (ASTM) guidelines for glochidia (ASTM 2006a, b). Survival was assessed at 24 h for a subsample of ~50 of the 150 glochidia in each of 3 replicates per temperature. A saturated NaCl solution was used to stimulate a shell-closure response that was observed with an Olympus SZ61 microscope (Olympus America, Center Valley, Pennsylvania) and QCapture Pro 5.1 digital photographic software (Quantitative Imaging Corporation, Burnaby, British Columbia, Canada).

Juveniles of 7 mussel species were used to evaluate thermal sensitivity. Because of limited availability, *L. complanata* was omitted from juvenile testing, and *A. varicosa* was not tested at the 17°C acclimation temperature. *Lampsilis siliquoidea*, *P. alatus*, and *L. recta* individuals ranged in age from 3 to 8 wk. Shell lengths were 1386 μm for *L. siliquoidea*, 1377 μm for *P. alatus*, and 947 μm for *L. recta*. Individuals of the

remaining species (*E. lineolata*, *M. nervosa*, *A. varicosa*, and *V. delumbis*) ranged in age from <1 to 4 wk. Shell lengths were 335 μm for *E. lineolata*, 364 μm for *M. nervosa*, 398 μm for *A. varicosa*, and 363 μm for *V. delumbis*. Individuals within a species differed in age by 1 to 3 wk at most.

Juveniles were acclimated to the test acclimation temperature by adjusting their shipping temperature upon arrival by 2.5°C/d, with a ≥ 24 h acclimation period once the target temperature was attained. Experiments were 96-h nonaerated static renewal tests with 90% reconstituted hard water renewal at 48 h. Tests were conducted according to ASTM guidelines for juveniles (ASTM 2006b). Survival was assessed visually with an Olympus SZ61 microscope to detect foot movement outside of the shell, foot movement within the shell, or the presence of a heart beat for the 7 mussels in each of 3 replicates per temperature. Controls had 10 mussels in each replicate.

Quality assurance and control were ensured by conducting all tests according to the Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (ASTM 2006b). Glochidial survival differed among species at the common control temperature (20°C). Therefore, control survival was deemed acceptable for a species if it did not decrease substantially from initial survival at the start of the test (average decline in control survival from initial survival upon arrival in laboratory until 24-h assessment was 5.3%, range 0–15.6%, $n = 24$ tests). Tests were conducted in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Electron Corp., Marietta, Ohio, and Isotemp Model 146E, Fisher Scientific, Dubuque, Iowa). National Institute of Standards and Technology (NIST)-certified thermometers were used for daily temperature monitoring. Target test temperatures were $\pm 1^\circ\text{C}$ ($n = 866$) for 98.6% of trials, with a maximum departure of 2°C. Mean water-quality conditions across all tests were: 103.9 mg CaCO₃/L alkalinity, 149.6 mg CaCO₃/L hardness, 564.2 $\mu\text{S}/\text{cm}$ conductivity, 8.44 pH, and 7.28 mg/L dissolved O₂ ($n = 27$ for alkalinity and hardness, $n = 223$ for all other variables).

Statistical analysis

The effects of temperature treatments on mussels were analyzed with SAS Proc Mixed (version 9.1.3; SAS Institute Inc., Cary, North Carolina). The proportion of individuals that survived (p_{surv}) was arcsin(x)-transformed before analysis. Significant temperature treatment effects ($p < 0.05$) were further analyzed through a pairwise comparison of differences in survival among the 20°C nonacclimated control and

experimental temperatures within an acclimation temperature using Tukey's post hoc test.

The LT50 was defined as the temperature that caused mortality in 50% of the exposed population, and the LT05 was the temperature that caused mortality in 5% of the exposed population. Survival data were used to generate LT50s and LT05s with logistic regression (Agresti 1996). p_{surv} was analyzed as the response variable, and species, acclimation temperature, and experimental temperature were the independent variables. The relationship between the experimental temperature and p_{surv} for a particular species at a given acclimation temperature (22 or 27°C) was analyzed with a generalized linear model, assuming that p_{surv} followed a Bernoulli distribution, and its logit was a linear function of the experimental temperatures. Differences between LT50 and LT05 values for both acclimation temperatures were analyzed for each species with a fixed-effect model that included acclimation temperature as a class variable and experimental temperature as a continuous variable. Survival curves and values of LT50 and LT05 for each curve and their differences were calculated with the SAS procedure NLMIXED.

Results

Glochidia

At the 17°C acclimation temperature, only *V. delumbis* were adversely affected by experimental temperatures (Fig. 2A). Survival at 32°C was significantly lower than survival at all other experimental temperatures and the 20°C control ($p < 0.0001$).

At the 22°C acclimation temperature, survival of *L. siliquoidea* was similar at all experimental temperatures and the 20°C control (all $p > 0.05$; Fig. 3A). Survival of *L. complanata* and *A. varicosa* was significantly lower at 37°C than at all other experimental temperatures and in the 20°C control ($p < 0.0001$). *Ellipsaria lineolata* survival was significantly lower at 34°C and 37°C than at all other experimental temperatures and in the 20°C control ($p < 0.0001$), and survival of *M. nervosa* was significantly lower at 31, 34, and 37°C than in all other experimental temperatures and in the 20°C control (all $p < 0.0001$, except *M. nervosa* 31°C $p = 0.0132$). *Ligumia recta* survival was significantly lower at 31°C ($p = 0.0160$) than at 22 and 28°C and in the 20°C control and lower at 37°C ($p < 0.0001$) than at 22, 25, and 28°C and in the 20°C control, and *P. alatus* survival was lower at 28 ($p = 0.0003$), 31, 34, and 37°C (all $p < 0.0001$) than in the 20°C control. *Villosa delumbis* survival was significantly lower at 31°C ($p < 0.0001$) than in the 20°C control and lower at 34 and 37°C (all $p < 0.0001$) than at 22, 25, and 28°C and in the 20°C control.

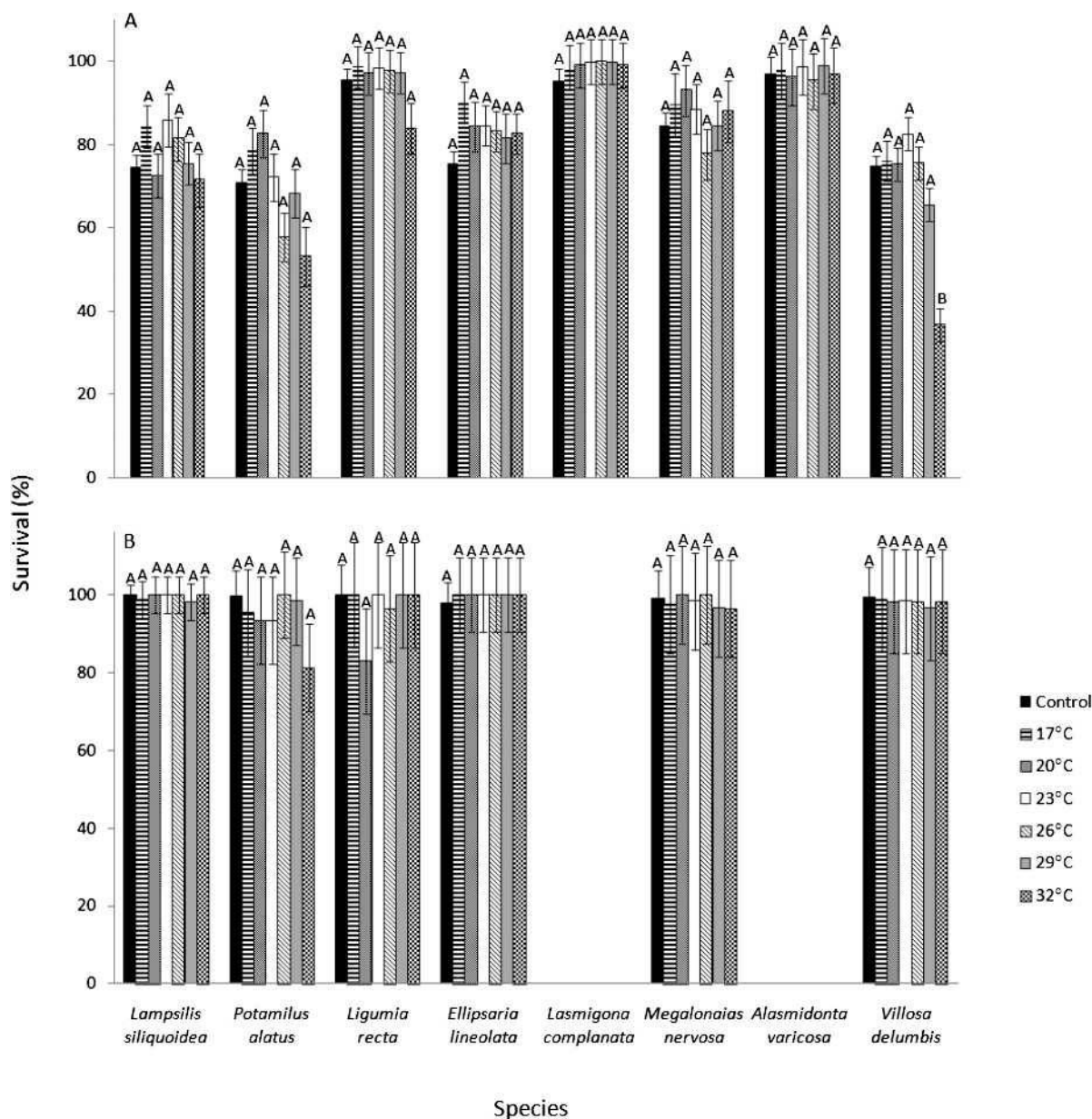


FIG. 2. Mean (± 1 SE, $n = 3$) survival of glochidia of 8 species (A) and juveniles of 6 species (B) of freshwater mussels held at an acclimation temperature of 17°C and subjected to 6 experimental temperatures (17, 20, 23, 26, 29, and 32°C) or held in a nonacclimated 20°C control. Bars representing temperature treatments within a species with the same uppercase letters are not significantly different ($p > 0.05$).

At the 27°C acclimation temperature (Fig. 4A), *L. siliquoidea* and *L. complanata* had significantly lower survival at 39°C and 42°C than at any other experimental temperature and in the 20°C control ($p < 0.0001$). *Alasmidonta varicosa* survival was significantly lower at 36°C ($p = 0.0046$) than in the 20°C control and lower at 39 and 42°C ($p < 0.0001$) than in all other experimental temperatures and in the 20°C control. Survival of *L. recta*, was significantly lower at

33 and 36°C than in the 20°C control or at 27 and 30°C and lower at 39 and 42°C than in all other experimental temperatures and the 20°C control (all $p < 0.0001$). *Megaloniais nervosa* survival was significantly lower at 27°C ($p = 0.0128$) and 33°C than in the 20°C control and was lower at 36, 39, and 42°C (all $p < 0.0001$) than at all other experimental temperatures and in the 20°C control. Survival of *V. delumbis* was significantly lower at all temperatures except 30°C

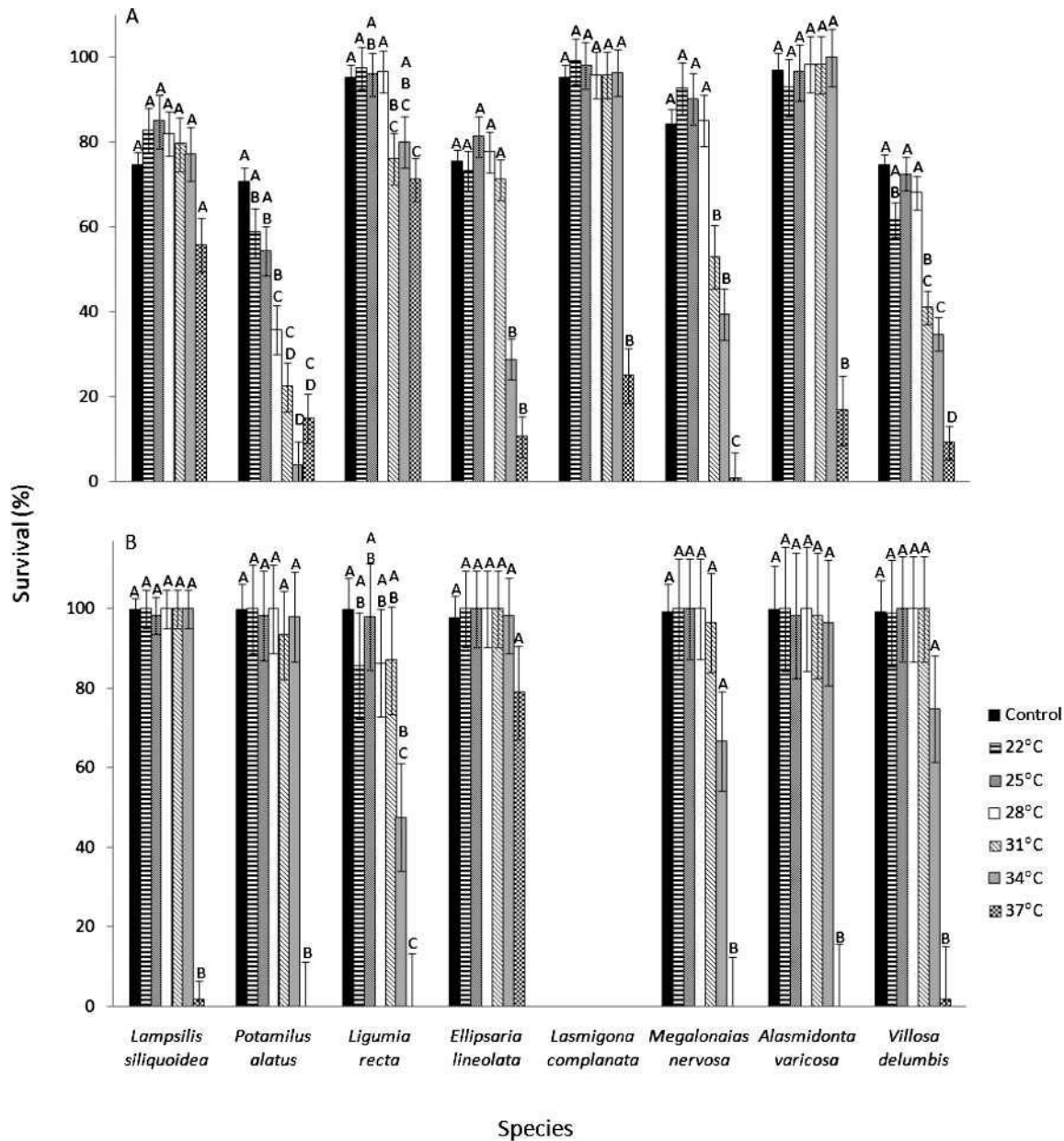


FIG. 3. Mean (± 1 SE, $n = 3$) survival of glochidia of 8 species (A) and juveniles of 6 species (B) of freshwater mussels held at an acclimation temperature of 22°C and subjected to 6 experimental temperatures (22, 25, 28, 31, 34, and 37°C) or held in a nonacclimated 20°C control. Bars representing temperature treatments within a species with the same uppercase letters are not significantly different ($p > 0.05$).

than in the 20°C control ($p < 0.0001$) and lower at 39°C and 42°C than in all other experimental temperatures (all $p < 0.0001$). *Potamilus alatus* and *E. lineolata* were the most thermally sensitive species, with survival significantly lower at all experimental temperatures than in the 20°C control ($p < 0.0001$). Significant decreases in survival relative to in the 20°C control at the 27°C acclimation temperature and not at the 17 or 22°C acclimation temperatures for *P. alatus*, *E. line-*

olata, *M. nervosa*, and *V. delumbis* were not indicative of an acclimation effect. The 27°C temperature caused significant mortality in these 4 species because, unlike the other 2 temperatures, it was essentially a temperature treatment rather than an acclimation temperature.

Alasmidonta varicosa generally re-opened ~1 min after initial shell closure when NaCl was added. For this reason, *A. varicosa* is not recommended as a

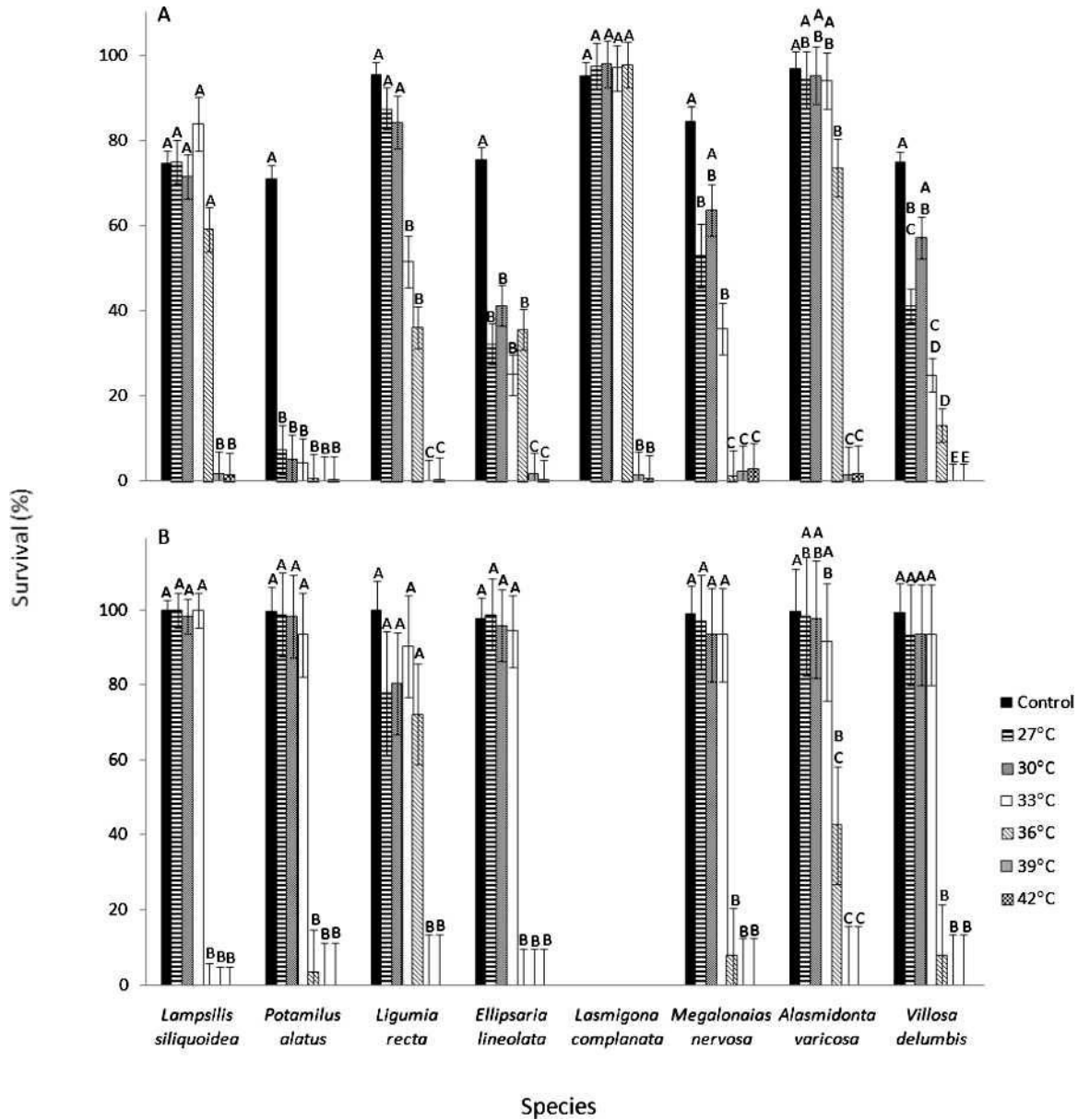


FIG. 4. Mean (± 1 SE, $n = 3$) survival of glochidia of 8 species (A) and juveniles of 6 species (B) of freshwater mussels held at an acclimation temperature of 27°C and subjected to 6 experimental temperatures (27, 30, 33, 36, 39, and 42°C) or held in a nonacclimated 20°C control. Bars representing temperature treatments within a species with the same uppercase letters are not significantly different ($p > 0.05$).

model species in laboratory testing with glochidia unless photographs can be taken immediately after NaCl addition.

Juveniles

At the 17°C acclimation temperature, survival of all species was similar among all experimental temperatures ($p > 0.05$; Fig. 2B). At the 22°C acclimation temperature, survival of *E. lineolata* did not differ

significantly among experimental temperatures ($p > 0.05$; Fig. 3B). Survival of *L. siliquoidea*, *P. alatus*, *M. nervosa*, *A. varicosa*, and *V. delumbis* was significantly lower at 37°C than at any other experimental temperature and the 20°C control ($p < 0.0001$ for all species), and survival was similar at all other experimental temperatures. Survival of *L. recta* was significantly lower at 34°C ($p = 0.0020$) than in the 20°C control and lower at 37°C (all $p < 0.0001$) than at 22, 25, 28, and 31°C and in the 20°C control.

At the 27°C acclimation temperature, mortality for all species was 100% at 39 and 42°C ($p < 0.0001$ for all species; Fig. 4B). Survival of *L. recta* did not differ among all other experimental temperatures or in the 20°C control, whereas survival of *L. siliquoidea*, *P. alatus*, *E. lineolata*, *M. nervosa*, and *V. delumbis* also was significantly lower at 36°C than at lower experimental temperatures and in the 20°C control ($p < 0.0001$ for all species). *Alasmidonta varicosa* survival was lower at 36°C ($p = 0.0120$) than in the 20°C control, but similar to survival at 27, 30, and 33°C (all $p > 0.05$).

Thermal tolerance

LT50s for glochidia (24 h) and juvenile (96 h) freshwater mussels were calculated for the 22 and 27°C acclimation temperatures (Table 1) but not for the 17°C acclimation temperature because of lack of sufficient mortalities. Overall mean LT50s ranged from 21.4 to 42.6°C with a mean of 33.1°C. Glochidial LT50s ranged from 21.4 to 42.6°C with a mean of 31.6°C. No differences in thermal tolerance were associated with change in acclimation temperature, but thermal tolerances did differ among some species (Table 1). At the 22°C acclimation temperature, *P. alatus* had a significantly lower LT50 than *L. complanata* and *A. varicosa*. At the 27°C acclimation temperature, *P. alatus* had a significantly lower LT50 than *L. siliquoidea* and *L. recta*. Juvenile LT50s ranged from 32.5 to 38.8°C with a mean of 34.7°C. No changes in thermal tolerance were associated with acclimation temperature, and juvenile LT50s did not differ among species. LT50s differed between glochidia and juveniles for *P. alatus*, and juveniles were significantly more thermally tolerant than glochidia at the 22°C ($p = 0.0029$) and 27°C ($p = 0.0004$) acclimation temperatures. Juvenile *V. delumbis* were more thermally tolerant than *V. delumbis* glochidia at the 27°C acclimation temperature ($p = 0.0334$). Thermal tolerances did not differ between life stages for any other species ($p > 0.05$).

Overall LT05s for glochidia (24 h) and juvenile mussels (96 h) at the 22 and 27°C acclimation temperatures ranged from 15.6 to 34.1°C with a mean of 27.8°C (Table 1). Glochidia LT05s ranged from 15.6 to 30.3°C with a mean of 25.0°C. Juvenile LT05s ranged from 23.7 to 34.1°C with a mean of 29.4°C. At the 22°C acclimation temperature, LT05s differed between juveniles of *L. recta* and *E. lineolata*, but tolerances did not differ between glochidia or juveniles of any other species (Table 1). No changes in LT05s were associated with acclimation temperature for glochidia or juveniles. For the 3 species with LT05 data, values did not differ between life stages

(all $p > 0.05$). The average difference between LT50 and LT05 was 10.6°C (6.8–19.1°C) for glochidia and 5.3°C (1.9–8.8°C) for juveniles within a species. Thus, a temperature increase of 10.6°C theoretically could reduce the survival of an average population of glochidia from 95% to only 50% survival, and a temperature increase of only 5.3°C could have the same consequence in an average population of juveniles.

Discussion

We are the first to report acute lethal thermal tolerances for the early life stages of a range of freshwater mussel species. Our results show that small increases in temperature can lead to significant reductions in survival of freshwater mussels. Dimock and Wright (1993) reported a 96-h LT50 of 31.5°C for 1-wk-old juvenile *Utterbackia imbecillis* (Say) and 33°C for 1-wk-old *Pyganodon cataracta* (Say) in the only other published study of acute thermal tolerances of early life stages of freshwater mussels. These results are similar to the results for juveniles in our study.

Acclimation temperature did not affect thermal tolerance in freshwater mussels. The acclimation period that we used was longer and more conservative than the 3°C/h temperature change recommended in the ASTM mussel testing guide (ASTM 2006b), but it might have been too short to establish a true acclimation (Ansell et al. 1980a). In a review of thermal tolerance studies for 50 aquatic species, de Vries et al. (2008) reported that acclimation periods generally exceeded 96 h. Future tests with a longer acclimation period might help determine any latent effect of acclimation on thermal tolerance of juvenile mussels.

Freshwater mussel species respond uniquely to different thermal regimes, and these differences correspond with variable filtration and excretion rates. Therefore, changes in thermal regime that alter species composition can affect ecological processes (Spooner and Vaughn 2008, 2009, Vaughn et al. 2008). We found significant differences in glochidial LT50s among species, but no differences in juvenile LT50s among species. In contrast, we found differences in juvenile LT05s between 2 species, but no differences in glochidial LT05s among species. The relatively wide range of LT50s for glochidia might be attributable to the fact that survival of this life stage is variable among species, even within the range of thermal tolerance (Zimmerman and Neves 2002, Cope et al. 2008). Morphological characteristics and trait-based differences might play a role in the different thermal tolerances among adults of different species

TABLE 1. Experimental temperatures causing 50% (LT50) and 5% (LT05) mortality (with 95% confidence intervals) in glochidia (24 h) and juvenile (96 h) mussels at 22 and 27°C acclimation temperatures. LT50 or LT05 values among species within a life stage and acclimation temperature with the same letters are not significantly different ($p > 0.05$). ND = value could not be determined, * = no test run for *Lasmigona complanata* juveniles.

Species	LT50				LT05			
	22°C acclimation		27°C acclimation		22°C acclimation		27°C acclimation	
	Glochidia	Juveniles	Glochidia	Juveniles	Glochidia	Juveniles	Glochidia	Juveniles
<i>Lampsilis siliquoidea</i>	ND	35.6 A (32.8–38.3)	32.8 AC (26.7–38.8)	34.4 A (32.3–36.5)	ND	31.3 AB (26.2–36.3)	ND	32.5 A (29.7–35.3)
<i>Potamilus alatus</i>	24.2 A (17.7–30.6)	35.0 A (32.6–37.4)	21.4 B (15.4–27.5)	34.1 A (31.4–36.7)	ND	31.2 AB (26.7–35.8)	ND	28.8 A (23.4–34.1)
<i>Ligumia recta</i>	42.6 AB (20.4–64.9)	32.5 A (28.7–36.4)	33.0 AC (29.1–36.9)	35.1 A (31.4–38.7)	23.5 A (6.4–40.6)	23.7 A (16.1–31.3)	24.9 A (16.6–33.2)	26.4 A (18.0–34.7)
<i>Ellipsaria lineolata</i>	31.0 AB (24.8–37.2)	38.8 A (30.5–47.2)	25.7 BC (17.2–34.2)	33.1 A (30.0–36.3)	ND	34.1 B (27.4–40.7)	ND	26.4 A (19.8–33.0)
<i>Lasmigona complanata</i>	37.8 B (29.4–46.3)	*	37.1 A (34.0–40.2)	*	27.9 A (18.9–36.9)	*	30.3 A (24.0–36.7)	*
<i>Megaloniais nervosa</i>	31.3 AB (26.9–35.6)	34.2 A (31.7–36.6)	28.4 BC (22.7–34.2)	34.0 A (30.9–37.1)	20.9 A (12.1–29.8)	30.0 AB (25.2–34.8)	15.6 A (0.7–30.5)	27.2 A (20.7–33.7)
<i>Alasmidonta varicosa</i>	38.0 B (29.0–46.9)	35.0 A (32.2–37.8)	36.1 A (32.8–39.4)	35.1 A (32.6–37.6)	27.6 A (18.3–36.9)	30.1 AB (24.7–35.5)	28.9 A (22.1–35.6)	30.8 A (26.2–35.5)
<i>Villosa delumbis</i>	28.4 AB (21.9–35.0)	34.6 A (31.8–37.3)	26.7 BC (20.3–33.1)	34.2 A (31.6–36.8)	ND	29.5 AB (24.1–35.0)	ND	29.3 A (24.2–34.3)

(Bartsch et al. 2000, Spooner and Vaughn 2008). Characteristics like shell shape, thickness, size, or physiology do not necessarily pertain to newly transformed juvenile mussels, but their influence should be investigated in future studies.

Acclimatization to environmental conditions occurs over time, so normal temperatures in an animal's natural habitat are rarely harmful (Ansell et al. 1980a). However, extreme thermal events can cause significant changes in aquatic community structure in relatively short amounts of time, and mollusk communities might be slow to recover if they have already experienced a gradual warming of their environment (Mouthon and Daufresne 2006). Summer maximum water temperatures in the US are variable but can range from 25°C in the upper midwest to 34–40°C in the south (Wellborn and Robinson 1996, Dyar and Alhadeff 1997, Wright et al. 1999, Spooner and Vaughn 2008). The chances of extreme thermal events increase in summer when the natural heat load can be increased by a variety of factors (Durrett and Pearson 1975, Ansell et al. 1980b, Parkin and Stahl 1981). For example, low flow conditions in an Oklahoma river produced temperatures that frequently ranged from 34 to 38°C (Schaefer et al. 2003), and thermal effluents from industry can increase the temperature of receiving waters by 4 to 8°C (Wellborn and Robinson 1996, Cooke et al. 2004, Encina et al. 2008). These temperatures are close to, or

above, the upper thermal tolerances for the early life stages of freshwater mussels (this study).

On average, the difference between the LT50 and the LT05 of juveniles within a given species was only 5.3°C, and the difference was only 10.6°C for glochidia of a given species. Over this relatively narrow span of temperatures, mortality in a mussel population could theoretically increase from 5% to 50%. However, mortality is not the only consequence of increased temperatures. The LT05s calculated in our study represent temperatures that are high enough to cause sublethal effects, such as changes in filtration rate or immune response, in bivalves (Chen et al. 2007, Loayza-Muro and Elias-Letts 2007). Warm temperatures create a higher demand on metabolic energy and can interfere with behavior, maintenance, and reproductive processes (Dudgeon and Morton 1984, Parker et al. 1984, Weaver et al. 1991, Roberts and Barnhart 1999, Bartsch et al. 2000). Temperature shifts can alter timing of reproduction (Barnett 1972), leading to decreased fertilization and recruitment success (Walther et al. 2002, Philippart et al. 2003). Changing temperatures also can lead to asynchrony, a mismatch between life-history events and environmental conditions (Visser and Holleman 2001, Philippart et al. 2003). For example, a mismatch between the timing of gravidity and glochidial release and the presence of necessary host fish could inhibit freshwater mussel reproduction.

Freshwater mussels are likely to encounter rising environmental temperatures from climate change, thermal effluents, drought, or landuse changes. Species living closest to their thermal limits might be most susceptible to changes in environmental temperatures (Tomanek and Somero 1999, Stillman 2003), and we have demonstrated that temperatures sometimes encountered in freshwater mussel habitat during summer in the temperate US are close to or above the upper thermal tolerances of early life stages of freshwater mussels. Mussels are exposed to a range of stressors, both chemical and nonchemical, and a bivalve that has already been weakened by thermal stress might be more susceptible to other adverse conditions (Sokolova 2004). Water-quality criteria should be developed with the understanding that thermal stress can arise from multiple sources simultaneously and can interact with other stressors. A single heat source might not be detrimental to aquatic organisms, but cumulative effects of combined inputs might be.

Freshwater mussels already are among the most imperiled organisms in the world, so it is crucial to identify the factors that contribute to population declines. Management of freshwater mussel populations can be difficult because mussel populations can persist under conditions of negative growth and appear stable when they are not (Strayer et al. 2004). Freshwater mussels are sedentary animals and must be able to tolerate local environmental conditions to survive. Because they are long-lived, recovery or establishment of populations might require more time than the interval between anthropogenic stressors. Therefore, some mussels might require direct management and human involvement via conservation, augmentation, translocation, or captive breeding (Hastie et al. 2003, Strayer et al. 2004). Habitat restoration, the creation of thermal buffers in riparian zones, and management strategies designed to maintain adequate flows during critical life-history periods could mediate some effects of increased temperatures and might restore entire communities (Hastie et al. 2003).

Acknowledgements

Funding for this research was provided by the US Environmental Protection Agency (EPA) grant agreement number DW-14-94814301 to WGC. The views expressed in this article do not necessarily represent the views of the US EPA. We thank Chris Eads and Renae Greiner at the North Carolina State University College of Veterinary Medicine for providing Atlantic Slope mussel species. We thank Andrea Crownhart

and Michael Pillow of Missouri State University for assistance in providing Interior Basin test organisms.

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Received: 21 September 2009

Accepted: 21 April 2010