Influence of sediment presence on freshwater mussel thermal tolerance

Jennifer M. Archambault^{1,4}, W. Gregory Cope^{2,5}, and Thomas J. Kwak^{3,6}

¹North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, Box 7617, North Carolina State University, Raleigh, North Carolina 27695 USA

²Department of Applied Ecology, Box 7617, North Carolina State University, Raleigh, North Carolina 27695 USA

³US Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, Box 7617,

North Carolina State University, Raleigh, North Carolina 27695 USA

Abstract: Median lethal temperature (LT50) data from water-only exposures with the early life stages of freshwater mussels suggest that some species may be living near their upper thermal tolerances. However, evaluation of thermal sensitivity has never been conducted in sediment. Mussels live most of their lives burrowed in sediment, so understanding the effect of sediment on thermal sensitivity is a necessary step in evaluating the effectiveness of the water-only standard method, on which the regulatory framework for potential thermal criteria currently is based, as a test of thermal sensitivity. We developed a method for testing thermal sensitivity of juvenile mussels in sediment and used the method to assess thermal tolerance of 4 species across a range of temperatures common during summer. Stream beds may provide a thermal refuge in the wild, but we hypothesized that the presence of sediment alone does not alter thermal sensitivity. We also evaluated the effects of 2 temperature acclimation levels (22 and 27°C) and 2 water levels (watered and dewatered treatments). We then compared results from the sediment tests to those conducted using the water-only standard methods. We also conducted water-only LT tests with mussel larvae (glochidia) for comparison with the juvenile life stage. We found few consistent differences in thermal tolerance between sediment and water-only treatments, between acclimation temperatures, between waterlevel treatments, among species, or between juvenile and glochidial life stages (LT50 range = 33.3-37.2°C; mean = 35.6°C), supporting our hypothesis that the presence of sediment alone does not alter thermal sensitivity. The method we developed has potential for evaluating the role of other stressors (e.g., contaminants) in a more natural and complex environment.

Key words: acute thermal sensitivity, Unionidae, LT50, benthic stream ecology, aquatic thermal stress, temperature

Anthropogenic activities, such as electrical power generation, land clearing, and urbanization, exacerbate thermal stress to aquatic organisms by contributing heated pointand nonpoint-source effluents, reducing riparian vegetation, modifying flow regimes, and altering stream geomorphology (LeBlanc et al. 1997, Hester and Doyle 2011). Climate change will further affect aquatic ecosystems through increased water temperature and changes in precipitation, including more frequent flooding and droughts (Bates et al. 2008). The first 12 y of the 21st century (2001–2012) ranked among the 14 warmest in the history of the instrumental record of global surface temperature (NOAA 2012). Drought results in degradation or loss of habitat caused by streambed drying, and flooding can increase runoff, pollution, sedimentation, and erosion. An average global temperature increase of just 1°C above

1990 levels could result in a loss of 8% of North American freshwater fish habitat, and an increase of 3° C above 1990 levels could lead to a 24% loss (IPCC 2007).

Freshwater mussels are especially vulnerable to disturbance because they are largely sedentary. Mussel populations have experienced steep declines worldwide, and only 70 (24%) of the nearly 300 North American species are considered stable (Williams et al. 1993). Declines have continued over the 2 decades since this conservation assessment. Given the many factors already affecting mussel survival (Downing et al. 2010), further alteration to freshwater systems associated with climate change could deepen this imperilment crisis (Galbraith et al. 2010).

Quantitative information on lethal temperatures (LTs) for mussels is available for <10 species, but these results suggest that some species already may be living near their

upper thermal limit, making them highly susceptible to increased thermal stress (Dimock and Wright 1993, Pandolfo et al. 2009, 2010a, b). However, these studies were done with the water-only standard method for toxicity testing (ASTM 2006b), and evaluation of the thermal sensitivity of these benthic organisms has never been conducted in sediment. Mussels burrow into the sediment, and sediments often establish a thermal gradient, so they are thought to provide cooler temperatures during periods of thermal stress (Somero 2002). Apart from the thermal refuge potentially provided, the effect of sediment itself on thermal sensitivity of mussels is unknown. However, the influence of sediment on mussel sensitivity to contaminants has been investigated (Maio et al. 2010, Wang et al. 2011).

The regulatory framework for potential thermal criteria is based on the water-only standard for testing, and standardized protocols for assessing thermal sensitivity in a more complex and realistic environment do not exist. We developed a method for conducting acute thermal experiments on juvenile mussels in the presence of sediment, thereby increasing the ecological relevance of laboratory trials. We used this method to test 3 hypotheses: 1) mussel thermal sensitivity is similar between wateronly and sediment exposures under similar test conditions, 2) mussels exhibit greater thermal sensitivity in dewatered conditions relative to watered conditions, and 3) acute thermal sensitivity is similar between acclimation temperatures, based on results of previous thermal research (Hicks and McMahon 2002, Pandolfo et al. 2009, 2010a, b). We tested thermal sensitivities of mussels in water-only exposures vs those in the presence of sediment using 2 temperatureacclimation (22 and 27°C) and water-level (watered and dewatered) treatments. For comparison with the juvenile life stage, we also tested the thermal sensitivities of the larvae (glochidia) of 3 mussel species.

METHODS

We tested thermal sensitivity of 5 species (juveniles of 4 species and glochidia of 3 species) representing 2 tribes in the family Unionidae: Amblema plicata (tribe Amblemini), and Lampsilis abrupta, Lampsilis cariosa, Lampsilis fasciola, and Lampsilis siliquoidea (all Lampsilini). All juveniles were propagated on fishes at the Alabama Aquatic Biodiversity Center (Marion, Alabama), Missouri State University (Springfield, Missouri), or North Carolina State University, College of Veterinary Medicine (Raleigh, North Carolina), with standard propagation and culture methods (Barnhart 2006). Juveniles and glochidia were shipped to us via overnight courier from these locations except for those propagated in North Carolina. These species are native to the southeastern and central USA in the Atlantic Slope and Interior basins, and they represent a range of conservation statuses, from secure to federally

endangered (US Fish and Wildlife Service 1985, Nature-Serve 2011, NC Wildlife Resources Commission 2011).

General experimental conditions and quality assurance

We developed a method for conducting thermal exposures to juvenile mussels in the presence of sediment, and we compared results from these exposures to standard water-only exposures. A standard protocol for conducting toxicity tests with mussels in the presence of sediment does not exist, so we used test conditions that followed ASTM (2006b) standards for water-only exposures to allow comparisons with previous research (Pandolfo et al. 2010b) and for quality assurance. All experiments were nonaerated static-renewal tests done with 90% reconstituted hard water renewed at 48 h in the 96-h juvenile tests (ASTM 2006a, b). Mean water-quality conditions among all tests were 108.2 mg CaCO₃/L alkalinity, 143.3 mg CaCO₃/L hardness, 534.9 µS/cm conductivity, 8.13 pH, and 7.52 mg/L dissolved O_2 (n = 21 for alkalinity and hardness, n = 167 for all other variables). We conducted tests in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific, Marietta, Ohio; Isotemp Model 146E, Fisher Scientific, Dubuque, Iowa). Thermometers for monitoring incubator temperature were certified for accuracy by the National Institute of Standards and Technology (NIST). We monitored sediment temperatures with partial-immersion thermometers (Fisherbrand® Red-Spirit®, Fisher Scientific, Pittsburgh, Pennsylvania) that met NIST tolerances for accuracy. Realized test temperatures were within $\pm 1^{\circ}$ C (*n* = 1206) of target temperatures for 97.1% of trials and $\pm 2^{\circ}C$ for 99.4% of trials, with a maximum departure of 3.5°C.

We used a nested-chamber static-renewal design for thermal exposure of juvenile mussels in sediment (Fig. 1). The nested-chamber design allowed us to use a sufficient water volume and sediment depth for testing, but reduced the total amount of sediment to be searched, which facilitated efficient recovery of juvenile mussels. The outer chamber was a 1-L glass beaker filled with 400 mL of contaminant-free Si-filter sand to achieve a sediment depth of 5 cm. The inner chamber, which held juvenile mussels, was constructed of a 5-cm length of 5-cm-diameter polyvinyl chloride (PVC) pipe joined to a 5×3.8 -cm PVC adapter coupling, with a layer of 400-µm Nitex® mesh fitted between the pipe section and adapter coupling. The inner chamber was buried partially and filled with sand to the level in the outer chamber. This design allowed mussels to burrow to a maximum depth of 2.5 cm. Whether the homogeneous particle size or lack of organic matter affects burrowing or thermal tolerance is not known, but use of this standardized sediment is necessary to avoid introduction of confounding factors, such as parasites, pathogens, or chemical toxicants. We drilled 18 holes in the PVC chambers to allow water exchange between the inner and

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outer chambers and to ensure a lack of thermal insulation by the PVC. Mean sediment temperatures in the inner chamber differed from target temperatures by $\leq 1.1^{\circ}$ C in all exposures. We conducted water-only tests according to the ASTM (2006b) guideline for laboratory toxicity tests with mussels. We used a 250-mL dish filled with 200 mL of reconstituted hard water and no sediment.

We examined the effects of water level in sediment exposures with 2 treatments (Fig. 1). Sediment in the watered treatment had 5 cm of overlying water with an average total water volume of 482 ± 15 mL (SD). A dewatered treatment meant to simulate drought conditions or stranding included only enough water to wet the sand and mitigate evaporative loss, with an average total water volume of 186 ± 11 mL.

Thermal exposures with juveniles

We conducted acute 96-h thermal exposures with juvenile mussels at 2 acclimation temperatures (22 or 27° C) held under 3 conditions: 1) watered sediment, 2) dewatered sediment, and 3) water-only (no sediment). Each condition included 7 temperature treatments: a control held at 20°C (ASTM 2006b), an acclimation temperature (22 or 27° C), and a series of 5 temperatures ranging from 27 to 37° C in the 22°C acclimation exposures and from 31 to 39° C in the 27°C acclimation exposures (Fig. 2). Acclimation and treatment temperatures followed Pandolfo et al. (2010b). These conditions encompassed a range of summer stream temperatures, and the highest temperatures ensured sufficient mortality for calculating median LTs (LT50s). We replicated each treatment combination $3\times$,



Figure 2. Schematic diagram of experimental design showing acclimation temperatures (22 and 27°C) and experimental temperature treatments for all glochidia and juvenile mussel exposures. All experiments used a control temperature of 20°C.

and each replicate contained 7 juvenile mussels, except for controls which included 10 juvenile mussels/replicate.

We used juveniles of A. plicata, L. abrupta, L. cariosa, and L. siliquoidea in thermal exposures. Lampsilis siliquoidea juvenile thermal tolerance was evaluated in water-only tests by Pandolfo et al. (2010b), so we tested this species only in sediment. All other species were tested in sediment and water-only exposures. Because of limited juvenile availability, L. fasciola was omitted from this part of the study, and A. plicata was not tested at the 22°C acclimation temperature in watered or dewatered sediment tests. Juveniles used in sediment tests ranged in age from 3 to 5 mo and were >3 mm in size to allow effective recovery from the sediment. Mean (SD) shell lengths were 4.08 ± 0.95 mm for *A. plicata*, 4.93 ± 0.85 mm for L. abrupta, 3.09 \pm 0.78 mm for L. cariosa, and 4.00 \pm 0.61 mm for L. siliquoidea. Smaller, younger juveniles were used in water-only tests in accordance with ASTM (2006b) guidelines. Mean shell lengths in water-only tests were 1.60 ± 0.32 mm for *A. plicata*, 0.59 ± 0.09 mm for *L.* cariosa, and 0.23 ± 0.02 mm for L. abrupta. Amblema plicata and L. cariosa ranged in age from 4 to 6 wk, and L. abrupta were <1 wk. Individuals within a species for a given test type differed in age by ≤ 1 wk.

We acclimated juveniles to the test acclimation temperature by adjusting their arrival temperature by 2.5°C/d, with a standard 24-h holding period once the acclimation temperature was attained (ASTM 2006b, Pandolfo et al. 2010b). The acclimation procedure we used is considerably more conservative than the recommended rate of 3°C/h (ASTM 2006b). Temperatures on arrival averaged 23 \pm 1°C from May through August and 18 \pm 1°C from October through February. At the end of each 96-h exposure, we assessed survival by viewing juveniles under a stereomicroscope. Juveniles that exhibited foot movement outside of the shell, foot movement inside the shell, or a detectable heart beat within 5 min of observation were considered alive (ASTM 2006b).

Water-only thermal exposures with glochidia

We evaluated thermal sensitivity in water-only exposures for glochidia of 3 species (*L. abrupta, L. cariosa,* and *L. fasciola*). All glochidia were harvested from females <24 h before initiation of each test. We acclimated glochidia by adjusting their arrival temperature by 1°C/h, with a 2-h holding period once the acclimation temperature was reached (ASTM 2006b). We conducted tests for 24 h in 80-mL beakers in accordance with the ASTM (2006a, b) guideline for glochidia. The experimental design, including acclimation and experimental temperature treatments, was the same as in tests with juveniles except that we used 150 glochidia in each replicate. We assessed viability at 24 h of a subsample of ~50 glochidia in each replicate by exposing glochidia to a saturated NaCl solu-

tion and viewing glochidia under a stereomicroscope. Glochidia that exhibited a shell-closure response to NaCl were considered viable (ASTM 2006b).

Statistical analysis

We analyzed the effects of temperature treatments on mussels with Comprehensive Environmental Toxicity Information Software (CETIS)TM (version 1.8.0.12; Tidepool Scientific, LLC, McKinleyville, California). The LT50 was defined as the temperature that caused mortality in 50% of the individuals in the exposed sample, and the LT05 was the temperature that caused mortality in 5% of the individuals in the sample. We used survival data to generate LT50s and LT05s with the trimmed Spearman– Karber method, and we computed 95% confidence intervals (CIs) based on the variance among the 3 replicates for each treatment combination. As is customary in toxicological studies, we considered LT values as statistically different among treatments if their 95% CIs did not overlap.

RESULTS

Thermal exposures with juveniles

We recovered 99.3% (n = 4205) of juvenile mussels from the sediment chambers across all sediment tests. We could not compute LT50s for *L. abrupta* and *L. cariosa* in watered sediment treatments at the 22°C acclimation because of a lack of within-treatment intermediate mortality (Table 1). We could not compute LT05s in a total of 9 tests because of a lack of partial mortality, and we could not estimate 95% CIs in 5 tests because of a lack of partial mortality (Table 2).

Juvenile mussel thermal sensitivity showed no strong differences between water-only and sediment exposures. Within a given acclimation temperature, LT50 values did not differ significantly between water-only and watered or dewatered sediment exposures for any species, except L. abrupta (Table 1). LT50 for L. abrupta at 27°C acclimation was slightly, but significantly, lower in the wateronly treatment than in the watered sediment treatment, but it did not differ from the LT50 in the dewatered treatment. Because of overall low mortality at the cooler treatment temperatures, we were unable to make firm conclusions about differences in LT05 among treatments in most tests (Table 2). LT05 for L. abrupta at 22°C acclimation was similar between water-only and watered sediment treatments (34.6 and 34.3°C, respectively; no CI available) but slightly lower in the dewatered sediment treatment (30.0°C; no CI available). At 27°C acclimation, LT05 for *L. abrupta* was slightly higher in the water-only treatment (34.7°C; no CI available) than in watered and dewatered sediment treatments (31.2 and 30.9°C, respectively; no CI available), but upper limits of 95% CIs for both sediment treatments approached the estimate for

4		22°C acclim	ation LT50			27°C acclin	nation LT50	
			Juveniles				Juveniles	
Species	Glochidia	Water-only	Watered	Dewatered	Glochidia	Water-only	Watered	Dewatered
Amblema plicata	I	$36.4^{A;1}$	I	I	I	$36.4^{A,B;1}$	37.2 ^{A;1}	$35.3^{\rm B;1}$
		(35.9 - 36.9)				(35.6 - 37.1)	(36.7 - 37.7)	(34.6 - 36.1)
Lampsilis abrupta	ND	$34.8^{A;1}$	ND	$35.1^{A;1}$	$37.2^{\rm B;1}$	$34.9^{A;2}$	$36.5^{\rm B;1}$	$35.0^{A,B;1}$
		(33.6 - 36.0)		(34.4 - 35.9)	(37.0 - 37.4)	(34.3 - 35.5)	(35.8 - 37.2)	(34.2 - 35.9)
Lampsilis cariosa	$35.8^{A;1}$	$36.8^{A;1}$	ND	$35.1^{\rm A,B;1}$	$33.3^{\rm B;2}$	$35.5^{A;1,2}$	$36.5^{A;1}$	$35.5^{A;1}$
	(35.3 - 36.2)	(35.6 - 37.9)		(33.8 - 36.3)	(32.7 - 33.8)	(35.0 - 36.0)	(36.0 - 36.9)	(34.8 - 36.2)
Lampsilis fasciola	$36.3^{A;1}$	I	I	I	$35.5^{\rm B;3}$	I	I	I
	(36.2 - 36.4)				(35.3 - 35.7)			
Lampsilis siliquoidea	ND	$35.6^{A,B;1}$	33.3^{A}	$35.4^{\rm A,B;1}$	$32.8^{A,B;1,2,3}$	$34.4^{A,B;1,2}$	$36.0^{\mathrm{B;1}}$	$35.3^{\rm B;1}$
		(32.8 - 38.3)	(32.4 - 34.2)	(34.1 - 36.7)	(26.7 - 38.8)	(32.3 - 36.5)	(35.4 - 36.5)	(34.9 - 35.7)

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		22°C acclim	ation LT05			27°C acclim	ation LT05	
			Juveniles				Juveniles	
Species	Glochidia	Water-only	Watered	Dewatered	Glochidia	Water-only	Watered	Dewatered
Amblema plicata	I	ND	I	Ι	I	ND	ND	34.8
								(30.1 - 35.6)
Lampsilis abrupta	QN	34.6	34.3	30.0	36.7	34.7	31.2^{A}	30.9^{A}
		(ND)	(ND)	(ND)	(ND)	(ND)	(6.4 - 33.9)	(0.2 - 33.7)
Lampsilis cariosa	31.1^{A}	22.2	ND	26.7^{A}	23.8^{A}	ND	ND	ND
	(25.1 - 32.8)	(ND)		(0.2 - 30.4)	(13.4 - 27.8)			
Lampsilis fasciola	ND	I	I	I	35.0	I	I	I
					(ND)			
Lampsilis siliquoidea	ND	31.3^{A}	28.7^{A}	32.2^{A}	ND	32.5^{A}	ND	ND
		(26.2 - 36.3)	(17.0 - 31.1)	(23.9 - 33.8)		(29.7 - 35.3)		

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the water-only treatment (Table 2). LT05 for *L. cariosa* at 22°C acclimation was lower in the water-only treatment (22.2°C; no CI available) than in the dewatered sediment treatment (26.7°C), but the 95% CI for this estimate included the estimate for the water-only treatment.

Water level in sediment treatments had little effect on juvenile survival. For a given acclimation temperature, LT50 differed between watered and dewatered sediment treatments for only *A. plicata*, for which LT50 was slightly, but significantly lower in the dewatered treatment (Table 1). For a given acclimation temperature, LT05s did not differ between watered and dewatered sediment treatments for either species for which estimates and CIs were available (*L. abrupta*, *L. siliquoidea*; Table 2). At 22°C acclimation, LT05 for *L. abrupta* was slightly lower in the dewatered treatment, but CIs were not available.

Acclimation temperature had little effect on juvenile mussel survival. Pairwise comparisons of LT50 values between acclimation temperatures showed significant differences for only *L. siliquoidea*, which was more thermally sensitive in the watered treatment at the 22°C acclimation (Table 1). Lack of mortality in the cooler temperatures precluded firm conclusions about differences in LT05 between acclimation temperatures, but available estimates for *L. abrupta* were similar, regardless of treatment, and showed no consistent trends with regard to acclimation temperature (Table 2).

We found few differences among species in juvenile temperature tolerance. Within a given treatment combination, significant differences in LT50 among species were seen only for the water-only treatment at the 27°C acclimation, but these differences were small (Table 1). Lack of mortality precluded firm conclusions about differences in LT05 among species, but the few available estimates showed no significant differences among species (Table 2).

Thermal exposures with glochidia

We could not compute LT50 for *L. abrupta* at 22°C acclimation because of a lack of partial mortality, and we were unable to compute LT05 estimates or CIs for several species for the same reason. In general, thermal tolerance of glochidia was similar to that of juveniles, and we observed few consistent differences among treatments or species. LT50 was higher for *L. abrupta* glochidia at 27°C acclimation than for *L. abrupta* juveniles in any treatment (Table 1). A similar trend was seen for *L. abrupta* LT05, but our glochidial estimate had no CI (Table 2). *Lampsilis cariosa* showed an opposite pattern, with significantly lower LT50 for glochidia at 27°C acclimation. Values of LT05 for *L. cariosa* showed no significant dif-

ferences between glochidia and juveniles. The most consistent pattern in glochidial thermal tolerance was a significantly lower LT50 at 27°C acclimation than 22°C for both species for which estimates were available (*L. cariosa* and *L. fasciola*; Table 1). Our estimate of LT05 was substantially lower at 27°C acclimation than 22°C for *L. cariosa*, but CIs around these estimates were wide and overlapping (Table 2). LT50 of glochidia differed significantly at 27°C acclimation among all 3 species for which data were available, with *L. cariosa* having the lowest value, but no differences among species were observed at 22°C (Table 1). Because of a lack of CIs, no firm conclusions were possible about differences in LT05 among species, but our estimate of LT05 was substantially lower for *L. cariosa* than for the other species (Table 2).

DISCUSSION

To our knowledge, we are the first to report acute lethal thermal sensitivities for juvenile freshwater mussels held in sediment in laboratory tests. Overall, thermal tolerance in sediment exposures was similar to tolerance in water-only exposures, supporting our hypothesis that the presence of sediment alone does not affect acute lethal thermal sensitivity. Furthermore, the water-only LT50s for L. siliquoidea juveniles reported by Pandolfo et al. (Table 1; 2010b) did not differ significantly from LT50s in our sediment tests. Acute lethal temperatures (LT50) of early mussel life stages for several other species ranged from 31.5 to 38.8°C (Dimock and Wright 1993, Pandolfo et al. 2010b), which is similar to the range observed in our study (33.3-37.2°C) among sediment and water-only exposures. These previous studies did not address the influence of sediment, but their results suggest that overall patterns of thermal tolerance may be similar across a wide range of mussel species. Cooler sediment temperatures in natural settings may buffer mussels from temperature extremes to some extent (Somero 2002), but our results show that laboratory studies of acute thermal tolerance provide useful measures regardless of whether sediment is present. Therefore, these data are directly relevant to the establishment of modern thermal water-quality criteria that would be protective of mussels. Water-quality criteria for temperature are currently species-specific, based solely on fish (USEPA 1986), and >25 y old, so they would benefit from review and augmentation with recent findings.

Our hypothesis that dewatering would result in greater thermal sensitivity of juvenile mussels was not supported. We found only 1 instance of significantly lower thermal tolerance in dewatered conditions (*A. plicata*, 27°C acclimation), but the magnitude of the difference between the watered and dewatered response was small. These results suggest that juveniles may endure a dewatered stream bed (with pore water present) and a watered stream bed

equivalently at warm temperatures, at least for short periods (e.g., 96 h). However, our experimental conditions did not reflect a number of factors that may influence survival in the wild during drought. For example, direct sunlight probably sharply increases temperatures within the shell of stranded mussels compared to mussels that remain immersed, and stranded mussels are vulnerable to predation. Drought conditions are usually accompanied by low dissolved O₂ levels, which along with stranding, may be a primary source of mortality in dewatered streams (Gagnon et al. 2004, Golladay et al. 2004). A severe drought in 2000 caused mussel density to decline as much as 83% in some southeastern USA streams. All species declined at similar rates and mussels survived primarily in sections of the stream bed that remained watered (Haag and Warren 2008). Moreover, fish may be more sensitive than invertebrates to thermal stress, and fish declines could lead to mussel declines via changes in host-fish availability (Pandolfo et al. 2012). Burlakova et al. (2011) concluded that the most important environmental factors influencing freshwater mussel diversity included climatic (precipitation and evaporation) and hydraulic variables (discharge), and the importance of these factors may increase with climate change.

Effects of acclimation temperature were largely absent, and the few differences were not consistent. These findings support our hypothesis that acclimation temperature has little effect on results of acute thermal trials for the early life stages of mussels (see also Pandolfo et al. 2010b). Acclimation temperature also had no effect on the upper lethal thermal limits of the marine Brown Mussel (Perna perna; Hicks and McMahon 2002). However, in a review of temperature tolerance for 50 aquatic organisms, including 16 mollusks, required acclimation periods were typically >96 h (de Vries et al. 1998). Recent thermal research with adult freshwater mussels, for which no ASTM standard guideline exists, detected differences in temperature sensitivity between 15 and 25°C acclimation temperatures when mussels were fed and held for 7 d before testing (Galbraith et al. 2012). Holding and acclimating early life stages of mussels for longer periods without feeding, as prescribed by ASTM (2006b) guidelines, may substantially increase mortality in acute tests. Our acclimation period was longer and the rate of change was slower than the 3°C/h recommended by the ASTM (2006b) guidelines, but it still may have been too brief to allow the mussels to establish true acclimation or, conversely, may be unimportant in acute exposures, especially because the 2 acclimation temperatures were relatively high and proximate (22 and 27°C). Further research is needed on the effect of acclimation period in thermal tolerance tests with juvenile mussels.

Guidelines are available for acute tests, but guidelines for conducting chronic exposures with the early life stages of freshwater mussels are needed. In one chronic study (Ganser 2012), 7-d LT50 values for juvenile L. abrupta (mean = 33.6°C, 95% CI = 32.5-34.6) and L. siliquoidea (mean = 32.5° C, $31.5-33.5^{\circ}$ C) were substantially but not significantly lower than our results from 96-h tests with those species, and the LT50 values after 28 d were significantly lower, based on comparisons of 95% CIs (L. abrupta, mean = 27.2°C, 95% CI = 26.3-28.2°C; L. siliquoidea, mean = 25.3°C, 95% CI = 24.1-26.7°C). These findings show the potential for an increase in mussel thermal sensitivity with longer exposures and demonstrate the need for more studies of this nature. In addition, potential relationships between acute and chronic sensitivities would be instructive. For example, the 7-d LT50s estimated by Ganser (2012) were similar to our 96-h LT05s, suggesting that prediction of chronic median lethal temperatures from acute test results may be possible.

CONCLUSIONS

We developed and applied a new method for conducting thermal toxicity tests in sediment with juvenile freshwater mussels. Construction of the treatment chambers was simple and low cost, contaminant-free substrate was commercially available and inexpensive, and we achieved nearly 100% recovery of test organisms. Overall, this sediment testing method was simple, efficient, and reproducible. Our findings and those of other researchers (Newton and Bartsch 2007, Maio et al. 2010, Wang et al. 2011) may assist the ASTM or others in developing amended guidelines for toxicity testing with freshwater mussels. This method also may guide future thermal research by providing a foundation for simulating benthic complexity in laboratory experiments.

Water-only tests appear to reflect the acute thermal tolerance of juvenile mussels in sediment exposures, and juveniles appear to exhibit similar thermal tolerance under watered and dewatered conditions when tests are done in temperature-controlled incubators. However, because the lethal temperatures that we observed in the laboratory are regularly exceeded in surface waters of the southeastern and central USA, we suggest that more complex interactions are involved in estimating thermal sensitivity in natural systems and, ultimately, in mitigating mortality during periods of excessive heat. For example, the maximum temperatures occurring at 5 and 15 cm below the sediment/water interface in streams in the North Carolina Piedmont were, on average, 1.9 and 2.9°C cooler, respectively, than the surface water temperature from July to October 2011 (T. J. Pandolfo, North Carolina State University, unpublished data). Freshwater mussels burrow to 10 cm and as deep as 20 cm depth (Schwalb and Pusch 2007), suggesting that they may experience thermal buffering in natural, complex stream sediments. Physiological factors that influence thermal sensitivity of other bivalves, such as induced thermotolerance (Jackson et al. 2011) and interactive effects of temperature with dissolved

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 O_2 concentration (Polhill and Dimock 1996, Portner et al. 2006, 2007, Peck et al. 2007) remain uninvestigated for freshwater mussels. Additional topics related to mussel thermal sensitivity that warrant future research include physiological variables that may alter thermal tolerance, daily temperature flux of surface and pore waters, and the role of other stressors, such as contaminants.

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LITERATURE CITED

- ASTM (American Society for Testing and Materials). 2006a. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-88a. American Society for Testing and Materials International, West Conshohocken, Pennsylvania.
- ASTM (American Society for Testing and Materials). 2006b. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. American Society for Testing and Materials International, West Conshohocken, Pennsylvania.
- Barnhart, M. C. 2006. Buckets of muckets: a compact system for rearing juvenile freshwater mussels. Aquaculture 254: 227–233.
- Bates, B. C., Z. W. Kundzewicz, S. Wu, and J. P. Palutikof (editors). 2008. Climate change and water. Technical Paper of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change Secretariat, Geneva, Switzerland.
- Burlakova, L. E., A. Y. Karatayev, V. A. Karatayev, M. E. May, D. L. Bennett, and M. J. Cook. 2011. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: patterns of diversity and threats. Diversity and Distributions 17:394–407.
- de Vries, P., J. E. Tamis, A. J. Murk, and M. G. D. Smit. 2008. Development and application of a species sensitivity distribution

for temperature-induced mortality in the aquatic environment. Environmental Toxicology and Chemistry 27:2591–2598.

- Dimock, R. V., and A. H. Wright. 1993. Sensitivity of juvenile freshwater mussels to hypoxic, thermal, and acid stress. Journal of Elisha Mitchell Scientific Society 109:183–192.
- Downing, J. A., P. van Meter, and D. A. Woolnough. 2010. Suspects and evidence: a review of the causes of extirpation and decline in freshwater mussels. Animal Biodiversity and Conservation 33:151–185.
- Gagnon, P., S. W. Golladay, W. K. Michener, and M. C. Freeman. 2004. Drought responses of freshwater mussels (Unionidae) in coastal plain tributaries of the Flint River Basin, Georgia. Journal of Freshwater Ecology 19:667–679.
- Galbraith, H. S., C. J. Blakeslee, and W. A. Lellis. 2012. Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia:Unionoida). Freshwater Science 31:83–92.
- Galbraith, H. S., D. E. Spooner, and C. C. Vaughn. 2010. Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. Biological Conservation 143:1175–1183.
- Ganser, A. M. 2012. The effects of elevated water temperatures on adult and juvenile freshwater mussels. MS Thesis, University of Wisconsin-La Crosse, La Crosse, Wisconsin.
- Golladay, S. W., P. Gagnon, M. Kearns, J. M. Battle, and D. W. Hicks. 2004. Response of freshwater mussel assemblages (Bivalvia:Unionidae) to a record drought in the Gulf Coastal Plain of southwestern Georgia. Journal of the North American Benthological Society 23:494–506.
- Haag, W. R., and M. L. Warren. 2008. Effects of severe drought on freshwater mussel assemblages. Transactions of the American Fisheries Society 137:1165–1178.
- Hester, E. T., and M. W. Doyle. 2011. Human impacts to river temperature and their effects on biological processes: a quantitative synthesis. Journal of the American Water Resources Association 47:571–587.
- Hicks, D. W., and R. F. McMahon. 2002. Temperature acclimation of upper and lower thermal limits and freeze resistance in the nonindigenous Brown Mussel, *Perna perna* (L.), from the Gulf of Mexico. Marine Biology 140:1167–1179.
- IPCC (Intergovernmental Panel on Climate Change). 2007. Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the 4th Assessment Report of the Intergovernmental Panel on Climate Change. M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson (editors). Cambridge University Press, Cambridge, UK.
- Jackson, S. A., K. R. Uhlinger, and J. S. Clegg. 2011. Duration of thermal tolerance and tissue-specific expression of hsp/ hsc70 in the Eastern Oyster, *Crassostrea virginica* and the Pacific Oyster, *Crassostrea gigas*. Aquaculture 317:168–174.
- LeBlanc, R. T., R. D. Brown, and J. E. FitzGibbon. 1997. Modeling the effects of land use change on water temperature in unregulated urban streams. Journal of Environmental Management 49:445–469.
- Maio, J., M. C. Barnhart, E. L. Brunson, D. K. Hardesty, C. G. Ingersoll, and N. Wang. 2010. An evaluation of the influence of substrate on the response of juvenile freshwater mussels (Fatmucket, *Lampsilis siliquoidea*) in acute water exposures to ammonia. Environmental Toxicology and Chemistry 29: 2112–2116.

- NatureServe. 2011. NatureServe Explorer: an online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. (Available from: http://www.natureserve.org/explorer)
- NC Wildlife Resources Commission. 2011. North Carolina atlas of freshwater mussels and endangered fish. North Carolina Wildlife Resources Commission, Raleigh, North Carolina. (Available from: http://www.ncwildlife.org/Wildlife_Species_Con /WSC_FWMussels_EndFish_Atlas.htm)
- Newton, T. J., and M. R. Bartsch. 2007. Lethal and sublethal effects of ammonia to juvenile *Lampsilis* mussels (Unionidae) in sediment and water-only exposures. Environmental Toxicology and Chemistry 26:2057–2065.
- NOAA (National Oceanic and Atmospheric Administration). 2012. NOAA National Climatic Data Center, State of the Climate. National Oceanic and Atmospheric Administration, Washington, DC. (Available from: http://www.ncdc.noaa .gov/sotc/)
- Pandolfo, T. J., W. G. Cope, and C. Arellano. 2009. Heart rate as a sublethal indicator of thermal stress in juvenile freshwater mussels. Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology 154:347–352.
- Pandolfo, T. J., W. G. Cope, and C. Arellano. 2010a. Thermal tolerance of juvenile freshwater mussels (Unionidae) under the added stress of copper. Environmental Toxicology and Chemistry 29:691–699.
- Pandolfo, T. J., W. G. Cope, C. Arellano, R. B. Bringolf, M. C. Barnhart, and E. Hammer. 2010b. Upper thermal tolerances of early life stages of freshwater mussels. Journal of the North American Benthological Society 29:959–969.
- Pandolfo, T. J., T. J. Kwak, and W. G. Cope. 2012. Thermal tolerances of freshwater mussels and their host fishes: species interactions in a changing climate. Walkerana: the Journal of the Freshwater Mollusk Conservation Society 15: 69–82.
- Peck, L. S., S. A. Morley, H. O. Pörtner, and M. S. Clark. 2007. Thermal limits of burrowing capacity are linked to oxygen

availability and size in the Antarctic clam *Laternula elliptica*. Oecologia (Berlin) 154:479–484.

- Polhill, J. B., and R. V. Dimock. 1996. Effects of temperature and pO₂ on the heart rate of juvenile and adult freshwater mussels (Bivalvia: Unionidae). Comparative Biochemical Physiology 114A:135–141.
- Pörtner, H. O., L. S. Peck, and T. Hirse. 2006. Hyperoxia alleviates thermal stress in the Antarctic bivalve, *Laternula elliptica*: evidence for oxygen limited thermal tolerance. Polar Biology 29:688–693.
- Pörtner, H. O., L. Peck, and G. Somero. 2007. Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 362:2233–2258.
- Schwalb, A. N., and M. T. Pusch. 2007. Horizontal and vertical movements of unionid mussels in a lowland river. Journal of the North American Benthological Society 26:261–272.
- Somero, G. N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrative and Comparative Biology 42:780–789.
- USEPA (US Environmental Protection Agency). 1986. Quality criteria for water. EPA 440/5-86-001. Office of Water Regulations and Standards, Washington, DC.
- US Fish and Wildlife Service. 1985. Recovery plan for the Pink Mucket Pearly Mussel (*Lampsilis orbiculata* (Hildreth, 1828)). US Fish and Wildlife Service, Atlanta, Georgia. (Available from: Fish and Wildlife Reference Service, 1776 E. Jefferson Street, 4th floor, Rockville, Maryland 20852 USA.)
- Wang, N., R. A. Consbrock, C. G. Ingersoll, and M. C. Barnhart. 2011. Evaluation of influence of sediment on the sensitivity of a unionid mussel (*Lampsilis siliquoidea*) to ammonia in 28-day water exposures. Environmental Toxicology and Chemistry 30:2270–2276.
- Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of the freshwater mussels of the United States and Canada. Fisheries 18(9):6–22.