

# Survival and behaviour of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient

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## SUMMARY

1. Freshwater mussels (Unionidae) are a highly imperilled faunal group. One critical threat is thermal sensitivity, because global climate change and other anthropogenic activities contribute to increasing stream temperature and altered hydrologic flow that may be detrimental to freshwater mussels.
2. We incorporated four benthic environmental components – temperature, sediment, water level (a surrogate for flow) and a vertical thermal gradient in the sediment column – in laboratory mesocosm experiments with juveniles of two species of freshwater mussels (*Lampsilis abrupta* and *Lampsilis radiata*) and tested their effects on survival, burrowing behaviour and byssus production.
3. Increasing temperature diminished burrowing behaviour significantly in both species ( $P < 0.01$ ), and the dewatered treatment significantly reduced burrowing in *L. radiata*, compared with that in the watered treatment. Increasing temperature also significantly reduced byssus production in both species ( $P < 0.01$ ). Median lethal temperatures (LT50) ranged from 29.9 to 35.6 °C. Mussels did not burrow beneath the top stratum of sediment (0–2.5 cm) and thus did not use the available thermal refuge.
4. Our findings suggest that rising stream water temperature and dewatering may directly impact freshwater mussel abundance by causing mortality and may have indirect impacts via sublethal effects. Reduced burrowing capacity may hamper ability to escape predation or unfavourably high or low flows, and decreased byssus production may inhibit attachment and dispersal capabilities in juveniles.

**Keywords:** burrowing, byssus, climate change, freshwater, mesocosm

## Introduction

Freshwater mussels are an imperilled fauna (Lydeard *et al.*, 2004). Approximately half of the world's unionid mussel diversity occurs in North America, where 71.7% of species are endangered, threatened or of special concern (Williams *et al.*, 1993). Freshwater mussels are integral to stream ecology and surface-water quality (Vaughn & Hakenkamp, 2001; Howard & Cuffey, 2006; Vaughn, Nichols & Spooner, 2008; Haag, 2012; Spooner

& Vaughn, 2012). Because they perform many ecological services and often comprise a substantial proportion of benthic biomass (Vaughn & Hakenkamp, 2001; Vaughn, Gido & Spooner, 2004; Spooner & Vaughn, 2008, 2012; Vaughn *et al.*, 2008), identifying and quantifying threats to mussels are important for conserving the biodiversity and ecological integrity of freshwater systems, two environmental benchmarks that typify management goals (Downing, Van Meter & Woolnough, 2010; Kwak & Freeman, 2010). Two such threats are elevated stream

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temperature and altered hydrologic flow due to climate change and other anthropogenic activities.

Thermal inputs to freshwater systems are common. Anthropogenic activities such as electric power generation, land-clearing and urbanisation may have deleterious consequences for freshwater habitats by contributing to additional heated point- and non-point-source effluents (Hester & Doyle, 2011), thus exacerbating thermal stress to freshwater mussels and other organisms. Moreover, the global climate is warming at a much faster pace than at any other time in recent geologic history (IPCC, 2007). However, despite extensive thermal research associated with fisheries and fish populations, research on thermal stressors to mussels has gained momentum only in the past decade. Few investigators have evaluated the effects of thermal stress on native freshwater mussels, and to date quantitative information on lethal temperatures is limited to about 15 species (Dimock & Wright, 1993; Pandolfo *et al.*, 2010; Archambault, 2012; Archambault, Cope & Kwak, 2013). Studies with most of these species were restricted to the water-only standard method for toxicity testing (ASTM, 2006a) and thus did not incorporate any environmental components that may affect mussel thermal sensitivity *in situ*. In an earlier study, we developed and applied a new method for conducting thermal tests in sediment with juvenile freshwater mussels (Archambault, 2012). We found that acute (96-h) median lethal temperatures (LT50) were similar among experiments conducted in water only and in sediment. Because stream temperatures regularly exceed these 96-h LT50s (USGS, 2012), we suggested that more complex factors may be influential in mussel thermal sensitivity in streams, and ultimately in mitigating mortality during periods of excessive heat.

Few studies have focused on potential sublethal effects of thermal stress in freshwater mussels. Burrowing behaviour is central to the ecology of these endobenthic organisms [e.g. escaping high flow (Schwalb & Pusch, 2007); bioturbation, nutrient cycling (Vaughn & Hakencamp, 2001)], but little is known about the effects of environmental stressors on burrowing (e.g. Nichols & Wilcox, 1997). Waller, Gutreuter & Rach (1999) considered the effects of common stream temperatures on righting and burrowing behaviours, but studies quantifying the effects of extreme temperatures on burrowing behaviour are rare (e.g. Bartsch *et al.*, 2000), and to our knowledge, our recently published study is the only one to do so with juvenile mussels (Archambault *et al.*, 2013). Similarly, research on stressors to byssus production has concentrated primarily

on efforts to control the non-native zebra mussel (*Dreissena polymorpha*) (Clarke & McMahon, 1996; Cope, Bartsch & Marking, 1997). Byssus is a thread-like secretion from glands in the foot of bivalve molluscs, used by native freshwater mussels for attachment and drift (Waite, 1983; Cope *et al.*, 1997; Bradley, 2011). Although some investigators have attempted to elucidate the role of byssus in unionid ecology, few have explored potential stressors to byssus production (e.g. Archambault *et al.*, 2013). Clarke & McMahon (1996) found that invasive zebra mussels produced more byssus at higher temperatures, with the highest rate at 30 °C, which is near their upper lethal limit. Changes in byssus production associated with elevated stream temperature in unionids may reduce the ability of young mussels to disperse, or conversely, to retain position within a stream bed.

We postulated that thermal gradients typically found in stream substrata may influence lethal and sublethal thermal sensitivity endpoints in freshwater mussels and may be especially important in mitigating thermal stress in juveniles. Building on our research that established a reliable and repeatable method for conducting thermal toxicity tests with freshwater mussels in sediment (Archambault, 2012), we incorporated a vertical thermal gradient as an additional experimental ecological factor. In this research, we investigate the effects of thermal stress, thermal refuge, dewatering and acclimation temperature on survival, burrowing behaviour and byssus production in juvenile native unionids by incorporating multiple environmental variables toward enhancing ecological relevance in controlled laboratory experiments.

## Methods

Previously we developed a standardised method for conducting thermal exposures to freshwater mussels in sediment, with two acclimation temperatures (22 and 27 °C), five temperature treatments per acclimation group and two experimental water treatments (watered and dewatered) that served as surrogates for different flow regimes (moderate and severe drought conditions; Archambault *et al.*, 2013). In this study, we expanded upon our earlier design to include a vertical temperature gradient in the sediment and associated interstitial (pore) water. A standard protocol for conducting toxicity tests with freshwater mussels in sediment does not currently exist, but as far as practical exposures in sediment were conducted following the same guidelines as for water-only exposures (ASTM, 2006a), to

ensure data quality and comparability to other research.

### Test organisms

We tested two species of mussels in the Lampsilini tribe of the Unionidae family, *Lampsilis abrupta* (Say) and *Lampsilis radiata* (Gmelin). All juveniles were propagated via host-fish infection in facilities at Missouri State University (Springfield, MO, U.S.A.) or the Harrison Lake National Fish Hatchery (Charles City, VA, U.S.A.), using standard propagation and culture methods (Barnhart, 2006). Test species were chosen based on availability, native range and conservation status. *Lampsilis abrupta* is federally listed as endangered (USFWS, 1985) and occurs in the Interior Basin of the central United States. *Lampsilis radiata* has a native range primarily in the Atlantic Slope Basin and occurs in some northern catchments of the Interior Basin. It is considered a globally secure species and has a wide distribution, but is classified as imperilled in several states (NatureServe, 2012).

### Experimental design

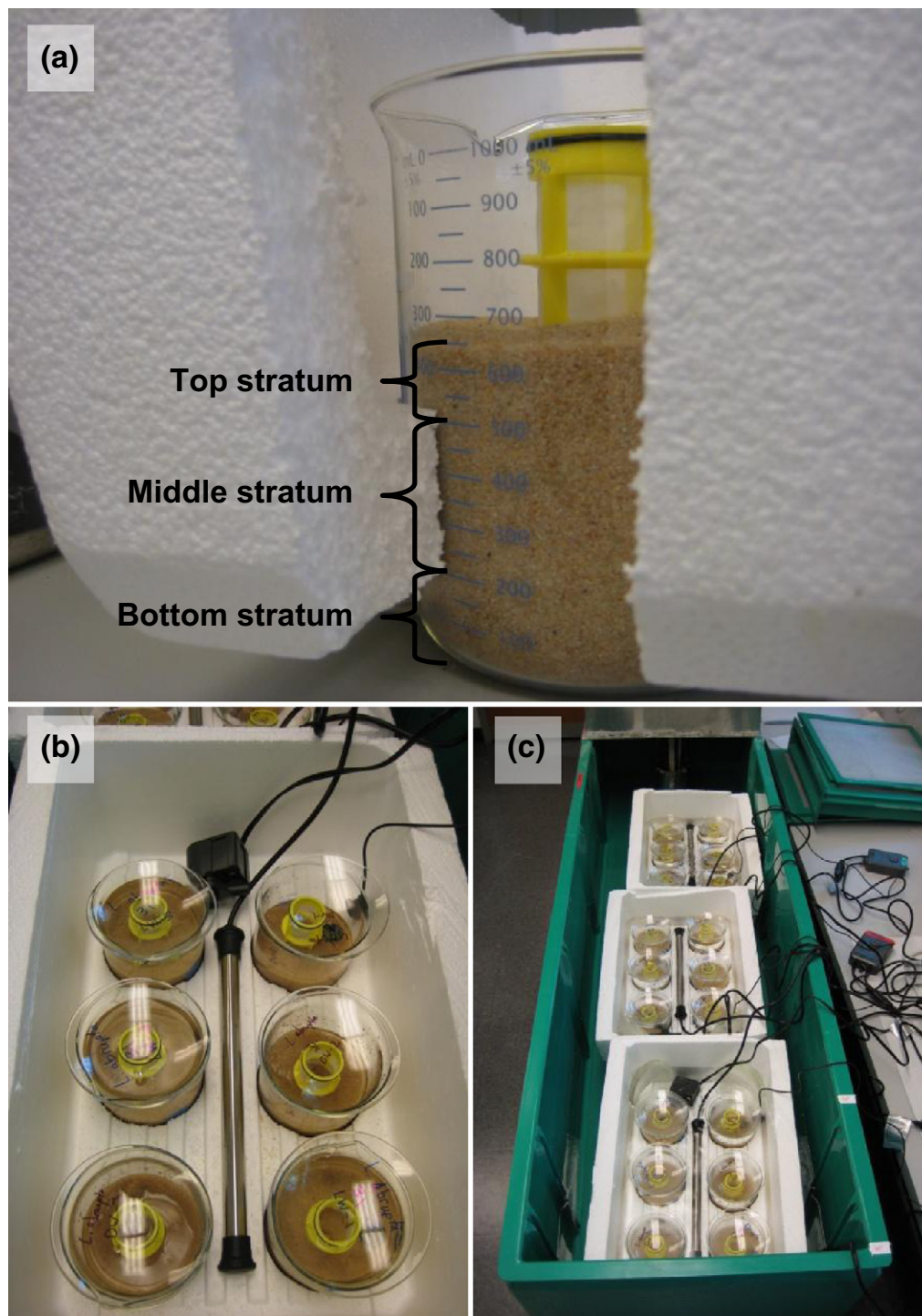
We used a nested-chamber, static-renewal design for thermal exposure of juvenile mussels in sediment. The nested-chamber design allowed the use of sufficient water volume and sediment depth to test for sediment effects on thermal sensitivity, while reducing the total amount of sediment to be searched, which allowed for efficient recovery of juvenile mussels. The outer chamber was a 1-L glass beaker filled with 650 mL of silica sand to achieve a sediment depth of 8 cm. A 12.7-cm-long by 4.4-cm-diameter cylindrical irrigation filter composed of 100- $\mu$ m nylon mesh and plastic frame comprised the inner chamber (Fig. 1; Archambault, 2012). We examined the effects of water level in the sediment exposures with two treatments by controlling the amount of reconstituted hard water (ASTM, 2006b) added to the test chambers. A watered treatment included approximately 4 cm (350 mL) of overlying water. A dewatered treatment served to simulate drought conditions and included enough water to wet the sand and mitigate evaporative loss [ $\leq 0.5$  cm (50 mL) overlying water].

We developed and constructed a novel apparatus to achieve a vertical sediment temperature gradient. Three replicate chambers for each of the two water-level treatments were sealed into holes cut in the bottom of a 42.5-L insulated foam cooler (Lifoam Industries, LLC,

Hunt Valley, MD, U.S.A.) for each of six temperatures, consisting of the acclimation temperature and five corresponding experimental temperatures. The coolers were floated in temperature-controlled artificial streams (Model LS-700, Frigid Units, Inc., Toledo, OH, U.S.A.) set to the test acclimation temperature (22 or 27 °C); thus, the cooler containing the acclimation temperature treatment served as a control within the artificial streams (Fig. 1). A submersible thermostatically controlled heater (IHC Model HTS1 or JH500, Aquatic Ecosystems, Inc., Apopka, FL, U.S.A.), a water pump (Maxi-Jet® Pro, Aquatic Ecosystems, Inc.) and approximately 4-L of deionised water were placed in each cooler. The bath circulated water around the outsides of the beakers, and the contents of the beakers (i.e. mussels, sediment and water) were isolated from direct contact with the water bath. The top 2.5 cm of sediment and overlying water in each chamber were exposed to the treatment temperatures inside the coolers. The middle stratum of sediment (2.5–6 cm) in each chamber was encased by the foam bottom of the coolers and served as a thermal gradient zone. The bottom stratum of sediment (6–8 cm) in each chamber protruded from the bottom of the cooler and was exposed to the acclimation temperature of the artificial stream (Fig. 1). Beakers were covered with watch glasses, and coolers were loosely covered with plastic wrap and a foam lid to reduce evaporation and heat exchange with ambient laboratory air temperatures to minimise thermal variation in the experiment. Large holes were cut in the foam lids to maintain a natural, ambient light/dark cycle (14:10 h) throughout the experiment. Three replicates in each of the two water level treatments were also held in a light- and temperature-controlled environmental chamber (Precision Model 818, Thermo Fisher Scientific, Marietta, OH, U.S.A.) at 20 °C without a vertical thermal gradient as experiment-wide controls.

Commercially available, contaminant-free filter sand (Southern Products and Silica Co., Inc., Hoffman, NC, U.S.A.) served as the substratum for the experiments. This silica sand is widely used in drinking water filtration and meets or exceeds the current American Water Works Association Standard for Filter Material (Southern Products & Silica Co., Inc., 2011). Before use, the sand was dry-sieved to a more uniform size range of 500 – 850  $\mu$ m and was heated to 200 °C in a drying oven to ensure the lack of organisms and low starting moisture content. Using this substratum may have practical limitations (e.g. it is not known whether the lack of organics or varied particle sizes may affect burrowing), but it





**Figure 1** To create a vertical sediment temperature gradient, experimental chambers were sealed into coolers with the bottom protruding under the cooler and the top held inside the cooler (a – cut-away side view of chamber and sediment strata). Experimental treatment temperatures were maintained inside the coolers using submersible thermostatically controlled heaters (b), while coolers were floated in artificial streams (c), exposing the bottom sediment stratum to the experimental acclimation temperature.

does not introduce any confounding influences (e.g. parasites, pathogens or chemical toxicants), making it well suited for this application. Schwalb & Pusch (2007)

noted that the natural sediment in their unionid study area 'consist[ed] almost exclusively of sand (mean particle size  $[D_{50}] = 0.42 \pm 0.12$  mm)', indicating that the

substratum we used sufficiently represents at least some natural sediments.

### Test conditions

We conducted acute (96 h) thermal exposures in the watered and dewatered sediment treatments that consisted of seven temperature treatments as follows: a control held at 20 °C (ASTM, 2006a), an acclimation temperature (22 or 27 °C) and five experimental temperatures, four of which were similar between the two acclimation groups. Optimal acclimation and test temperatures were informed by results of Pandolfo *et al.* (2010), encompassed a range of probable summer stream temperatures and ensured sufficient mortality in the highest treatments for calculating LT50s. Test temperatures in the 22 °C acclimation exposures ranged from 27 to 37 °C, and test temperatures in the 27 °C acclimation exposures ranged from 31 to 39 °C. Similar temperature treatments between the two acclimation regimes facilitated the identification and analysis of any acclimation-related effects.

Mussels used in the thermal tests ranged in age from 12 to 17 months. Average shell lengths were 7.58 mm ( $\pm$  1.63 mm, SD) for *L. abrupta* and 4.78 mm ( $\pm$  0.72 mm) for *L. radiata*. Mussels within a species for a given test type differed in age by no more than 2 weeks. Juvenile mussels were acclimated to the test acclimation temperature by adjusting their arrival temperature by 2.5 °C day<sup>-1</sup>, with a standard minimum 24-h acclimation period once the target temperature was attained (ASTM, 2006a; Pandolfo *et al.*, 2010). The acclimation procedure used here is considerably more conservative than the recommended rate of 3 °C h<sup>-1</sup> (ASTM, 2006a). Shipping temperatures averaged 19.3 °C ( $\pm$  3.5 °C, SD) from June through August 2012. Experiments were non-aerated static-renewal tests with water renewed (90% volume) at 48 h (ASTM, 2006a). Seven mussels were in each of three replicates per treatment, with 10 mussels per replicate in controls.

Burrowing data were recorded upon completion of the 96-h thermal exposures in all tests. The number of mussels visible on the sediment surface in each chamber was recorded. Mussels were considered not burrowed if they were lying flat or relatively flat on the sediment surface and no burrowing attempt was apparent. Mussels were considered burrowed if they were visibly upright and in position for siphoning at the sediment–water interface, as indicated by the observation of mantle tissue or the anterior edge of the shell, or if they were not visible beneath the sediment–water interface. Those

that were burrowed in the siphoning position as described were recorded as burrowed and siphoning. Burrowing depth of mussels was further assessed by extruding the sediment within the mussel enclosure (inner chamber) into the three depth strata [top (0–2.5 cm), middle (2.5–6 cm) and bottom (6–8 cm)], inspecting it with a magnifying lamp, and recording the number of mussels present in each layer. The presence of byssal threads on juvenile mussels in each chamber at the end of tests was assessed visually as detected or not detected using a magnifying lamp.

Quality assurance and control were ensured by conducting all tests according to the Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (ASTM, 2006a), as modified for sediment testing. Thermometers used for daily temperature monitoring in the control incubator were certified for accuracy by the National Institute of Standards and Technology (NIST). Daily temperature of circulating water in the coolers and artificial streams was monitored with partial-immersion thermometers (Fisherbrand® Red-Spirit®, Fisher Scientific, Pittsburgh, PA, U.S.A.) that met NIST tolerances for accuracy. Target test temperature in water baths and artificial streams was  $\pm$  1 °C ( $n$  = 466) for 90% of trials and  $\pm$  2 °C for 97% of trials. Sediment temperature within treatment beakers was monitored with iButton® iBCod (Model 22L) submersible temperature data loggers and OneWireViewer software (version 0.3.15.50; Alpha Mach, Inc., Mont St-Hilaire, QC, Canada), with one logger placed in each of the three depth strata in one watered and one dewatered replicate per temperature treatment. The temperature loggers were placed at the surface in the top stratum, at approximately 4 cm (half of the sediment depth) in the middle stratum and at bottom of the beaker (8 cm depth) in the bottom stratum. Sediment temperature in the 20 °C controls was monitored only in the top and bottom strata. Mean water-quality conditions among all tests were 102.5 mg CaCO<sub>3</sub> L<sup>-1</sup> alkalinity, 137.5 mg CaCO<sub>3</sub> L<sup>-1</sup> hardness, 464.5  $\mu$ S cm<sup>-1</sup> conductivity, 8.30 pH and 7.27 mg L<sup>-1</sup> dissolved oxygen ( $n$  = 4 for alkalinity and hardness,  $n$  = 32 for all other variables). The lowest dissolved oxygen concentration in each test was observed in the hot-test treatment and was sufficiently high to support mussel respiratory demand (mean 6.32 mg L<sup>-1</sup>, range 5.98–6.54 mg L<sup>-1</sup>).

### Statistical analyses

The lethal effects of temperature treatments on mussels were analysed using survival data to calculate LT50s

and LT05s with the Trimmed Spearman–Kärber method (Comprehensive Environmental Toxicity Information Software (CETIS)<sup>TM</sup>, v 1.8.0.12, Tidepool Scientific, LLC, McKinleyville, CA, U.S.A.). The LT50 was defined as the temperature that caused mortality in 50% of the individuals in the exposed sample, and the LT05 caused mortality in 5% of the sample. LTs were calculated based on the treatment temperatures assigned to the surface layer of sediment and overlying water. LT50s and their 95% confidence intervals (CI) were compared between acclimation temperatures, test types (watered versus dewatered) and species, to detect significant differences when 95% CIs did not overlap (i.e.  $\alpha = 0.05$ ).

The effects of temperature, water level, thermal refuge and acclimation treatment on burrowing and byssus production were analysed with logistic regression (SAS PROC LOGISTIC; SAS version 9.2; SAS Institute, Inc., Cary, NC, U.S.A.). The most plausible, parsimonious models explaining burrowing and byssus production for each species with the fewest parameters were selected from all possible models using Akaike's information criterion adjusted for low sample sizes (AIC<sub>C</sub>; Burnham & Anderson, 2002). An additional analysis of the effect of age on byssus production was performed for *Lampsilis abrupta* by including a numeric term for age in the logistic regression model and comparing data from this study and an earlier study in which younger mussels (5 months old) from the same cohort were used (Archambault *et al.*, 2013). Because of the nature of the byssus data (i.e. one datum per replicate), analysis of interactive effects was not possible, and only main effects on byssus production were interpreted.

## Results

We achieved 100% ( $n = 597$ ) recovery of juvenile mussels from the sediment chambers among all experiments. Temperature monitoring in the sediment columns revealed consistent temperatures throughout the vertical column in control (20 °C) and acclimation (22 and 27 °C) treatments with an average differential of 0.3 °C, and establishment of a vertical thermal gradient in all other temperature treatments during each experiment (Fig. 2). All mussels were recovered from the top stratum (0–2.5 cm) of sediment upon test termination, and most were burrowed in an upright position for siphoning.

### Survival

Acute (96 h) LT50s ranged from 29.9 to 35.6 °C, with a grand mean of 32.8 °C, and watered and dewatered

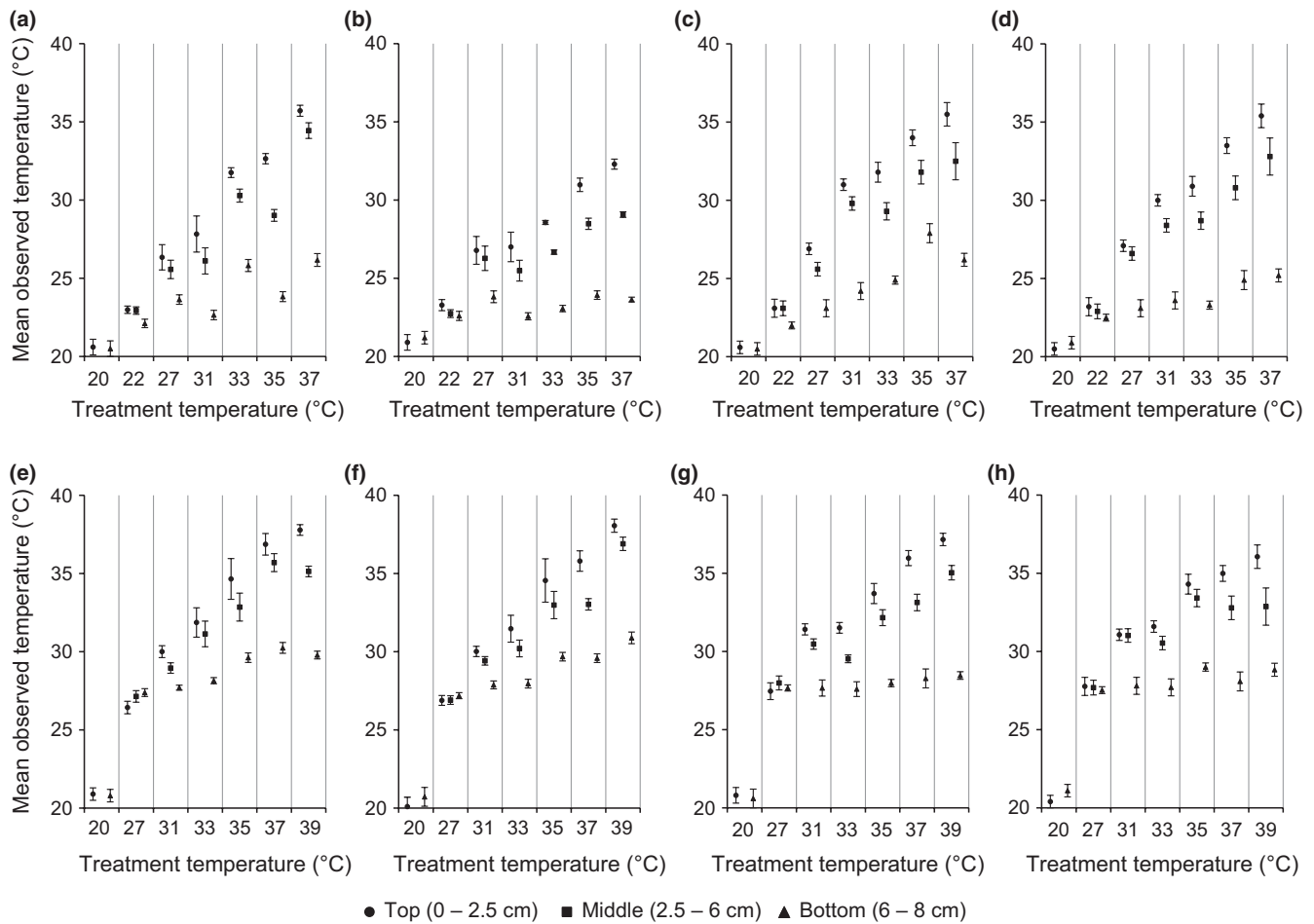
treatment means of 31.5 and 35.2 °C, respectively (Table 1). No significant effects of acclimation temperature or water level were detected in *L. abrupta*, and no significant effect of acclimation temperature was detected for *L. radiata*. In the 22 °C acclimation temperature tests, *L. radiata* was more thermally sensitive in the watered treatment, compared with the dewatered treatment. No significant difference was detected between species for a given acclimation and water level treatment combination. LT50s could not be determined in two cases due to lack of partial mortality responses (Table 1).

LT05s ranged from 18.7 to 32.5 °C, with a watered treatment mean of 26.2 °C, a dewatered treatment mean of 27.5 °C and a grand mean of 26.8 °C (Table 1). LT05s could not be determined from survival data in one case due lack of mortality, and 95% CIs could not be estimated in three cases. No significant difference was detected among tests that could be compared within or between species for a given acclimation temperature and water level (Table 1).

### Burrowing behaviour

We found a negative effect of elevated temperature on burrowing behaviour in both *L. abrupta* and *L. radiata* (Table 2; Fig. 3). Increasing temperature significantly reduced burrowing in both species, and effects of water level were apparent in *L. radiata* (Table 2). The most parsimonious logistic regression model explaining burrowing behaviour in *L. abrupta* included treatment temperature and acclimation temperature (Table 2). Treatment temperature significantly affected the burrowing behaviour of *L. abrupta* ( $P < 0.01$ ) (Fig. 3). For a given acclimation, every degree rise in temperature decreased the odds of burrowing by a factor of 0.917, or approximately 8%. Acclimation temperature was included in the model, but was not significant ( $P = 0.13$ ), and a model that contained only temperature was equally plausible (i.e., equivalent AIC<sub>C</sub> values).

The most parsimonious logistic regression model explaining burrowing behaviour in *L. radiata* included the main effects from all three experimental factors and two interactions (Table 2; Fig. 3). The water-level treatment interacted significantly with acclimation temperature ( $P = 0.02$ ), but not with treatment temperature ( $P = 0.07$ ). Despite the interactive treatment effects, increasing temperature always reduced burrowing, regardless of the water-level and acclimation treatment combination [i.e. when values for acclimation temperature (22 or 27 °C) and water level (0 or 1) were included



**Figure 2** Mean observed sediment temperatures ( $\pm$  SE) in the top, middle and bottom sediment strata for each of seven treatment temperatures in the 22 °C acclimation (a, *Lampsilis abrupta*, watered treatment; b, *L. abrupta*, dewatered treatment; c, *Lampsilis radiata*, watered treatment; d, *L. radiata*, dewatered treatment) and 27 °C acclimation (e, *L. abrupta*, watered treatment; f, *L. abrupta*, dewatered treatment; g, *L. radiata*, watered treatment; h, *L. radiata*, dewatered treatment) vertical thermal gradient experiments.

**Table 1** Median lethal temperatures (LT50) causing 50% mortality and protection-level lethal temperatures (LT05) causing 5% mortality (with 95% confidence intervals) in juvenile mussels at 22 and 27 °C acclimation temperatures in watered and dewatered sediment exposures (96 h)

Species	LT50				LT05			
	22 °C Acclimation		27 °C Acclimation		22 °C Acclimation		27 °C Acclimation	
	Watered	Dewatered	Watered	Dewatered	Watered	Dewatered	Watered	Dewatered
<i>Lampsilis abrupta</i>	31.9 A (30.7–33.3)	ND	33.3 AB (32.1–34.7)	35.6 B (34.7–36.5)	25.1 (ND)	18.7 (ND)	27.0 A (17.3–29.8)	31.3 A (29.1–32.6)
<i>Lampsilis radiata</i>	29.9 A (29.1–30.7)	34.8 B (34.1–35.6)	31.0 A (29.9–32.2)	ND	27.1 A (22.2–28.9)	ND	25.6 A (14.3–28.7)	32.5 (ND)

LT50 and LT05 values with the same letter for a given species are not significantly different. LT50 values between species for a given acclimation temperature and water-level treatment did not differ in any case. LT05 values between species for a given acclimation temperature and water level did not differ in any case that could be compared.

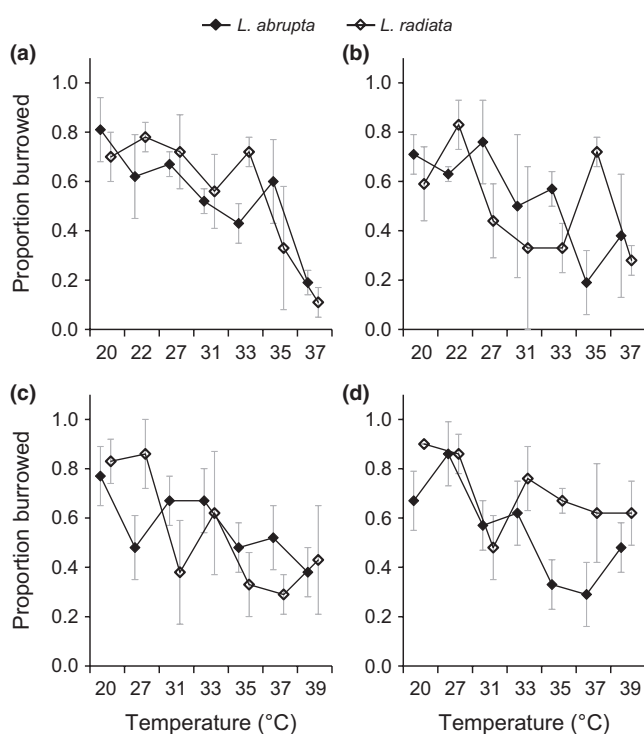
ND = value could not be determined.



**Table 2** Burrowing behaviour of *Lampsilis abrupta* and *L. radiata* tested in vertical-gradient thermal exposure experiments, as explained by the most parsimonious logistic regression models, selected using Akaike's information criterion, corrected for small samples (AICc)

Species	Model	Partial slope <i>P</i> -value						
		$x_1$ (temp)	$x_2$ (water)	$x_3$ (acc)	$x_4$ (temp*water)	$x_5$ (temp*acc)	$x_6$ (water*acc)	$x_7$ (temp*water*acc)
<i>Lampsilis abrupta</i>	$y = 1.5615 - 0.0870x_1 + 0.0527x_3$	<0.01		0.13				
<i>Lampsilis radiata</i>	$y = 2.8958 - 0.1272x_1 - 5.6491x_2 + 0.0487x_3 + 0.0539x_4 + 0.1728x_6$	<0.01	<0.01	0.35	0.07		0.02	

Let  $t$  = treatment temperature,  $w$  = water level, and  $a$  = acclimation temperature. Then let  $x_1 = t$ ;  $x_2 = w$ ;  $x_3 = a$ ;  $x_4 = t * w$ ;  $x_5 = t * a$ ;  $x_6 = w * a$ ; and  $x_7 = t * w * a$ . Coefficients for water level are for the dewatered treatment with respect to watered (reference level), and in all models,  $y$  = logit (burrowed/exposed), or the log odds of burrowing.



**Figure 3** Mean ( $\pm$ SE) proportion of mussels that were burrowed at the end of the acute (96 h) exposures in the (a) 22 °C acclimation, watered; (b) 22 °C acclimation, dewatered; (c) 27 °C acclimation, watered; and (d) 27 °C acclimation, dewatered experiments.

in the logistic model, the slope of the temperature coefficient ( $\beta_1$ ) remained negative, regardless of the combination].

Of the mussels that were burrowed at the end of the 96-h acute exposures, we observed a substantial proportion in position for siphoning surface water. In *L. abrupta*, 87% of burrowed mussels were observed in an upright siphoning position, and in *L. radiata*, 82% of those burrowed were in siphoning position. The mean

percentage of burrowed mussels that were siphoning in juveniles of both species was 84%. In both species, more than half of the chambers had 100% of the burrowed mussels in an upright siphoning position.

#### Byssus production

Increasing temperature significantly reduced byssus production in both species (Table 3). The most plausible logistic regression model explaining byssus production in *L. abrupta* included treatment temperature and water level (Table 3). After controlling for water level, each degree increase in temperature reduced the odds of byssus production ( $P < 0.01$ ) by a factor of 0.826, or approximately 17%. Water level was included in the model as a negative effect, but it did not significantly affect byssus production ( $P = 0.13$ ). In a second analysis that included mussel age and data from a previous study (Archambault *et al.*, 2013), age explained a significant amount of the variation in byssus production ( $P < 0.01$ ), causing a reduction by a factor of 0.776, or approximately 22%, per month (Table 4). In addition to showing an effect of age, the most plausible model of the larger data set also revealed significant effects of dewatering ( $P < 0.01$ ) and acclimation ( $P = 0.04$ ) on byssus production, in addition to temperature ( $P < 0.01$ ) (Table 4).

The most plausible logistic regression model explaining byssus production in *L. radiata* included treatment temperature and acclimation temperature; however, the effect of acclimation temperature on byssus production was not significant ( $P = 0.13$ ) (Table 3). Treatment temperature significantly reduced byssus production in *L. radiata* ( $P < 0.01$ ). Every unit increase in temperature (°C) reduced the odds of byssus production by a factor of 0.803, or approximately 20%.



**Table 3** Byssus production behaviour of *Lampsilis abrupta* and *L. radiata* in vertical gradient thermal exposures, as explained by the most parsimonious logistic regression models, selected using Akaike's information criterion, corrected for small samples (AIC<sub>C</sub>)

Species	Model	Partial slope <i>P</i> -value			Odds ratio estimate		
		<i>x</i> <sub>1</sub> (temp)	<i>x</i> <sub>2</sub> (water)	<i>x</i> <sub>3</sub> (acc)	<i>x</i> <sub>1</sub> (temp)	<i>x</i> <sub>2</sub> (water)	<i>x</i> <sub>3</sub> (acc)
<i>Lampsilis abrupta</i>	$y = 3.5481 - 0.1908x_1 - 1.3608x_2$	<0.01	0.13		0.826 (0.718–0.951)	0.256 (0.044 – 1.496)	
<i>Lampsilis radiata</i>	$y = 3.3183 - 0.2194x_1 + 0.1620x_3$	<0.01		0.13	0.803 (0.719–0.896)		1.176 (0.953–1.451)

Let *x*<sub>1</sub> = treatment temperature, *x*<sub>2</sub> = water level, and *x*<sub>3</sub> = acclimation temperature. Coefficients for water level are for dewatered with respect to watered (reference level); in all models, *y* = logit (byssus), or the log odds of byssus occurring; and odds ratios are point estimates, with 95% confidence intervals in parentheses.

**Table 4** Byssus production behaviour of *Lampsilis abrupta* in 2011 (age 5 months) and 2012 (age 17 months) thermal exposures, as explained by the most parsimonious logistic regression models, selected using Akaike's information criterion, corrected for small samples (AIC<sub>C</sub>).

A highly significant effect of age on byssus production was detected

Model	Partial slope <i>P</i> -value				Odds ratio estimate			
	<i>x</i> <sub>1</sub> (temp)	<i>x</i> <sub>2</sub> (water)	<i>x</i> <sub>3</sub> (acc)	<i>x</i> <sub>4</sub> (age)	<i>x</i> <sub>1</sub> (temp)	<i>x</i> <sub>2</sub> (water)	<i>x</i> <sub>3</sub> (acc)	<i>x</i> <sub>4</sub> (age)
$y = 12.9959 - 0.1894x_1 - 2.2558x_2 - 0.1995x_3 - 0.2660x_4$	<0.01	<0.01	0.04	<0.01	0.827 (0.760–0.900)	0.105 (0.037–0.297)	0.819 (0.679–0.989)	0.766 (0.695–0.845)

Let *x*<sub>1</sub> = treatment temperature, *x*<sub>2</sub> = water level, *x*<sub>3</sub> = acclimation temperature and *x*<sub>4</sub> = age. Coefficients for water level are for dewatered with respect to watered (reference level); *y* = logit (byssus), or the log odds of byssus occurring; and odds ratios are point estimates, with 95% confidence intervals in parentheses.

## Discussion

Laboratory research offers substantial scientific inference and provides repeatability in a controlled setting, such as strict application of treatment factors, replication and quality control, that may not be attainable by research conducted in the field. However, laboratory experiments often lack the ecological complexity and realism of field research, and their applicability may be diminished or lost when multiple field conditions must be considered (Odum, 1984). In many fields of study, mesocosm experiments are a useful tool for increasing environmental complexity while maintaining experimental control. Although mesocosm experiments have become increasingly common in terrestrial and aquatic ecological research, we are aware of relatively few such experiments incorporating freshwater mussels (e.g. Downing, Van Leeuwen & Di Paolo, 2000; Spooner & Vaughn, 2006; Allen & Vaughn, 2009) and only three that examined temperature effects on mussels (Spooner & Vaughn, 2008, 2012; Block, Gerald & Levine, 2013).

We successfully established an ecologically realistic vertical thermal gradient within the sediment column in our laboratory mesocosm tests with juvenile mussels. Within the five temperature treatments of each acclimation group, temperatures in the middle stratum (4-cm) averaged 1.8 °C cooler than temperatures in the top stratum. Similar conditions were recorded in a study

conducted at 20 sites in streams of the Tar River Basin within the Piedmont region of NC, U.S.A., suggesting that our laboratory methods produced conditions that mimic natural conditions in at least some freshwater mussel habitats. At the Tar River Basin sites, the maximum temperature 5 cm beneath the sediment–water interface from July to October 2011 averaged 1.9 °C cooler than that in the water column 10 cm above the sediment–water interface (T. Pandolfo, unpubl. data). These data support the increased realism of our experimental design; however, it is impossible to capture the wide variation of field conditions in this study alone. For example, Gough, Gascho Landis & Stoeckel (2013) observed much cooler temperatures just 2.5 cm below the surface compared with surface temperatures (average difference, 5.7 °C) in Opintlocco Creek (Alabama, U.S.A.) during a summer drought when the stream was drying.

Juvenile mussels regularly burrow deeper than 5 cm and even as deep as 20 cm (<3-cm size class; Schwalb & Pusch, 2007), but the mussels in our study never descended below the top stratum of sediment (2.5 cm). Schwalb & Pusch (2007) found that mussel surface densities of three unionid species were positively correlated with temperature and day length within the ranges they studied (13.5–26.2 °C, and 12.0–16.8 h daylight, respectively) and negatively correlated with flow discharge, with discharge as the dominant factor, explaining 53% of burrowing behaviour. Negishi *et al.* (2011) found

juvenile (<20 mm) *Pronodularia japonensis* (Lea), a freshwater unionid, near the surface (0–3 cm) in spring and summer and found that 70% of all mussels studied – both juveniles and adults – descended to greater depths in winter (3–8 cm). Amyot & Downing (1997) also observed seasonal vertical movements, finding that *Elliptio complanata* (Lightfoot) had a peak surface abundance in July that was closely correlated with water temperature. Our experiments were conducted from June through August, and mussels were deliberately exposed to warm temperatures, so the lack of deeper burrowing in our observations may have been related to environmental cues, such as day length, water treatment temperature or flow. A recent study during a 15-week drought in Alabama, U.S.A., found that when mussels in a creek responded to being stranded away from water by burrowing, they burrowed to only 3–4 cm depth (Gough *et al.*, 2013). If freshwater mussels, including juveniles, are more likely to remain near the surface in warmer summer months because of environmental cues, as reported in previous field research (Amyot & Downing, 1997; Schwalb & Pusch, 2007; Negishi *et al.*, 2011), they may have limited drive or ability to escape excessively low flows, high water temperatures or drying streams when such conditions are most likely to occur. The drive to escape unfavourable environmental conditions may be diminished by possible entrainment of their molecular clock by these environmental cues into a seasonal shallow burrowing pattern and may explain why mussels in our study did not seek out more favourable conditions in the cooler refugia of the middle and bottom strata of sediment.

Another factor that may have precluded deeper burrowing by juvenile mussels in our study is the ratio of substrate particle size to body size. The mean body size of all mussels in our experiments (5.90 mm  $\pm$  1.80 mm) compared with the substrate particle size (0.500–0.850 mm) yields a body/particle size ratio range of 11.8 (smaller particles) to 6.9 (larger particles). The large particles relative to body size and lack of fine particles or substrate heterogeneity may have been difficult for small mussels to negotiate to any appreciable depth. Troia & Ford (2010) suggested that vertical movements in unionids may not be affected by particle size, but their finding is from one species, and related research has not been conducted with juveniles. Gough *et al.* (2013) found that three mussel species burrowed only shallowly regardless of whether the substratum was dominated by sand or clay/silt. Deeper burrowing may also be affected by limited dissolved oxygen in more organic sediments. Because we used sterile sand, we did not measure subsurface dissolved oxygen, but such mea-

surement is appropriate in field studies and experiments using sediment with organic content.

### Survival

Evidence is mounting that freshwater mussels have a predictable range of thermal tolerances, regardless of exposure conditions. Among the few published studies on acute lethal temperatures for juvenile freshwater mussels, researchers reported findings similar to our 96-h LT50 mean (32.8 °C) and range (29.9–35.6 °C), further substantiating the predictable range of upper lethal thermal limits and suggesting that many freshwater mussel species respond similarly [31.5 and 33.0 °C, Dimock & Wright (1993); 35.8 °C (32.5–38.8 °C), Pandolfo *et al.* (2010); 35.6 °C (33.3–37.2 °C), Archambault (2012)]. Despite the similarity of lethal responses within and among species, mussels that fall into tolerant or sensitive thermal guilds (Spooner & Vaughn, 2008) may differ in their sublethal responses to thermal stress.

Our finding that *Lampsilis radiata* was more thermally sensitive in the watered treatment, compared with dewatered, at the 22 °C acclimation, is counterintuitive based on our previous thermal tolerance experiments in incubator settings, where we found either no difference in survival between water-level treatments, or greater thermal sensitivity of species in the dewatered treatments (Archambault, 2012). Moreover, field studies of mussel response to severe drought conditions support the inference that extreme drought conditions are clearly more detrimental to survival and mussel community composition than moderate drought conditions (Haag & Warren, 2008; Galbraith, Spooner & Vaughn, 2010; Gough *et al.*, 2013). The apparent greater tolerance in the dewatered experimental treatments here may have been related to microscale thermal environments within test chambers (e.g. the dewatered chambers may have retained heat less effectively than the watered chambers in a climate-controlled laboratory).

No effects of acclimation temperature on survival for a given species and water level were detected in our study. Acclimation effects on survival were also largely absent in previous studies (Pandolfo *et al.*, 2010; Archambault, 2012; Archambault *et al.*, 2013) that used the same acclimation period used here. These findings together further support the hypothesis that acclimation of juveniles may be unimportant in acute lethal tests when acclimation temperatures are relatively high and proximate in range (22 and 27 °C), despite the use of more conservative protocols for acclimation than those recommended by the ASTM (2006a).

### Burrowing behaviour

The negative effects of increased temperature and dewatering on burrowing behaviour are consistent with previous burrowing results for five mussel species evaluated in incubator-based temperature exposures with no vertical gradient present (Archambault *et al.*, 2013) and also support the findings of Bartsch *et al.* (2000) that adult unionids took longer to right themselves in sediment and had lower survival after emersion for up to 60 minutes in high (45 °C) air temperatures.

This is the second study in which we regularly observed juvenile mussels in siphoning position at the sediment–water interface. In a previous study, we observed a grand mean of 53% of burrowed juveniles siphoning among all experiments, and means for individual species ranged from 32 to 68% (Archambault *et al.*, 2013). Several previous studies have reported that juvenile unionids remain burrowed in the sediment for the first 2–4 years of life, garnering their nutrition primarily from sediment pore water by employing a pedal feeding strategy (Yeager, Cherry & Neves, 1994; Balfour & Smock, 1995; Strayer *et al.*, 2004; Schwalb & Pusch, 2007). It is plausible that the lack of organic material and microbes in the filter sand used here may have affected this behaviour, but nor was food available in the overlying water. It is also likely that juvenile freshwater mussels may regularly siphon at the sediment–water interface, a finding relevant to future research and applications on diet, contaminant exposure or other ecological processes with juvenile mussels.

### Byssus production

Like burrowing, byssal thread production was negatively affected by elevated temperatures. Increasing temperature reduced byssus production by 17–20% per degree Celsius. This finding supports previous research that evaluated the byssus production of three species, including *L. abrupta*, under similar conditions, and found an 18–35% reduction of byssus per degree Celsius (Archambault *et al.*, 2013). In earlier thermal experiments conducted in uniform temperature incubators (i.e. no thermal gradient), we also observed that dewatering affected byssus production and was the factor with the most drastic negative effect, reducing byssus in dewatered treatments by 93–99%, compared with watered, among three species evaluated (Archambault *et al.*, 2013). An environmental condition common to streams in the south-eastern United States during summer is the combination of warm temperatures and very low flows

due to seasonal drought, followed by flashy stream conditions caused by strong thunderstorms (NOAA, 2012). Our findings suggest that this combination may reduce byssus production, thereby inhibiting attachment, and then sweep juveniles downstream, resulting in mortality or dispersal to potentially unsuitable habitats.

In a review of freshwater mussel byssus literature, Haag (2012) reported that byssus production in most species generally senesces by 1 or 2 years of age. Our analysis of the apparent effect of age explained a significant amount of the variation in byssus production of *L. abrupta*. This finding supports the currently accepted concept that small, very young juveniles are the most prolific producers of byssus in most species and that production tapers with age and growth (Bradley, 2011; Haag, 2012). Moreover, this model substantiates the negative impacts of high temperature and dewatering on byssus production in native freshwater bivalves, and therefore the impacts on their attachment and drift capabilities.

In conclusion, we observed a burrowing pattern in juveniles that remained near the sediment surface, consistent with field research suggesting that mussels are associated with the sediment–water interface during warmer months (Amyot & Downing, 1997; Negishi *et al.*, 2011; Gough *et al.*, 2013). Our findings suggest that the availability of deep, hyporheic thermal refuges may not mitigate mortality or sublethal effects of thermal stress. Moreover, the availability of shallow thermal refuges may be critical to mitigating thermal stress (e.g. Gough *et al.*, 2013). Other environmental factors, such as daily temperature fluctuations and subsurface dissolved oxygen, are also likely important to mussel survival and behaviour, warranting future study. Several recent studies, including our research here, have highlighted the sensitivity of freshwater mussels to excessive temperatures and drought in both lethal and sublethal contexts (Dimock & Wright, 1993; Haag & Warren, 2008; Spooner & Vaughn, 2008; Pandolfo, Cope & Arellano, 2009; Pandolfo *et al.*, 2010; Archambault, 2012; Galbraith, Blakeslee & Lellis, 2012; Gough *et al.*, 2013). The impetus for most of these studies is the status of current climate trends of warming and increased stochasticity of precipitation patterns. While future research can refine understanding of these topics, we suggest it is more important for the knowledge to be promptly applied by resource managers concerned by the ongoing decline in mussel abundance and diversity and the loss of ecological integrity. Continued efforts by the research community to incorporate ecological complexity into laboratory and mesocosm experiments are needed so that we may continue to gain confidence in

our ability to predict accurately the effects of increasing temperature, drought and other stressors on freshwater mussels under natural conditions.

## Acknowledgments

Funding for this research was provided by the US Geological Survey (USGS) National Climate Change and Wildlife Science Centre through Research Work Order no. 171. We thank Chris Barnhart at Missouri State University for providing *Lampsilis abrupta* and Brian Watson at the Harrison Lake National Fish Hatchery for providing *Lampsilis radiata*. Kevin Gross provided valuable insight and assistance with statistical analyses. We thank Bobby Cope, Jeremy Leonard, Tamara Pandolfo and Angela White for laboratory and field assistance. The North Carolina Cooperative Fish and Wildlife Research Unit is jointly supported by North Carolina State University, North Carolina Wildlife Resources Commission, US Geological Survey, US Fish and Wildlife Service, and Wildlife Management Institute. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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(Manuscript accepted 13 November 2013)