Determining Optimum Temperature for Growth and Survival of Laboratory-Propagated Juvenile Freshwater Mussels

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ARTICLE

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Abstract

The effects of temperature on growth and survival of laboratory-propagated juvenile freshwater mussels of two federally endangered species, the Cumberlandian combshell Epioblasma brevidens and oyster mussel E. capsaeformis, and one nonlisted species, the wavy-rayed lampmussel Lampsilis fasciola, were investigated to determine optimum rearing temperatures for these species in small water-recirculating aquaculture systems. Juveniles 4–5 months old were held in downweller buckets at five temperatures. Growth and survival of juveniles were evaluated at 2-week intervals for 10 sampling events. At the end of the 20-week experiment, mean growth at 20, 22, 24, 26, and 28°C was, respectively, 0.75, 2.22, 3.27, 4.23, and 4.08 mm for Cumberlandian combshell; 1.35, 3.73, 3.81, 4.90, and 4.70 mm for oyster mussel; and 2.09, 3.96, 4.99, 5.13, and 4.87 mm for wavy-rayed lampmussel juveniles. Generally, temperature was positively correlated with growth of juveniles. Final mean maximum growth occurred at 26°C for all three species, although no significant differences in growth were detected between 26°C and 28°C. The relationship between temperature and survival of juveniles was less clear. Final survival was 82.5, 89.0, 91.0, 89.5, and 93.5% for Cumberlandian combshell; 73.0, 83.5, 78.0, 78.0, and 68.1% for oyster mussel; and 75.0, 89.5, 87.0, 86.5, and 89.5% for wavy-rayed lampmussel juveniles at the five temperature treatments, respectively. Based on the species used in this study, results indicate that 26°C is the optimum temperature to maximize growth of juvenile mussels in downweller bucket systems. The ability to grow endangered juveniles to larger sizes will improve survival in captivity and upon release into the wild and will reduce time spent in hatcheries. As a result, hatcheries can increase their overall production and enhance the likelihood of success of mussel population recovery efforts by federal and state agencies, and other partners.

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Because of significant declines of mussel populations in recent decades (Williams et al. 1993; Neves et al. 1997; Neves 1999), and with the culture and release of laboratory-propagated mussels into the wild being used as a recovery method (USFWS 2003, 2004; Jones et al. 2005, 2006; Eckert and Pinder 2010), there is a growing need to improve culture methods, particularly grow-out of propagated juveniles. Water temperature is a significant environmental variable affecting growth and survival of juvenile mussels in captivity and in the wild, which also affects various reproductive processes in adults, such as gametogenesis, spawning, and larval brooding (Krebs 1972; Hastie et al. 2000; Zimmerman and Neves 2002; Gosling 2003; Hastie et al. 2003; Zimmerman 2003; Jones et al. 2005; Negishi and Kayaba 2010; Pandolfo et al. 2010b). Temperature can affect mussel developmental and physiological processes and also has specific effects on different life stages (Krebs 1972; Negishi and Kayaba 2010; Pandolfo et al. 2010b). Efforts to propagate and culture mussels in captivity require an understanding of the environmental factors that influence growth and survival at different life stages. Thus, defining the optimum temperature for production of laboratory-propagated juvenile mussels is critical for optimizing propagation and culture success and thereby has important implications for their conservation.

In the past few years, it has become clear that larger and older laboratory-propagated juveniles have a significantly increased chance of survival when released in the wild than do newly metamorphosed juveniles (Sarrazin and Legendre 2000; Hua et al. 2011). Though methods have been developed to produce thousands of newly metamorphosed juveniles, refinement of culture methods to grow these species to larger sizes is needed. The ability to grow juveniles of imperiled species to larger sizes improves survival of individuals while captive and upon release to the wild by decreasing the incidence of predation in both settings. In addition, enhancing grow out of cultured mussels increases detection probabilities for subsequent monitoring and, most importantly, improves population recovery (Zimmerman et al. 2003; Hua et al. 2011).

The purpose of this study was to determine the effect of temperature on the growth and survival of juvenile (>4 months old and ≥1.5 mm) mussels of two federally endangered species, Cumberlandian combshell Epioblasma brevidens and oyster mussel E. capsaeformis, and one nonlisted species, wavy-rayed lampmussel Lampsis fasciola, in captivity. The intent of this research was to determine optimum rearing temperatures to maximize growth and survival of juvenile mussels of these three mussel species in captivity.

METHODS

Gravid mussel collection.—Juveniles were produced by the Freshwater Mollusk Conservation Center (FMCC) at Virginia Polytechnic Institute and State University (Virginia Tech) in Blacksburg, and Virginia Department of Game and Inland Fisheries’ Aquatic Wildlife Conservation Center (AWCC) near Marion, Virginia, following standard propagation and culture methods for these organisms. Gravid females of each species were collected in May 2011 by snorkeling and using view scopes in the lower Clinch River, Hancock County, Tennessee. Gravid individuals were held and transported to the FMCC and AWCC in coolers containing river water with aeration.

After arriving at the facilities, gravid females were placed in holding systems with maintained water temperatures of 15°C to prevent early glochidial release before infestation of host fishes could be conducted. The holding system at the FMCC contained 50–80 mm of river substrate (pebble, gravel) and water from the facility’s pond; the holding system at the AWCC contained 50–80 mm of coarse limestone gravel substrate and water sourced from the South Fork Holston River. Mussels were fed daily with a premixed commercial algae diet (Nanno 3600 and Shellfish Diet 1800 from Reed Mariculture, Campbell, California).

Host fish collection and care.—Based on the results of previous studies (Zale and Neves 1982; Yeager and Saylor 1995), Black Sculpin Cottus baileyi were used as the host fish for the Cumberlandian combshell and oyster mussel, and Large-mouth Bass Micropterus salmoides were used as the host for the wavy-rayed lampmussel. Black Sculpin were collected using a backpack electrofisher (Model LR24, Smith-Root, Vancouver, Washington) and Largemouth Bass were obtained from a regional fish farm in Arkansas.

Black Sculpin were held and transported to each facility in 140-L coolers containing local stream water. Salt was added to coolers to increase salinity to 0.7‰ in order to reduce fish stress during transport. Water temperature was maintained at ambient stream levels during transportation, and dissolved oxygen was maintained using an aerator. Transport time ranged from 1 to 2 h. After arrival at culture facilities, fish were acclimated to laboratory conditions, regarding temperature and salinity, and were quarantined for 2–3 d at a salinity of 3.0‰ prior to being infested with glochidia.

Infestation with mussel glochidia and juvenile mussel collection.—Host fish were infested with mussel glochidia following FMCC established nonlethal laboratory protocols (Zale and Neves 1982; Neves 2004). At the FMCC, 180 Black Sculpin were separated into groups of 45 fish, which were placed into one of four 16-L containers with 3.5 L of conditioned water at 21°C under continuous aeration. Glochidia from two gravid oyster mussels were mixed into each of the four containers (eight gravid oyster mussels in total) and allowed 45 min to attach to host fish. After infestation, host fish were moved into water-recirculating aquaculture holding systems. Water quality variables were monitored bi-weekly in the host-fish holding systems. Similar host-fish infestation methods were used at AWCC to produce juvenile mussels.

Once juveniles began to excyst from host fish, tank water was siphoned daily through 300-µm and 150-µm mesh sieves. Collected juveniles were rinsed into a petri dish, counted, and placed into 18-L downweller-bucket culture systems for growth
and development (Barnhart 2006; Figure 1). Buckets were filled with 18 L of filtered (<5 μm) pond (FMCC) or river (AWCC) water maintained at 20–24°C, and bucket water was exchanged once per week. At each water exchange interval, buckets were cleaned and standard water quality parameters were tested. Juveniles were fed continuously with a premixed commercial algae diet. Young juveniles experience a mortality bottleneck at 4–8 weeks of age and are susceptible to flatworm predation at small sizes (Henley et al. 2001; Jones et al. 2005); therefore, to remove any confounding factors, juveniles were cultured for 4–5 months to the desired initial size of 1–2 mm before the culture experiment was initiated. A summary of gravid mussel collection, captive holding conditions, and host fish infestation protocols are given for each species in Table 1.

Test conditions.—Juvenile mussels were acclimated to 20°C before testing and then allowed to acclimate to treatment temperatures gradually over a 24-h period. Temperature was controlled by a water bath surrounding the buckets that was held constant (± 0.5°C) through the use of heaters or chillers, and monitored daily using a temperature data logger (Onset Computer Corporation, HOBO Pendant Logger Model UA-001-08). Water quality testing in each bucket was conducted biweekly for ammonia (salicylate method, Hach Method 8155), nitrite (diazo method, Hach Method 8507), nitrate (cadmium reduction method, Hach Method 8171), dissolved oxygen, pH, and specific conductivity (YSI Professional Plus Multiparameter Meter). Total hardness (mg of Ca/L as CaCO₃ plus mg of Mg/L as CaCO₃) via the titration method (Hach Method 8213) and total alkalinity (Hach Model AL-AP; mg of phenolphthalein alkalinity/L plus mg total methyl orange alkalinity/L as CaCO₃) were tested on the source water once a week.

Mussels in each bucket were fed 500 mL daily (21 mL/h) of a premixed commercial algae formula (mean cell concentration, about 1.0–2.0 × 10⁶ μm³/mL) delivered continuously from a 1-L water bottle through a drip valve. Eighty water samples were taken randomly from the buckets over the course of the experiment in order to quantify the algal cell concentrations using a Coulter counter (Beckman Coulter, Multisizer 3) located at the AWCC. Algal concentrations also were measured by a hemocytometer and compared with those from the Coulter counter. Feeding bottles were cleaned, juvenile mussel holding chambers were rinsed, and bucket water was completely exchanged once a week. Air bubbles were removed from culture chambers and pumps and power sources and water levels were checked daily, as per the FMCC protocols.

Testing of Cumberlandian combshell and oyster mussel juveniles began 18 November 2011 and finished 4 April 2012. Testing of wavy-rayed lampmussel juveniles began 22 November 2011 and finished 12 April 2012. Mussels in buckets were sampled at 2-week intervals for 20 weeks to provide a total of 10 sampling events. Random samples of 10 of the 40 juveniles in each chamber were measured under a microscope (Olympus American, Model SZ40) to assess mean growth (i.e., mean length at time t minus initial length). All live individuals and shells within a chamber were counted to assess survival rates since the start of experiment. Shells of dead mussels were removed and documented. A summary of test conditions is given in Table 2.

Experimental design and statistical analyses.—Five temperature treatments were tested (20, 22, 24, 26, and 28°C), covering the range of normal (20–24°C) to upper (26–28°C) temperatures that mussels experience in the wild during the warmer months of the annual growth period in the Clinch River. The test was conducted in recirculating downweller bucket aquaculture systems, in which each bucket was independent of others and served as one experimental unit (EU) (Barnhart 2006).
TABLE 1. Summary of gravid female mussel, host-fish collection, and host-fish infestation methods at the Freshwater Mollusk Conservation Center (FMCC) and Aquatic Wildlife Conservation Center (AWCC) in 2011 used to produce juveniles used in this study. All gravid females were collected from the Lower Clinch River, Tennessee.

<table>
<thead>
<tr>
<th>Experimental detail</th>
<th>Cumberlandian combshell</th>
<th>Oyster mussel</th>
<th>Oyster mussel</th>
<th>Wavy-rayed lammp Mussel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility</td>
<td>AWCC</td>
<td>AWCC</td>
<td>FMCC</td>
<td>AWCC</td>
</tr>
<tr>
<td>Mussel collection month</td>
<td>June</td>
<td>June</td>
<td>May</td>
<td>July</td>
</tr>
<tr>
<td>Mussel holding system</td>
<td>150-L circular fiberglass tank</td>
<td>150-L circular fiberglass tank</td>
<td>300-L living stream</td>
<td>150-L circular fiberglass tank</td>
</tr>
<tr>
<td>Host fish species</td>
<td>Black Sculpin</td>
<td>Black Sculpin</td>
<td>Black Sculpin</td>
<td>Largemouth Bass</td>
</tr>
<tr>
<td>Fish collection site</td>
<td>Middle Fork Holston, Virginia</td>
<td>Middle Fork Holston, Virginia</td>
<td>South Fork Holston, Virginia</td>
<td>Regional Fish Farm, Arkansas</td>
</tr>
<tr>
<td>Fish holding system</td>
<td>AHAB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AHAB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Quarantine tank</td>
<td>RPS&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Infestation month</td>
<td>June</td>
<td>June</td>
<td>May</td>
<td>July</td>
</tr>
<tr>
<td>Number gravid mussels used</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Number of fish used</td>
<td>64</td>
<td>84</td>
<td>180</td>
<td>78</td>
</tr>
<tr>
<td>Infestation temperature (°C)</td>
<td>22–24</td>
<td>22–24</td>
<td>21</td>
<td>22–24</td>
</tr>
<tr>
<td>Duration of infestation (min)</td>
<td>60</td>
<td>60</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Infested fish recirculating aquaculture holding system</td>
<td>AHAB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AHAB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76-L Tanks&lt;sup&gt;b&lt;/sup&gt;</td>
<td>RPS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days till first excystments</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Number available</td>
<td>1,000</td>
<td>500</td>
<td>500</td>
<td>1,000</td>
</tr>
</tbody>
</table>

<sup>a</sup>AHAB = Aquatic Habitats, Inc. Z-Hab System.  
<sup>b</sup>A 2,000-L closed recirculating system made up of twenty 76-L tanks and two sumps.  
<sup>c</sup>RPS = recirculating propagation system.

RESULTS

Cumberlandian Combshell

For the five temperature treatment conditions we tested, final growth at 138 d for Cumberlandian combshell juveniles ranged from 0.75 mm at 20°C to 4.23 mm at 26°C (Table 3; Figure 2). Analysis of simple effects (i.e., separating the data by sampling events and conducting one-way ANOVAs at each time step) revealed significant differences in growth between temperature treatments at each of the 10 sampling events. Results of the mixed model analysis for growth indicated that the fixed effects of temperature, time, and temperature × time interaction were all significant.

Contrasts of differences in treatment means (effect size) for final growth (i.e., mean shell length at final sample minus initial mean shell length) revealed that growth at 20°C was significantly lower than that of all of the other treatment temperatures. Growth at 22°C was significantly lower than growth at 24, 26, and 28°C, and growth at 24°C was significantly lower than growth at 26°C and 28°C. No significant difference in juvenile growth was detected between 26°C and 28°C (P = 0.36).

Cumberlandian combshell juvenile survival ranged from 82.5% to 93.5% (Table 3; Figure 3). Examination of simple effects for temperature treatments at individual sampling events showed some significance (P = 0.05) of temperature on survival at the fourth (day 54) sampling event; however, survival was not
TABLE 2. Experimental design and test conditions for culture temperature tests of Cumberlandian combshell, oyster mussel and wavy-rayed lampmussel juveniles at the Freshwater Mollusk Conservation Center, November 2011–April 2012.

<table>
<thead>
<tr>
<th>Statistical analysis</th>
<th>Test conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental design</td>
<td>Randomized, repeated measures</td>
</tr>
<tr>
<td>Test system</td>
<td>Downweller buckets with six chambers</td>
</tr>
<tr>
<td>Test duration (weeks)</td>
<td>20</td>
</tr>
<tr>
<td>Test bucket volume (L)</td>
<td>18</td>
</tr>
<tr>
<td>Water renewal</td>
<td>Every 7 d</td>
</tr>
<tr>
<td>Initial age of juveniles</td>
<td>Cumberlandian combshell: 4.5 months</td>
</tr>
<tr>
<td></td>
<td>Oyster mussel: 5 months</td>
</tr>
<tr>
<td></td>
<td>Wavy-rayed lampmussel: 4.5 months</td>
</tr>
<tr>
<td>Initial size of juveniles (mm; mean ± SE)</td>
<td>Cumberlandian combshell: 2.2 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Oyster mussel: 1.5 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Wavy-rayed lampmussel: 1.8 ± 0.03</td>
</tr>
<tr>
<td>Chambers/species/bucket</td>
<td>1</td>
</tr>
<tr>
<td>Juveniles/chamber</td>
<td>40</td>
</tr>
<tr>
<td>Buckets (replicates)/ treatment</td>
<td>5</td>
</tr>
<tr>
<td>Juveniles/treatment</td>
<td>200</td>
</tr>
<tr>
<td>Feeding (each bucket/day)</td>
<td>0.05 mL Nanno 3600: 0.15 mL shellfish diet</td>
</tr>
<tr>
<td></td>
<td>1800: 500 mL conditioned water</td>
</tr>
<tr>
<td>Algal cell concentration in bucket</td>
<td>Mean range: 1.0 – 2.0 × 10^6 um^3/mL</td>
</tr>
<tr>
<td>Flow</td>
<td>Submersible pump^a, maximum flow = 590 L/h</td>
</tr>
<tr>
<td>Test water</td>
<td>Pond water filtered to &lt;5 μm, mean hardness = 200 mg/L as CaCO₃, alkalinity = 184 mg/L as CaCO₃</td>
</tr>
<tr>
<td>Test temperatures (°C)</td>
<td>20, 22, 24, 26, or 28</td>
</tr>
<tr>
<td>Water quality</td>
<td>Bi-weekly testing of ammonia, nitrite, nitrate, dissolved oxygen, pH, and specific conductivity</td>
</tr>
<tr>
<td>Sampling interval (d)</td>
<td>14</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Growth (mean length at time t minus mean initial length) and survival (proportion survival)</td>
</tr>
</tbody>
</table>

^aAquarium Systems Mini-jet model MN-606.

affected by treatment temperature at any other sampling event. Survival was not affected by temperature (P = 0.13), while the effects of time and temperature × time interaction were significant. Contrasts of differences in treatment means for final survival showed that survival was significantly lower at 20°C than at 24°C (P = 0.05) and 28°C (P = 0.03). The remaining final survival estimates were not significantly different between other treatment temperatures.

TABLE 3. Final growth and survival (mean ± SE) of Cumberlandian combshell (CC), oyster mussel (OM), and wavy-rayed lampmussel (WL) juveniles cultured in five temperature treatments. Values followed by different subscripts are significantly different (P < 0.05); the lowercase letters z–w indicate differences within species, the capital letters A–C differences within temperature treatments. The final sampling event occurred at 138, 138, and 141 d for CC, OM, and WL juveniles, respectively.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Species</th>
<th>Growth (mm)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>CC</td>
<td>0.75 ± 0.04 zA</td>
<td>2.22 ± 0.13 yA</td>
<td>3.27 ± 0.16 xA</td>
</tr>
<tr>
<td>OM</td>
<td>1.35 ± 0.09 zB</td>
<td>3.73 ± 0.10 yB</td>
<td>3.81 ± 0.14 yB</td>
</tr>
<tr>
<td>WL</td>
<td>2.09 ± 0.12 zC</td>
<td>3.96 ± 0.13 yB</td>
<td>4.99 ± 0.09 xC</td>
</tr>
</tbody>
</table>
FIGURE 2. Mean growth versus time for (a) Cumberlandian combshell, (b) oyster mussel, and (c) wavy-rayed lampmussel juveniles cultured in one of five temperature treatments. Growth measurements were taken at 2-week intervals for 20 weeks to provide a total of 10 sampling events.

FIGURE 3. Mean survival versus time for (a) Cumberlandian combshell, (b) oyster mussel, and (c) wavy-rayed lampmussel juveniles cultured in one of five temperature treatments. Survival was assessed at 2-week intervals for 20 weeks to provide a total of 10 sampling events.
Oyster Mussel

Final growth increment at 138 d for oyster mussel juveniles ranged from 1.35 to 4.90 mm across all temperature treatments (Table 3; Figure 2). Analysis of simple effects of temperature at each sampling event revealed significant differences in growth between temperature treatments at each of the 10 sampling events. Mixed-model analysis for growth indicated that the effects of temperature, time, and temperature × time interaction were all significant.

Contrasts of differences in treatment means for final growth revealed that growth at 20°C was significantly lower than growth at all other treatment temperatures. Significantly lower growth also was observed for the 22°C and 24°C treatments than growth at 26°C and 28°C. Growth was similar between 22°C and 24°C (P = 0.63) and between 26°C and 28°C (P = 0.25).

Substantial mortality was observed in one of the EUs of the 28°C treatment during the fourth sampling event (day 54), causing it to be a significant outlier for the survival analysis and violating the homogeneity of variance assumption. It is unlikely that the mortality observed was caused by temperature because (1) no other EU within the treatment experienced similar mortality, (2) mortality occurred in a single early sampling event, and (3) mortality ceased in this bucket for all further sampling events (sample events 5–10). It is possible that this single mortality event may have been induced by human error during sampling efforts (e.g., handling stress). Data from this outlier unit were removed from the mixed-model analysis of survival from the fourth sampling event forward (days 54–138) to reduce model variance and thereby to meet the assumption of homogeneity of variance. Final survival of oyster mussel juveniles ranged from 68.1% to 83.5% (Table 3; Figure 3). Analysis of simple effects for temperatures by time under this model revealed no significant differences in survival between any temperature treatments. Time was significant, whereas the effects of temperature (P = 0.16) and temperature × time interaction (P = 0.71) were not significant. Contrasts of differences in treatment means for final survival uncovered significantly lower survival at 22°C than at 28°C (P = 0.03). Final survival contrasts between all other temperature treatments were not significant.

Wavy-Rayed Lampmussel

Final growth at 141 d for wavy-rayed lampmussel juveniles ranged from 2.09 to 5.13 mm (Table 3; Figure 2). Analysis of simple effects for temperatures by time under this model revealed significant differences in growth between temperature treatments at each sampling event. All fixed effects for growth were significant.

Contrasts of differences in treatment means for final growth revealed that growth at 20°C was significantly lower than growth at all other temperature treatments. Significantly lower growth was observed at 22°C than at 24, 26, and 28°C. Growth at 24°C did not differ statistically from growth at 26°C (P = 0.44) and 28°C (P = 0.51). No significant differences in growth were detected between 26°C and 28°C (P = 0.15).

Wavy-rayed lampmussel juvenile final survival ranged from 75.0% to 89.5% (Table 3; Figure 3). Statistically significant differences in survival between some temperature treatments were detected at the second (day 29, P = 0.04) and sixth (day 86, P = 0.03) sampling events. No significant differences in survival between temperature treatments at other sampling events were revealed by examination of simple effects under this model. Survival was not affected by temperature (P = 0.10), while time and the temperature × time interaction effects were significant. Contrasts of differences in treatment means for final survival revealed significantly lower survival at 20°C than at 22°C (P = 0.04) and 28°C (P = 0.02). Final survival means were not significantly different between any of the other temperature treatment comparisons.

Algal Concentrations and Water Quality

Algal cell concentrations within buckets ranged from 1.54 to 2.06 × 10^6 µm^3/mL (mean = 1.80 × 10^6 µm^3/mL) and did not differ among temperature treatments (P = 0.23). Temperatures within treatments did not vary greatly from target temperatures (±0.2°C). Ammonia (mean, 0.01 mg/L as NH3), nitrite (0.005 mg/L as NO2), and nitrate (0.2 mg/L as NO3) concentrations within buckets stayed within acceptable levels. Water in buckets had a mean dissolved oxygen concentration of 7.33 mg/L, pH of 8.46, and specific conductivity of 393 µS/cm. The source pond water had a total hardness as CaCO3 range from 193.76 to 209.09 mg/L (mean = 201.42 mg/L) and alkalinity as CaCO3 was 174.76–193.68 mg/L (mean = 184.22 mg/L).

DISCUSSION

Previous experimental and observational studies have examined the direct effects of numerous factors affecting growth and survival rates of freshwater bivalves in captivity and the wild. Factors that have been found to correlate with mussel growth and survival rates include, but are not limited to, substrate type and size (Hinch et al. 1986; Liberty et al. 2007), flows and sediment load (Beaty 1997; Zimmerman 2003; Jones et al. 2005; Liberty et al. 2007; Rypel et al. 2008), toxicant exposure (Pandolfo et al. 2010a), mussel density (Hanson et al. 1988; Beaty 1997; Beaty and Neves 2004; Negishi and Kayaba 2009), food availability (Hanlon 2000), sampling frequency (Beaty 1997; Zimmerman 2003; Liberty et al. 2007), maturity of larvae (Jones et al. 2005), and temperature (Hanson et al. 1988; Buddensiek 1995; Beaty 1997; Hanlon 2000; Zimmerman and Neves 2002; Zimmerman 2003; Liberty 2004; Hanlon and Neves 2006; Pandolfo et al. 2010a, 2010b; Negishi and Kayaba 2010). These studies have helped define requirements for mussel propagation and culture by advancing understanding of factors affecting growth and survival and have shown that mussels are useful biological indicators of environmental change. Providing optimal temperatures for laboratory-propagated mussels is critical for propagation and culture success.

Due to their small size (<10–20 mm), juvenile mussels are difficult to detect in the wild, restricting field investigations to
adult life stages and making it difficult to examine effects of temperature and other factors on early life stages (Negishi and Kayaba 2010). Though growth rates of juveniles from field-based studies are uncommon, researchers have begun to close this knowledge gap by utilizing laboratory-propagated juveniles for experimental studies, as we have done. To our knowledge, no other studies have been published that directly tested the effects of temperature on the growth and survival of older and larger (>4 months, ≥1.5 mm) laboratory-propagated endangered juveniles with the goal of determining an optimal rearing temperature for maximizing culture success.

We found that temperature had a positive correlation with growth of Cumberlandian combshell, oyster mussel, and wavy-rayed lampmussel juveniles, which agreed with conclusions from previous studies regarding the effect of temperature on juvenile mussel growth (Buddensiek 1995; Beaty 1997; Hanlon 2000; Hanlon and Neves 2006). Further, the positive relationship between temperature and growth and the magnitude of growth varied between juveniles of these three species. These observed differences in juvenile mussel growth demonstrated that growth among these species varies in relation to water temperature. In contrast to previous studies, temperature was neither positively nor negatively associated with survival (Buddensiek 1995; Beaty 1997). The relationship between temperature and survival of juveniles was less clear within the time-scale and temperature treatments of this study. Even though survival did not statistically differ over time between treatments for all three species of juveniles, a few significant treatment comparisons between final survivals (at sampling event 10) were detected. Generally, it appeared that lowest survival occurred at 20°C, although some pairwise comparisons were not significant.

Prior to our study, we set a biologically important effect size for final growth between temperature treatments at 1 mm. For monitoring release and population success, juveniles are individually tagged in the laboratory with a Hallprint shellfish tag before being released into the wild. This tagging procedure requires that individuals be a minimum size of 10 mm because of the size of the tags (8 × 4 mm oval tag size). Thus, a difference in 1 mm between individuals can influence how soon juveniles can be tagged in the laboratory and then released to sites selected for population restoration. In addition to size influencing when juveniles can be released, survival of overwintering juveniles may be directly correlated with size, significantly improving the survival of individuals when released to the wild (Buddensiek 1995; Hanlon 2000; Sarrazin and Legendre 2000; Hanlon and Neves 2006; Hua et al. 2011). Greater size will also increase detection probability during monitoring efforts of released individuals and enhance the overall likelihood of population recovery success (Hua et al. 2011).

One goal of this study was to determine the optimum temperature for maximizing growth of juveniles in captivity. We found that maximum growth in shell length after approximately 4.5 months for Cumberlandian combshell, oyster mussel, and wavy-rayed lampmussel juveniles occurred at 26°C. However, growth at 26°C did not differ statistically nor biologically (difference < 1.0 mm) from growth at 28°C. Therefore, differences in final survivals within species were assessed to make evaluations between these two temperatures.

While Cumberlandian combshell and wavy-rayed lampmussel juveniles experienced highest final survival at 28°C, oyster mussel juveniles had the lowest survival at this temperature treatment. It is not clear whether high mortality at 28°C was due to approaching an upper thermal limit for oyster mussel juveniles, sampling stress, or factors other than temperature. Sampling procedure involves handling juveniles to obtain shell measurements and to estimate survival data; this requires short-term exposure to air, which can cause stress (Liberty et al. 2007). Several studies have reported lower mortality in juveniles that were sampled less frequently (Beaty 1997; Zimmerman 2003; Liberty et al. 2007). Considering that differences in survival of juveniles over time were not significant for the three species in our study, perhaps sampling frequency or other factors contributed to final mortality rather than temperature alone. With no statistical or biological difference detected between the 26°C and 28°C in growth and survival within species, and due to the unknown source of additional mortality at 28°C for oyster mussel juveniles, we incorporated conclusions of previous studies on water temperature relationships into our assessment of optimum rearing temperature for these species.

Water temperature is one of the most important environmental variables affecting growth and survival of juvenile mussels in captivity (Zimmerman 2003; Jones et al. 2005; Pandolfo et al. 2010a, 2010b). Several laboratory experiments have described the effects of temperature on growth and survival of freshwater bivalves during early life stages (i.e., newly transformed juveniles and <1-year-old juveniles; Buddensiek 1995; Beaty 1997; Hanlon 2000; Zimmerman 2003; Hanlon and Neves 2006; Pandolfo et al. 2010a, 2010b). Buddensiek (1995) found that growth rates and mortality of juvenile eastern pearlshell Margaritifera margaritifera were positively correlated with temperature. Similarly, Beaty (1997) reported a positive relationship between temperature and growth and survival of newly transformed rainbow Villosa iris. Hanlon (2000) also reported a positive relationship between temperature and growth in juvenile wavy-rayed lampmussels but showed seasonal variation in survival to suggest temperature is negatively associated with mortality. Hanlon (2000) further suggested that the relationship between temperature and survival is not always clear and that opposing study results may be due to resource availability at different experimental scales (i.e., streams are less likely to be food-limiting at higher temperatures than in a laboratory-scale experiment).

Two other studies examined temperature effects on survival during early life stages and determined acute lethal temperatures (LT50s) for glochidia and laboratory-propagated juveniles. The aims of these studies were to determine upper thermal limits of early life stages to provide insight into any effects that rising maximum water temperatures—due to global climate change—may have on mussel populations. Pandolfo et al. (2010b)
reported acute lethal thermal tolerances for glochidia of eight species and juveniles of seven species, ranging in age from <1–8 weeks old. They reported that mean LT50s in 96-h tests were 34.7°C for juveniles, and 31.6°C in 24-h tests for glochidia. Pandolfo et al. (2010b) concluded that the survival of these early life stages can decline significantly with small increases in temperature. Dimock and Wright (1993) also reported acute thermal tolerances for 1-week old juveniles of two freshwater mussel species and reported LT50s between 31.5°C and 33°C. Because our study goal was to determine optimum production temperatures, our experiment did not cover the upper temperature ranges (i.e., >30°C) considered in these studies, suggesting why we likely did not observe a clear relationship between temperature and survival for Cumberlandian combshell, oyster mussel, and wavy-rayed lampmussel juveniles.

Temperature has a significant effect on aquatic organism growth and survival rates in hatchery settings due to its influence on physiological processes such as respiration, filtration, and excretion rates (Zimmerman and Neves 2002; Spooner 2007; Spooner and Vaughn 2008; Pandolfo et al. 2010a, 2010b; Fitzgibbon and Battaglene 2012). These metabolic activities of mussels generally increase with higher water temperatures, i.e., within the natural range (Hanlon 2000; Spooner and Vaughn 2008; Vaughn et al. 2008). Typically, oxygen and food resources can become limiting with increasing water temperature (Hanlon 2000). The availability of dissolved oxygen in a system is negatively related to temperature and dependent on the water system (Hastie et al. 2003). The availability of food in a closed system is limited by the amount of supplemental diet dispensed to individuals exhibiting higher feeding rates in systems cultured at higher temperatures. Therefore, a combination of increased dissolved oxygen demand and feeding rates with lower availability of these resources at higher temperatures can strongly influence growth and survival. In addition, total ammonia concentrations have been shown to increase with increased excretion rates of mussels due to higher temperatures (Spooner and Vaughn 2008). Although ammonia toxicity (total ammonia) from increased water temperatures is negligible between 3°C and 30°C for fish in freshwater systems, early life stages of mussels are more sensitive to total ammonia concentrations than are other aquatic organisms (USEPA 1998, cited by Randall and Tsui 2002; Wang et al. 2007a, 2007b).

In healthy non-degraded streams, juveniles generally do not face issues with food and oxygen availability or ammonia toxicity because of the continuous influx of freshwater and high turnover rate. Conversely, experiments that are confined to small recirculating aquaculture systems, compared with streams, have a higher likelihood of encountering (if not managed properly) limited food and oxygen or increased ammonia levels at higher temperatures because of their lack of a continuous influx of freshwater (Hanlon 2000). As a consequence, juveniles may experience increased levels of mortality. Because of the possible occurrence of food and oxygen limitations and sublethal ammonia levels in small recirculating systems, researchers have been cautious about culturing juveniles at higher temperatures. These general temperature relationships were taken into consideration, even though food quantity was not a limiting factor in our experiment, and our experimental culture systems did not experience any abnormal dissolved oxygen or total ammonia levels.

Based on our analyses of final growth and survival and previously described temperature relationships, we believe that the optimal rearing temperature for maximum growth and survival in captivity is around 26°C for Cumberlandian combshell, oyster mussel, and wavy-rayed lampmussel juveniles. We believe our findings can be applied by researchers to improve laboratory culture methods for juveniles of other species of mussels. Present culture temperatures for juvenile mussels are set based on research manager discretion and source water temperatures, and sometimes overwintering juveniles in captivity are held below growing temperatures (i.e., <15°C). Researchers also have been cautious about culturing juveniles, particularly those of endangered species, at temperatures consistently exceeding 24°C because of concern of increased mortality. Results suggested that a simulated winter season is not necessary for continued mussel growth or survival. However, because some biologists believe laboratory-propagated mussels need to experience lower overwintering temperatures to be better adapted to natural conditions upon release, further investigation is needed to determine whether long-term survival after release is affected by the absence of a cooling-off period in captivity.

Determination of an optimum rearing temperature has clear implications for culturing of laboratory-propagated juveniles, and ultimately for conservation efforts. The culture and release of laboratory-propagated juveniles has been identified by federal species recovery plans and other documents as an approach to increasing the viability of existing populations or reintroducing species within their historical ranges (Williams et al. 1993; Neves et al. 1997; Neves 1999; USFWS 2003, 2004). Optimizing temperature to maximize growth and survival of mussels in hatchery settings reduces the length of time juveniles are held in the laboratory, allowing biologists to grow endangered juveniles to larger sizes more quickly and maximizing production levels relative to costs. Decreasing holding time is important because it reduces mortality in captivity (i.e., subjects them to less handling stress) and frees up space in hatcheries, thereby increasing the overall number of individuals produced for population recovery efforts by resource managers.

Understanding the relationship between temperature and mussel growth and survival across all life stages is important for optimizing propagation and culture success and, by extension, recovery of imperiled species. Our findings support previous conclusions that higher temperatures increase growth rates but neither supported nor contradicted conclusions on the relationship between temperature and survival. Upper thermal limits (i.e., >50% mortality over the duration of this experiment) were not observed for juveniles of the three species in our study. This experiment should be repeated with newly transformed
juveniles to determine if temperature affects growth and survival differently for younger and smaller juveniles. Furthermore, additional testing of growth and survival of juveniles within these temperature ranges (20–28°C) over a larger temporal scale and at higher temperature ranges (>28°C), is needed to reveal a clear relationship between temperature and survival and to understand and predict the potential effects of persistent high water temperatures on mussel populations due to global climate change (Hastie et al. 2003; Pandolfo et al. 2010b).

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