

REGULAR ARTICLE

ASSESSMENT OF BURROWING BEHAVIOR OF FRESHWATER JUVENILE MUSSELS IN SEDIMENT

Nile E. Kemble^{1*}, John M. Besser¹, Jeff Steevens¹, and Jamie P. Hughes²

¹ U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201 USA

² Veterans United, Columbia, MO 65203 USA

ABSTRACT

Standard laboratory sediment toxicity methods have been adapted for conducting toxicity tests with juvenile freshwater mussels. However, studies looking at juvenile mussel burrowing behavior at the water-sediment interface are limited. Juvenile mussels burrow in sediment for the first 0 to 4 yr of life but also may inhabit the sediment-water interface. The objective of this study was to evaluate burrowing behavior of various species and ages of juvenile freshwater mussels in three control sediments: West Bearskin Lake, Spring River, and coarse commercial sand. Species tested included (1) Fatmucket (*Lampsilis siliquoidea*), (2) Notched Rainbow (*Villosa constricta*), (3) Washboard (*Megaloniais nervosa*), (4) Rainbow (*Villosa iris*), (5) Arkansas Fatmucket (*Lampsilis powellii*), and (6) Oregon Floater (*Anodonta oregonensis*). Greater than 95% of the mussels burrowed into test sediment within 15 min. Across species, life stage, and substrate type, most mussels were recovered from the upper layers of sediment (91% at a sediment depth of 3.4 mm or less), and only 2% of the mussels were recovered at a depth >5.1 mm. No mussels were recovered from a depth >6.8 mm. There was no difference in mussel burrowing depth at 4 h versus 24 h across species, age, and sediment type. Two ages of Fatmucket burrowed to a significantly greater depth in the West Bearskin Lake sediment compared to the Spring River sediment or Coarse Sand. However, there was no significant difference in mean depth across sediment type with the other five species of mussels tested. Based on species and age of mussels tested, juvenile mussels up to an age of at least 20 wk and a length of at least 5 mm readily burrow into sediment and likely would be exposed to contaminants in whole sediment and associated pore water throughout a laboratory sediment toxicity test.

KEY WORDS: freshwater mussel, Unionidae, behavior, benthic ecology, Fatmucket, Notched Rainbow, Washboard, Oregon Floater, Rainbow, Arkansas Fatmucket, control sediments

INTRODUCTION

Freshwater mussels of the family Unionidae are widely distributed throughout North America. Mussels have been reported from lakes and streams, on substrata varying from mud and clay to sand and coarse gravel, and they are often associated with vegetation (Clarke 1973). Freshwater mussels are among the most imperiled groups of fauna in North America (Ricciardi and Rasmussen 1999; Lydeard et al. 2004; Strayer et al. 2004). North America has the world's most diverse freshwater mussel fauna, with more than 300 taxa, but over 70% of species are considered extinct, endangered,

threatened, or of special concern (Williams et al. 1993). The decline in the U.S. mussel fauna has been attributed to a variety of factors, including habitat modification, introduction of exotic species, over-utilization, and contaminants (Watters 1999; Wang et al. 2007a, 2007b, 2013; Bringolf et al. 2007; Okay and Karacik 2008; Cope et al. 2008; Downing et al. 2010; Besser et al. 2015).

The three main life stages of freshwater mussels are glochidia, juveniles and adults, and each life stage uses a different habitat. Glochidia are primarily found in the water column while in the free-living stage (Cope et al. 2008). Juvenile mussels reportedly burrow in sediment for the first 0 to 4 yr of life after transformation (Strayer et al. 2004; Schwalb

*Corresponding Author: nkemble@usgs.gov

and Pusch 2007; Cope et al. 2008). Numerous studies have documented the burrowing behavior of older juvenile and adult mussels (Lewis and Riebel 1984; Hull et al. 1998; Watters et al. 2001; Archambault et al. 2014; Block et al. 2013; Hazelton et al. 2014); they have been observed using their shell and foot to burrow into sediment. Though mussels generally are considered to be sessile, several studies have documented both vertical and horizontal movements (Kat 1982; Amyot and Downing 1991; Downing et al. 1993; Balfour and Smock 1995; Amyot and Downing 1997; Schwalb and Pusch 2007; Allen and Vaughn 2009). Populations of Eastern Elliptio (*Elliptio complanate*) were found to move up to 3 m/yr, and this movement was nondirectional (Balfour and Smock 1995). Horizontal movement of up to 15 cm/wk has been documented for Painter Mussels (*Unio pictorum*) and Duck Mussels (*Anodonta anatina*) in a river setting (Schwalb and Pusch 2007). Mussels may burrow completely or partially in sediment throughout the year, depending on water temperature (seasonal migration) and reproductive activity (Amyot and Downing 1991, 1997; Watters et al. 2001; Cope et al. 2008; Block et al. 2013).

Mussels spend much of their lives at or just below the sediment/water interface. This interface is a particularly important factor when assessing the environmental effects of chemical contaminants such as metals and persistent hydrophobic or nonpolar organic chemicals (e.g., oil and polychlorinated biphenyls). The sediment surface is the bioactive zone, where organisms interact with sediment and can receive the greatest exposure, whereas organisms may not be exposed to contaminants that are present in deeper sediments (National Research Council 2003). This upper layer of sediment is a microbially active layer and can have important redox properties that affect metal speciation and subsequent uptake and toxicity. Here mussels can be exposed to contaminants resulting in adverse effects on mussel recruitment, reproduction, or survival (Thorsen 2004; Cope et al. 2008; Hazelton et al. 2014).

Sediment toxicity bioassay methods, used to determine the bioavailability and toxicity of chemicals in sediment, rely on organisms that come in direct contact with the sediment. Standard laboratory organisms commonly used to conduct sediment toxicity tests are either epibenthic (e.g., amphipods) or create irrigated tubes on the sediment surface (e.g., midges) or into surficial sediments (e.g., mayflies). Juvenile freshwater mussels are an ideal candidate for use in toxicity bioassays because they are in direct contact with sediment. Standard bioassay methods using freshwater mussels in water-only bioassays have been developed (ASTM 2019a) and were modified in the current study to evaluate toxicity of field-collected sediments to mussels (Wang et al. 2013; Besser et al. 2011, 2015; Ingersoll et al. 2015; Schein et al. 2015). However, because freshwater mussels have a complex life history, they require specialized methods for laboratory culture (Neves 2004; Barnhart 2006), and there has been uncertainty about contaminant exposure and the role of bioavailability in laboratory toxicity tests with juvenile freshwater mussels. The

objective of this study was to improve our understanding of the burrowing behavior of juvenile mussels (3- to 20-wk-old mussels) and to determine whether species, age, or sediment type influences burrowing behavior.

METHODS

The six species evaluated included (1) Fatmucket (*Lampsilis siliquoides*; size 0.4–5.0 mm [about 3, 7, 10, and 20 wk posttransformation]); (2) Notched Rainbow (*Villosa constricta*; size 6.0–8.0 mm [about 20 wk posttransformation]); (3) Washboard (*Megaloniais nervosa*; size 1.0–1.5 mm [about 6 wk posttransformation]); (4) Rainbow (*V. iris*; size 1.4–2.5 mm [about 7 wk posttransformation]); (5) Arkansas Fatmucket (*L. powellii*; size 0.9–1.3 mm [about 4 wk posttransformation]); and, (6) Oregon Floater (*Anodonta oregonensis*; size 1.5–2.3 mm [about 4 wk posttransformation]). Starting lengths of mussels were determined to the nearest 0.1 mm with a digitizing system using video micrometer software (Image Caliper, Resolution Technology, Dublin, OH, USA). Test organisms were obtained from Missouri State University cultures (Chris Barnhart, Springfield, MO, USA).

We conducted exposures using three sediments with different physical properties. Sediments evaluated included two commonly used control sediments: (1) West Bearskin Lake sediment, a sand/silt/clay mixture (49% sand) with a total organic carbon (TOC) content of about 3% obtained from northeastern Minnesota (Ingersoll et al. 1998), and (2) Spring River sediment, a predominantly fine sand (82% sand) with a TOC content of about 1% obtained from southwest Missouri (Besser et al. 2011) as well as (3) a coarse commercial sand with a diameter of <0.5 mm (Granusil, no. 4030) purchased from Menards (Eau Claire, WI, USA). Sediments were selected because they have been used successfully as control sediments in previous sediment toxicity exposures (Kemble et al. 2013; Ingersoll et al. 1998; Besser et al. 2011, 2015).

Experiments were conducted in clear 60-mL Monoject plastic syringes (Covidien, Mansfield MO, USA), which were modified by cutting the top off, leaving the top open to produce a 3.5-cm diameter opening (Fig. 1). Before the start of an exposure, test sediment was homogenized with a plastic spoon in a stainless-steel bowl. The syringe handle was pulled back to the 30-mL mark on the syringe, and 40 mL of one of three control materials was placed inside the syringe using a small scoop. About 10 mL of overlying water was gently poured over the sediment to maintain a flat sediment-water interface. The source of the overlying water was well water diluted with deionized water to a hardness of about 100 mg/L (as CaCO₃), an alkalinity of 85 mg/L (as CaCO₃), and a pH of about 8.2. A 24-h equilibration period was used to let sediments settle out of the water column before the introduction of mussels.

Up to 20 mussels were placed into each of the substrates (e.g., typically five mussels/replicate syringe with a total of four replicates/species/treatment) for either 4 or 24 h under static conditions. This stocking rate provided ~306 mm² of

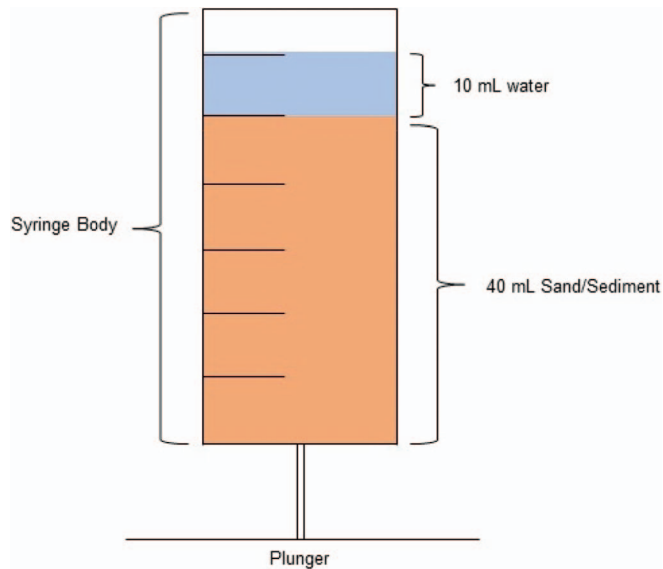


Figure 1. Design of the chambers (60-mL syringes) used to evaluate burrowing behavior of mussels in sediment.

surface area of sediment/mussel/chamber. For exposures using older Fatmucket Mussels and Notched Rainbow Mussels (5 mo old), we exposed one mussel/test chamber with additional replicate chambers/substrate tested (five replicates chamber/species). Using a pipette, mussels were stocked below the water surface of a syringe. We observed test chambers after stocking and recorded the time to complete burrowing. Chambers were then placed in a water bath at 23°C with a light intensity of about 500 lux (16L:8D photoperiod in the 24-h exposures). We did not feed mussels during the exposures. Average burrowing depth was the endpoint evaluated in exposures.

Water quality analysis was conducted on overlying water siphoned off at the end of the exposures. Given the small volume of overlying water, we were able to measure only dissolved oxygen, conductivity, pH, and total ammonia in most of the exposures. Ranges of the water quality parameters in the exposures were dissolved oxygen, 6.6 to 8.7 mg/L; conductivity, 238 to 935 μ S/cm; pH, 7.86 to 8.50; total ammonia, 0.11 to 13.0 mg N/L; and unionized ammonia, 0.004 to 0.548 mg N/L (Appendix 1). The wide ranges of some water quality parameters are a result of using both artificial and natural sediments as a substrate.

At the end of the exposures, we recovered mussels from select sediment sections by siphoning off the overlying water with a pipette, then gently pressing the syringe plunger to the first 1-mL mark until a 1.7-mm section of sediment was exposed at the top of the syringe. The extruded sediment was then scraped off the top of the syringe using a stainless-steel spatula into a glass dish. Sediment in the glass dish was gently rinsed using a squirt bottle to break the small clump of sediment apart so the mussels could be counted in that section. We repeated this process using 1.7-mm sections of sediment until all the mussels had been recovered from the syringe.

Average burrowing depth was calculated using the midpoint of a sampling section of sediment (i.e., 0.85 used for the 0–1.7 mm section) from a syringe. For the exposures with younger mussels (e.g., 3 wk old), sections of sediment were scraped into a 150- μ m sieve and rinsed gently with test water. Material remaining on the sieve was rinsed into a small Petri dish. A microscope was used for counting mussels in the Petri dish from each 1.7-mm section.

Statistical analyses were performed using SAS statistical software (SAS/STAT version 9.2; SAS, Cary, NC, USA). Average burrowing depth of mussels was determined by (1) species, (2) mussel age, (3) sediment type, (4) study duration, or (5) a combination of these four. Differences in average burrowing depth of mussels were determined by analysis of variance (ANOVA). Burrowing depth data were transformed before ANOVA to improve normality as indicated by Shapiro–Wilk test (United States Environmental Protection Agency 2000; ASTM International 2019b). If transformations (square root or log) did not improve normality, data were rank transformed before analysis (Conover and Iman 1981).

RESULTS

Nearly all mussels burrowed into sediments after being stocked into the chamber, except one 20-wk-old Notched Rainbow collected on the surface of the West Bear Lake sediment after 24 h. Most mussels (90%) burrowed within 15 min of introduction to the test chambers. Shell size and age of mussels had no effect on the time it took for a mussel to completely burrow into a test sediment. Most mussels across species and age were recovered from the upper two layers of the three substrates (91% at sediment depths of 0.0 to 1.7 or 1.7 to 3.4 mm; Fig. 2, Appendix 2) with only 2% of mussels recovered at a depth >5.1 mm. No mussels were recovered from a sediment depth >6.8 mm. Mussels that were partially burrowed at the end of a bioassay were counted as being burrowed. Overall, there was a general trend for the older mussels to be recovered from a greater depth in sediment than younger mussels. However, this trend may be the result of fewer older mussels being tested and the fact that only one 20-wk-old mussel was tested per replicate. Similarly, we also observed a trend for mussels tested in the West Bear Lake treatment to be recovered at greater depths compared to the Spring River and Coarse Sand treatments (Fig. 3).

In our initial comparison of burrowing behavior with juvenile mussels we evaluated burrowing at two exposure times (4 and 24 h). However, our results showed there was no difference in average burrowing depth of mussels in any of the test sediments between the two study durations (Appendix 2). Therefore, all later exposures were conducted for 24 h only. Because there was no significant difference in mean mussel depth in the two study durations, we used replicate data from both the 4- and 24-h trials to determine mean burrowing depth where we had data for both exposure times.

Because the Fatmucket is a common test species it was the only species for which different age mussels were tested across

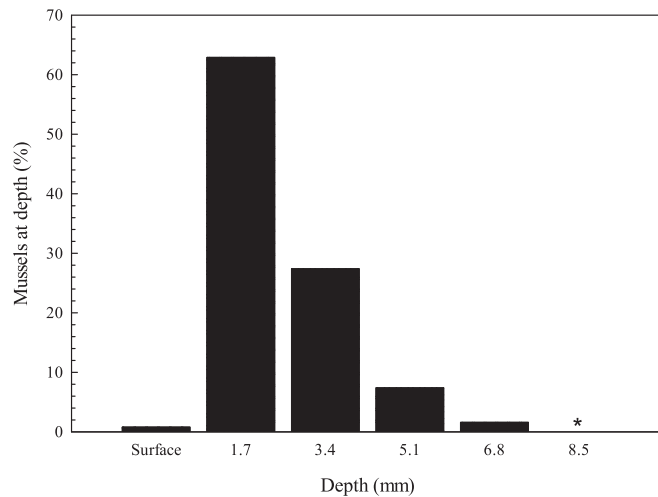


Figure 2. Percentage of all mussels recovered at a sampling depth. * = 0% recovered from a depth.

the three sediment types. The mean burrowing depth of 10- and 20-wk-old Fatmucket was significantly greater than the 3-wk-old Fatmucket in the West Bear Lake sediment. However, in the Coarse Sand and the Spring River sediment, only the 10-wk-old Fatmucket were recovered at a significantly greater depth compared to the other ages of Fatmucket tested (Appendix 3; Fig. 4).

Overall, 3- and 20-wk-old Fatmucket burrowed significantly deeper in the West Bear Lake sediment compared to the Spring River sediment and Coarse Sand. These age groups also burrowed more deeply than the 7- and 10-wk-old Fatmucket (Appendix 3). Mean burrowing depth of 7- and 10-wk-old Fatmucket was similar across all three sediment types (Appendix 3). We recovered 20-wk-old Fatmucket at a mean depth 3.3 times deeper in the West Bear Lake sediment than in either the Spring River sediment (0.85 mm)

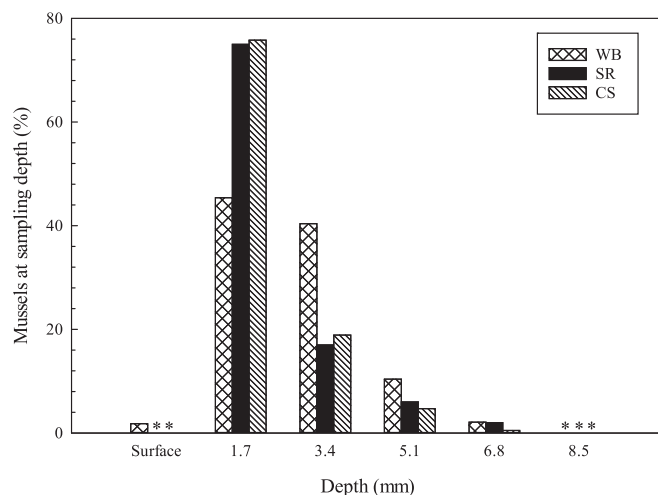


Figure 3. Percentage of mussels recovered 4 or 24 h at sampling depths by sediment type. * = 0% recovered from a sampling depth. WB = West Bear Lake sediment, SR = Spring River sediment, CS = Coarse Sand.

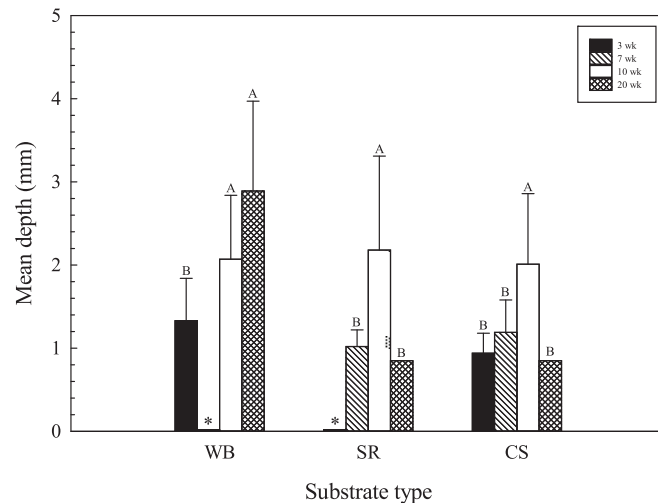


Figure 4. Percentage of Fatmucket mussels recovered at 4 or 24 h at sampling depths by sediment type. * = Age not tested. Different letters designate a significant difference in burrowing depth within a sediment type. WB = West Bear Lake sediment, SR = Spring River sediment, CS = Coarse Sand.

or Coarse Sand (0.85 mm). West Bear Lake sediment has more fines (higher silt and clay content) and is less dense than either the Spring River sediment or the Coarse Sand.

No significant difference in mean burrowing depth was detected between species (Arkansas Fatmucket, Rainbow, and Washboard) in multiple test sediments (Appendix 2). All 4-wk-old Arkansas Fatmucket were recovered at a depth of 3.4 mm or less in the three sediments. Individual Arkansas Fatmucket burrowed deeper in the Coarse Sand than in the two natural sediments, but there was no significant difference in average burrowing depth of 4-wk-old Arkansas Fatmucket based on sediment type (Appendix 2). Similar to the Arkansas Fatmucket exposures, 6-wk-old Washboard were recovered at deeper depths in the Coarse Sand than in the West Bear Lake or Spring River sediments, but there was no significant difference in burrowing depth of Washboard in the three sediments (Appendix 2). Similarly, we observed no significant difference in mean burrowing depth of 7-wk-old Rainbow across the three sediments (Appendix 2).

There was no general pattern in mussel burrowing behavior when we compared different species of similar-age mussels across the three sediment types (Fig. 5). However, when we compared age groups (i.e., 3–4 wk, 6–7 wk, and 20 wk), we observed differences in burrowing depth between the ages tested. The 20-wk-old mussels burrowed significantly deeper in the West Bear Lake sediment compared to mussels 7 wk old or younger. In the Spring River sediment, 10-week-old mussels burrowed significantly deeper than the 4-, 6-, and 20-wk-old mussels. In the Coarse Sand treatment, mean burrowing depth of 6- and 10-wk-old mussels was significantly deeper than mean depths of the 3- and 20-wk-old mussels (Appendix 2).

Mussels of similar ages tended to burrow to similar

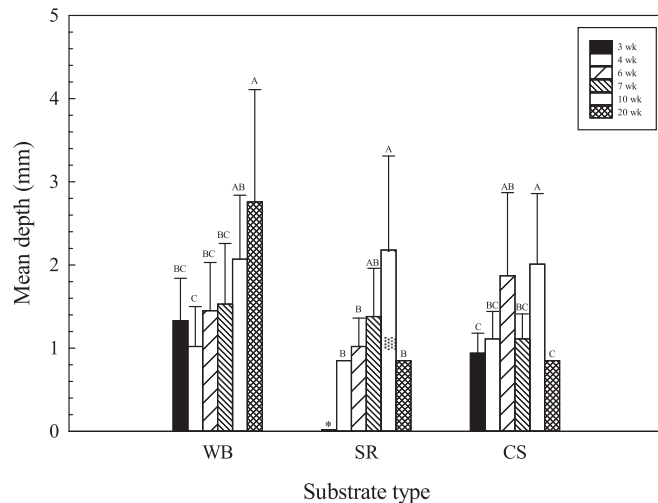


Figure 5. Mean mussel burrowing depth of all species tested at 4 and 24 h in three substrates by mussel age. Mean depths with different letters indicate a significant difference across age within a sediment type. * = Age not tested. WB = West Bear Lake sediment, SR = Spring River sediment. CS = Coarse Sand.

depths. Mean burrowing depth of the 3- to 4-wk-old mussels ranged from 0.85 to 1.33 mm in the West Bear Lake sediment and 0.94 to 1.11 mm in the Coarse Sand. A single 3-wk-old Fatmucket was recovered at a depth greater than 3.4 mm in the West Bear Lake sediment, but there was no difference in mean burrowing depth across the three species in the West Bear Lake treatment (Appendix 2). Similarly, we observed no significant difference in burrowing depth of the Arkansas Fatmucket and Fatmucket in the Coarse Sand. In exposures with 7-wk-old mussels, Rainbow were recovered at greater depths than the 7-wk-old Washboard in West Bear Lake and deeper than the Washboard and Fatmucket in Spring River. We recovered 18% of the Rainbow at a depth >3.4 mm in the West Bear Lake treatment, while 100% of the Washboard were recovered at <3.4 mm. However, there was no significant difference in mean burrowing depth of the two species (Appendix 2). Similarly, we recovered 10% of the 7-wk-old Rainbow at a depth of >3.4 mm in the Spring River sediment, while 100% of Fatmucket and Washboard were recovered at depths <3.4 mm. However, there was no significant difference in mean burrowing depth of the three species in the Spring River sediment (Appendix 2). In the Coarse Sand, 6-wk-old Washboard were recovered at a deeper average mean depth (1.85 mm) than either the 6-wk-old Fatmucket (1.19 mm) or 6-wk-old Rainbow (1.0 mm). Again, no significant difference in mean burrowing depth between the three species in the Coarse Sand was observed (Appendix 2). A similar pattern was observed with the older mussels. Notched Rainbow were recovered at a wider range of sediment depths compared to the 20-wk-old Fatmucket. However, there was no significant differences in mean burrowing depths of the two species in the West Bear Lake sediment (Appendix 2).

DISCUSSION

Based on the species and age of mussels tested in the current study, juvenile mussels up to an age of 20 wk readily burrow into sediment as was reported by Yeager et al. (1994). They reported 1- to 2-wk-old Rainbow burrowed within 20 min of being placed into the sediment with similar grain size characteristics of the natural sediments used in the current study. With the exception of one individual, mussels in the current study were completely burrowed within 15 min in all sediments and remained below the sediment surface for the duration of the study.

Burrowing depth varies based on the age of the mussel. The deepest we observed mussels in the syringes was 6.8 mm in the two natural sediments, similar to what Yeager et al. (1994) reported in feeding and burrowing studies in which juvenile Rainbow were recovered in the top 1 cm of sediment. Juvenile mussels have been recovered from much greater depths in field studies. Mussels 0–3 yr old were recovered from the top 8 cm of sediment (Neves and Widlak 1987) and Schwalb and Pusch (2007) recovered mussels up to a depth of 20 cm. However, juvenile mussels in these studies included older mussels, up to 3 yr in age. One potential limitation to burrowing depths observed in the current study was the size of the study chambers used (maximum depth, 36 mm). However, because the greatest observed burrowing depth was only 6.8 mm, we do not believe that space limitation in the syringe prevented the mussels from burrowing deeper. No mussels were recovered in the lowest sediment fraction of the syringe in any of our study Gough et al. (2013) and Archambault et al. (2014) also found mussels at shallow depths, indicating that space for vertical or horizontal movement was not a limitation. Gough et al. (2013) found that adult Pondhorn (*Unio tetrasmus*), Giant Floater (*Pyganodon grandis*), and Southern Fatmucket (*Lampsilis straminea*) burrowed to shallow depths (a few centimeters) instead of moving to greater depths with reducing water levels. Archambault et al. (2014) found that under thermal stress, juvenile Pink Mucket (*Lampsilis abrupta*) and Eastern Lampmussel (*Lampsilis radiata*) did not burrow below the top stratum of sediment (2.5 cm).

In the current study, we exposed six different species of mussels and found no difference in burrowing depth or behavior between species of similar age. However, burrowing behavior of a mussel community depends on the diversity of the community (Allen and Vaughn 2009). When a mussel community was manipulated (i.e., density and diversity were manipulated by increasing the number of mussels or the number of species within a treatment), Allen and Vaughn (2009) observed significant differences in shell exposure (i.e., shell above sediment surface) and both vertical and horizontal movement between species. However, all the exposures conducted here were single-species exposures and differences in burrowing depth and behavior may have resulted had we tested with multiple species in a syringe.

Previous studies have shown that mussel density may affect vertical movement. Mussel density in the current study

was reduced from five mussels per chamber to one per chamber when we tested with larger mussels. In contrast to Allen and Vaughn (2009), it is unlikely, given the size of mussels tested, that burrowing depth with any of the six species tested was affected by density within a sediment.

Mussels in the current study showed no general pattern in burrowing behavior across the three sediment types (Fig. 5). If the physical characteristic of the sediment is important in determining the distribution, then relative ability of a mussel to burrow in different sediment types may be important in establishing and maintaining suitable habitats for survival, growth, and reproduction (Kat 1982). This is especially important for juvenile mussels in habitats prone to water-flow alterations, sedimentation, and erosion. While a clay substrate proved more difficult for the Eastern Elliptio and Giant Floater (*Anodonata grandis*) to right on than sand or gravel, both species and the Eastern Lampmussel (*Lampsilis radiata*) burrowed significantly deeper in clay in 30 min than the other two substrates (Lewis and Riebel 1984). Lewis and Riebel (1984) concluded that the substratum particle size had an influence on the ability and speed of righting and burrowing of unionid mussels. However, in the current study, only the 20-wk-old mussels were generally found at deeper depths in the West Bearskin Lake substrate. It is unclear if this result is due to the fact that West Bearskin Lake is a much finer substrate or if our sampling method artificially increased depth by pushing mussels deeper as we scraped a sediment section.

Similar to water temperature, photoperiod is thought to play a role on vertical migration of mussels. Vertical migration of adult Fatmucket was found to be more correlated with day length than with water temperature in both field populations and artificial streams (Perles et al. 2003). In the current study, the photoperiod was the standard 16:8 (light:dark) for all exposures (ASTM 2019a, 2019b). This photoperiod corresponds to a mid-April to mid-July time frame, when high densities of adult mussels have been reported at the sediment-water interface (Amyot and Downing 1991; Balfour and Smock 1995; Amyot and Downing 1997; Watters et al. 2001). Juvenile mussels are thought to stay in the substrate for the first couple years of life. Longer exposures in the syringes, along with alternating the photoperiod, could be conducted to determine whether day length or duration have an effect on burrowing behavior of young mussels (i.e., daily movement or depth used by young mussels).

Rapid burrowing by young mussels, as observed in this study, might provide protection from strong currents in a stream; remaining burrowed also might reduce the chance of being dislodged and relocated to less suitable habitat. Mussels 1 to 14 d old have been recovered at depths about two to three times deeper than in the current study (Yeager et al. 1994). One potential explanation is that the present study was conducted under static conditions while the other studies were done under flow-through conditions. Adult Freshwater Pearl Mussel (*Margaritifera margaritifera*) will burrow as deep as necessary to avoid being dislodged by the current (Thoms and Berg 1985). Schwalb and Pusch (2007) reported no significant

difference in the distance moved among three species and found that the dynamics of surface densities of mussels could be explained by discharge, day length and water temperature, and those mussels may circumvent dislodgement in extreme flows by burrowing deeper into the sediment in riverine systems. These studies suggest that burrowing behavior is flexible in response to environmental conditions, so that may explain why we do not see a wide range of burrowing behavior in laboratory tests under controlled conditions.

Little research has been done evaluating juvenile mussel burrowing behavior. The current study examined burrowing behavior of several species of juvenile mussels under controlled conditions (i.e., testing with control sediments only, a set temperature). However, many of the factors that other investigators have found to affect older mussel burrowing behaviors could be evaluated with the methods and test apparatus used here. Study duration and species type did not affect burrowing depth of the mussel. However, additional studies with a longer duration may be needed to fully evaluate burrowing behavior over time. Also, due to the lack of mussel availability, we did not test all four ages with all the species. We also observed a general trend for older mussels to burrow to a greater depth than younger mussels. By burrowing to greater depths, older mussels may be exposed to different contaminants, or to lower levels of contaminants, than mussels remaining at or near the sediment-water interface, where contaminants tend to accumulate (Mulligan and Law 2013). Additional studies with older juveniles (>20 wk) in larger chambers also may help determine if and when juvenile mussels might inhabit the sediment-water interface and begin filtering overlying water. Additional studies could examine juvenile burrowing behavior in field-collected sediments evaluating mussel exposure of contaminants at the sediment/water interface to whole sediment and porewater throughout a sediment toxicity test. These additional studies would provide needed information about the benthic ecology of this imperiled group.

ACKNOWLEDGMENTS

We would like to thank the members of the Columbia Environmental Research Center Toxicology Branch along with Chris Barnhart and staff at Missouri State University for their technical support and for providing test organisms. We thank two anonymous reviewers for providing comments on the manuscript. Funding for this study was provided, in part, by the U.S. Environmental Protection Agency Region 5. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

LITERATURE CITED

- Allen, D. C., and C. C. Vaughn. 2009. Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society* 28:93–100.

- Amyot, J. P., and J. A. Downing. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. *Journal of the North American Benthological Society* 10:280–285.
- Amyot, J. P., and J. A. Downing. 1997. Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae). *Freshwater Biology* 37:345–354.
- Archambault, J. M., W. G. Cope, and T. J. Kwak. 2014. Survival and behavior of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient. *Freshwater Biology* 59:601–613.
- ASTM International. 2019a. Standard guide for conducting laboratory toxicity tests with freshwater mussels, ASTM Standard (E2455-06 2013b). ASTM International, West Conshohocken, Pennsylvania.
- ASTM International. 2019b. Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates, ASTM Standard (E1706-05 2010). ASTM International, West Conshohocken, Pennsylvania.
- Balfour, D. L., and L. A. Smock. 1995. Distribution, age structure and movement of the freshwater mussel *Elliptio complanata* (Mollusca: Unionidae) in a headwater stream. *Journal of Freshwater Ecology* 10:255–268.
- Barnhart, M. 2006. Buckets of mucklets: A compact system for rearing juvenile freshwater mussels. *Aquaculture* 254:227–233.
- Besser, J. M., W. G. Brumbaugh, N. E. Kemble, C. D. Ivey, J. L. Kunz, C. G. Ingersoll, and D. Rudel. 2011. Toxicity of nickel-spiked freshwater sediments to benthic invertebrates—Spiking methodology, species sensitivity, and nickel bioavailability. U.S. Geological Survey Scientific Investigations Report 2011–5225, 53 pp. plus appendixes.
- Besser, J. M., C. G. Ingersoll, W. G. Brumbaugh, N. E. Kemble, T. W. May, N. Wang, D. D. MacDonald, and A. D. Roberts. 2015. Toxicity of sediments from lead-zinc mining areas to juvenile freshwater mussels (*Lampsilis siliquoidea*), compared to standard test organisms. *Environmental Toxicology and Chemistry* 34:626–639.
- Block, J. E., G. W. Gerald, and T. D. Levine. 2013. Temperature effects on burrowing behaviors and performance in a freshwater mussel. *Journal of Freshwater Ecology* 28:375–384.
- Bringolf, R. B., W. G. Cope, C. B. Eads, P. R. Lazaro, M. C. Barnhart, and D. Shea. 2007. Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* 26:2086–2093.
- Clarke, A. H. 1973. The freshwater molluscs of the Canadian interior basin. *Malacologia* 13:1–509.
- Conover, W. J., and R. L. Iman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *American Statistician* 35:124–129.
- Cope, W. G., R. B. Bringolf, D. B. Buchwalter, T. J. Newton, C. G. Ingersoll, N. Wang, T. Augspurger, F. J. Dwyer, M. C. Barnhart, R. J. Neves, and H. Hammer. 2008. Differential exposure, duration, and sensitivity of unionoid bivalve life stages to environmental contaminants. *Journal of the North American Benthological Society* 27:451–462.
- Downing, J. A., Y. Rochon, M. Pêrusse, and H. Harvey. 1993. Spatial aggregation, body size, and reproductive success in the freshwater mussel *Elliptio complanata*. *Journal of the North American Benthological Society* 12:148–156.
- Downing, J. A., P. Van Meter, and D. A. Woolnough. 2010. Suspects and evidence: A review of causes and extirpation and decline in freshwater mussels. *Animal Biodiversity and Conservation* 33:151–185.
- Gough, H. M., A. M. Gascho-Landis, and J. Stockel. 2013. Behaviour and physiology are linked in the responses of freshwater mussels to drought. *Freshwater Biology* 57:2356–2366.
- Hazelton, P. D., B. Du, S. P. Haddad, A. K. Fritts, C. K. Chambliss, B. W. Brooks, and R. B. Bringolf. 2014. Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels. *Aquatic Toxicology* 151:27–35.
- Hull, P. J., R. G. Cole, R. G. Creese, and T. R. Healy. 1998. An experimental investigation of the burrowing behavior of *Paphies australis* (Bivalvia: Mesodesmatidae). *Marine and Freshwater Behaviour and Physiology* 31:167–183.
- Ingersoll, C. G., E. L. Brunson, F. J. Dwyer, D. K. Hardesty, and N. E. Kemble. 1998. Use of sublethal endpoints in sediment toxicity tests with the amphipod *Hyalella azteca*. *Environmental Toxicology and Chemistry* 17:1508–1523.
- Ingersoll, C. G., J. L. Kunz, J. P. Hughes, N. Wang, D. S. Ireland, D. R. Mount, R. J. Hockett, and T. W. Valenti. 2015. Relative sensitivity of an amphipod *Hyalella azteca*, a midge *Chironomus dilutus*, and a unionid mussel *Lampsilis siliquoidea* to a toxic sediment. *Environmental Toxicology and Chemistry* 34:1134–1144.
- Kat, P. W. 1982. Effects of population density and substratum type on growth and migration of *Elliptio complanata* (Bivalvia:Unionidae). *Malacological Review* 15:119–127.
- Kemble, N. E., D. K. Hardesty, C. G. Ingersoll, J. L. Kunz, P. K. Sibley, D. L. Calhoun, R. J. Gilliom, K. M. Kuivila, L. H. Nowell, and P. W. Moran. 2013. Contaminants in stream sediments from seven U.S. metropolitan areas: II. Sediment toxicity to the amphipod *Hyalella azteca* and the midge *Chironomus dilutus*. *Archives of Environmental Contaminants and Toxicology* 64:52–64.
- Lewis, J. B., and P. N. Riebel. 1984. The effects of substrate on burrowing in freshwater mussels (Unionidae). *Canadian Journal of Zoology* 62:2023–2025.
- Lydeard, C., R. H. Cowie, W. F. Onder, A. E. Bogan, P. Bouchet, S. A. Clark, K. S. Cummings, T. J. Frest, O. Gargominy, D. G. Herbert, R. Hershler, K. E. Perez, B. Roth, M. Seddon, E. E. Strong, and F. G. Thompson. 2004. The global decline of nonmarine mollusks. *BioScience* 54:321–330.
- Mulligan, T. G., and B. A. Law. 2013. Contaminants at the sediment–water interface: Implications for environmental impact assessment and effects monitoring. *Environmental Science and Technology* 47:5828–5834.
- National Research Council. 2003. Bioavailability of contaminants in soils and sediments: Processes, tools, and applications. National Academies Press. Washington, DC. 432 pages.
- Neves, R. 2004. Propagation of endangered freshwater mussels in North America. *Journal of Conchology, Special Publication* 3:69–80.
- Neves, R. J., and J. C. Widlak. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. *American Malacological Bulletin* 5:1–7.
- Okay, O. S., and B. Karacik. 2008. Bioconcentration and phototoxicity of selected PAHs to marine mussel *Mytilus galloprovincialis*. *Journal of Environmental Science and Health Part A* 43:1234–1242.
- Perles, S. J., A. D. Christian, and D. J. Berg. 2003. Vertical migration, orientation, aggregation, and fecundity of the freshwater mussel *Lampsilis siliquoidea*. *Ohio Journal of Science* 103:73–78.
- Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* 13:1220–1222.
- Schein, A., J. A. Sinclair, D. D. MacDonald, C. G. Ingersoll, N. E. Kemble, and J. L. Kunz. 2015. Evaluation of the toxicity of sediments from the Anniston PCB site to the mussel *Lampsilis siliquoidea*. Report prepared for U.S. Fish and Wildlife Service, Birmingham, Alabama.
- 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.
- Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54:429–439.
- Thoms, R. E., and T. M. Berg. 1985. Interpretation of bivalve trace fossils in fluvial beds of the basal Catskill formation (Late Devonian), eastern U.S.A. Pages 13–20 in H. A. Curran, editor. *Biogenic Structures: Their Uses in Interpreting Depositional Environments*. Society of Economic Paleontologists and Mineralogists, Special Publication Number 35, Tulsa, Oklahoma.

- Thorsen, W. A., G. Cope, and D. Shea. 2004. Bioavailability of PAHs: Effects of soot carbon and PAH source. *Environmental Science and Technology* 38:2029–2037.
- U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA 823-B-99-007, Duluth, Minnesota, and Washington, DC.
- Wang, N., C. G. Ingersoll, I. E. Greer, D. K. Hardesty, C. D. Ivey, J. L. Kunz, W. G. Brumbaugh, F. J. Dwyer, A. D. Roberts, T. Augspurger, R. J. Neves, and M. C. Barnhart. 2007a. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* 26:2048–2056.
- Wang, N., C. G. Ingersoll, D. K. Hardesty, C. D. Ivey, J. L. Kunz, T. W. May, F. J. Dwyer, A. D. Roberts, T. Augspurger, and C. M. Kane. 2007b. Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* 26:2036–2047.
- Wang, N., C. G. Ingersoll, J. L. Kunz, W. G. Brumbaugh, C. M. Kane, R. B. Evans, S. Alexander, C. Walker, and S. Bakaletz. 2013. Toxicity of sediments potentially contaminated by coal mining and natural gas extraction to unionid mussels and commonly tested benthic invertebrates. *Environmental Toxicology and Chemistry* 32:207–221.
- Watters, G. T. 1999. Morphology of the conglutinate of the Kidneyshell freshwater mussel, *Ptychobranchus fasciolaris*. *Invertebrate Biology* 118:289–295.
- Watters, G. T., S. H. O'Dee, and S. Chordas. 2001. Patterns of vertical migration in freshwater mussels (Bivalvia: Unionoida). *Journal of Freshwater Ecology* 16:541–549.
- Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18:6–22.
- Yeager, M. M., D. S. Cherry, and R. J. Neves. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). *Journal of the North American Benthological Society* 13:217–222.

Appendix 1. Overlying water quality data. NM: Not Measured, WB: West Bear Lake sediment, SR: Spring River sediment, CS: Coarse Sand.

Species	Age (wk)	Sediment	Exposure Time (h)	Temp (°C)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	pH	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)
Fatmucket	3	CS	4	23	8.6	342	NM	0.70	NM
Fatmucket	3	CS	24	23	8.5	238	NM	1.15	NM
Fatmucket	3	WB	4	23	8.1	486	NM	1.19	NM
Fatmucket	3	WB	24	23	7.7	383	NM	1.72	NM
Arkansas Fatmucket	4	WB	24	23	7.7	625	7.86	0.12	0.004
Arkansas Fatmucket	4	SR	24	23	7.1	774	7.98	0.45	0.020
Arkansas Fatmucket	4	CS	24	23	7.9	779	8.11	0.11	0.007
Oregon Floater	4	WB	24	23	7.8	593	8.07	0.38	0.021
Washboard	6	WB	24	23	8.2	583	NM	3.51	NM
Washboard	6	CS	24	23	8.6	725	NM	1.16	NM
Washboard	6	SR	24	23	8.1	533	NM	3.25	NM
Rainbow	7	CS	24	23	8.7	723	NM	0.15	0.000
Fatmucket	7	CS	24	23	8.3	709	NM	0.22	0.000
Fatmucket	7	SR	24	23	7.2	723	8.50	1.44	0.196
Rainbow	7	WB	24	23	7.4	581	NM	0.22	0.000
Rainbow	7	SR	24	23	7.2	747	NM	1.36	0.000
Fatmucket	10	WB	4	23	8.6	376	8.43	4.64	0.548
Fatmucket	10	CS	4	23	8.7	491	8.44	0.85	0.102
Fatmucket	10	SR	4	23	8.5	530	8.36	0.36	0.037
Fatmucket	10	WB	4	23	6.6	319	NM	5.37	NM
Fatmucket	10	WB	24	23	7.1	304	NM	5.93	NM
Fatmucket	10	CS	4	23	6.6	NM	NM	2.38	NM
Fatmucket	10	CS	24	23	7.2	735	NM	4.49	NM
Fatmucket	10	SR	4	23	6.7	401	NM	0.32	NM
Fatmucket	10	SR	24	23	7.1	445	NM	0.47	NM
Fatmucket	20	SR	4	23	NM	NM	NM	NM	NM
Fatmucket	20	CS	4	23	NM	NM	NM	NM	NM
Fatmucket	20	WB	4	23	8.0	334	NM	4.36	NM
Fatmucket	20	WB	24	23	7.3	347	NM	12.50	NM
Notched Rainbow	20	WB	4	23	8.0	338	NM	4.32	NM
Notched Rainbow	20	WB	24	23	7.3	339	NM	13.00	NM

Appendix 2. Raw mussel burrowing data. WB: West Bear Lake sediment, SR: Spring River sediment, CS: Coarse Sand.

Species	Age (wk)	Sediment	Exposure Time (h)	Rep	N	Percentage of Mussels at Burrowing Depths (mm)					
						Surface	0–1.7	1.7–3.4	3.4–5.1	5.1–6.8	6.8–8.5
Fatmucket	3	CS	4	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	4	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	4	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	3	CS	4	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	4	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	4	2	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	3	WB	4	3	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	3	WB	4	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	5	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	6	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	7	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	8	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	24	1	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	3	WB	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	24	3	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	3	WB	24	4	4	0.0	75.0	25.0	0.0	0.0	0.0
Fatmucket	7	CS	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	CS	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	CS	24	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	7	CS	24	4	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	7	SR	24	1	4	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	SR	24	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	7	SR	24	3	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	7	SR	24	4	4	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	20.0	40.0	40.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	20.0	40.0	40.0	0.0	0.0
Fatmucket	10	CS	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	CS	4	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	CS	4	3	5	0.0	40.0	40.0	0.0	20.0	0.0
Fatmucket	10	CS	4	4	5	0.0	0.0	20.0	80.0	0.0	0.0
Fatmucket	10	CS	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	CS	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	CS	4	3	5	0.0	20.0	80.0	0.0	0.0	0.0
Fatmucket	10	SR	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	SR	4	2	5	0.0	40.0	20.0	40.0	0.0	0.0
Fatmucket	10	SR	4	3	5	0.0	0.0	40.0	0.0	60.0	0.0
Fatmucket	10	SR	4	4	5	0.0	80.0	0.0	20.0	0.0	0.0
Fatmucket	10	SR	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	SR	4	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	SR	4	3	5	0.0	20.0	60.0	20.0	0.0	0.0
Fatmucket	10	WB	24	4	5	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	10	WB	24	5	5	0.0	0.0	60.0	20.0	20.0	0.0
Fatmucket	10	WB	24	6	2	0.0	0.0	100.0	0.0	0.0	0.0

Appendix 2, continued.

Species	Age (wk)	Sediment	Exposure Time (h)	Rep	N	Percentage of Mussels at Burrowing Depths (mm)					
						Surface	0–1.7	1.7–3.4	3.4–5.1	5.1–6.8	6.8–8.5
Fatmucket	10	CS	24	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	CS	24	5	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	CS	24	6	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	SR	24	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	SR	24	5	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	SR	24	6	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	20	SR	4	1	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	2	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	3	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	4	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	1	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	2	1	0.0	100.0	0.0	20.0	0.0	0.0
Fatmucket	20	CS	4	3	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	4	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	WB	4	1	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	4	2	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	4	3	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	4	4	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	4	5	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	1	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	2	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	WB	24	3	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	4	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	24	5	1	0.0	0.0	100.0	0.0	0.0	0.0
Rainbow	7	CS	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Rainbow	7	CS	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Rainbow	7	CS	24	3	5	0.0	80.0	20.0	0.0	0.0	0.0
Rainbow	7	CS	24	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Rainbow	7	WB	24	1	5	0.0	80.0	0.0	20.0	0.0	0.0
Rainbow	7	WB	24	2	5	0.0	80.0	20.0		0.0	0.0
Rainbow	7	WB	24	3	4	0.0	50.0	0.0	50.0	0.0	0.0
Rainbow	7	WB	24	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Rainbow	7	SR	24	1	4	0.0	100.0	0.0	0.0	0.0	0.0
Rainbow	7	SR	24	2	4	0.0	50.0	50.0	0.0	0.0	0.0
Rainbow	7	SR	24	3	5	0.0	20.0	80.0	0.0	0.0	0.0
Rainbow	7	SR	24	4	5	0.0	60.0	0.0	40.0	0.0	0.0
Arkansas Fatmucket	4	CS	24	1	4	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	CS	24	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Arkansas Fatmucket	4	CS	24	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Arkansas Fatmucket	4	CS	24	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	WB	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	WB	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	WB	24	3	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	WB	24	4	4	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	SR	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	SR	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	SR	24	3	4	0.0	100.0	0.0	0.0	0.0	0.0
Arkansas Fatmucket	4	SR	24	4	4	0.0	100.0	0.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	1	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	2	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	3	1	0.0	0.0	0.0	100.0	0.0	0.0

Appendix 2, continued.

Species	Age (wk)	Sediment	Exposure Time (h)	Rep	N	Percentage of Mussels at Burrowing Depths (mm)					
						Surface	0–1.7	1.7–3.4	3.4–5.1	5.1–6.8	6.8–8.5
Notched Rainbow	20	WB	24	4	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	5	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	1	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	2	1	0.0	0.0	100.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	3	1	0.0	100.0	0.0	0.0	0.0	0.0
Notched Rainbow	20	WB	24	4	1	0.0	0.0	0.0	0.0	100.0	0.0
Notched Rainbow	20	WB	24	5	1	100.0	0.0	0.0	0.0	0.0	0.0
Oregon Floater	4	WB	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Oregon Floater	4	WB	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Oregon Floater	4	WB	24	3	5	0.0	100.0	0.0	0.0	0.0	0.0
Oregon Floater	4	WB	24	4	5	0.0	20.0	80.0	0.0	0.0	0.0
Washboard Mucket	6	WB	24	1	5	0.0	20.0	80.0	0.0	0.0	0.0
Washboard Mucket	6	WB	24	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Washboard Mucket	6	WB	24	3	5	0.0	100.0	0.0	0.0	0.0	0.0
Washboard Mucket	6	WB	24	4	5	0.0	60.0	40.0	0.0	0.0	0.0
Washboard Mucket	6	CS	24	1	5	0.0	20.0	60.0	20.0	0.0	0.0
Washboard Mucket	6	CS	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Washboard Mucket	6	CS	24	3	5	0.0	20.0	40.0	40.0	0.0	0.0
Washboard Mucket	6	CS	24	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Washboard Mucket	6	SR	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Washboard Mucket	6	SR	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Washboard Mucket	6	SR	24	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Washboard Mucket	6	SR	24	4	5	0.0	100.0	0.0	0.0	0.0	0.0

Appendix 3. Fatmucket (FM) mussel burrowing data. WB: West Bear Lake sediment, SR: Spring River sediment, CS: Coarse Sand.

Species	Age (wk)	Sediment	Exposure			Percentage of Mussels at Burrowing Depths (mm)					
			Time (h)	Rep	N	Surface	0–1.7	1.7–3.4	3.4–5.1	5.1–6.8	6.8–8.5
Fatmucket	3	CS	4	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	4	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	4	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	3	CS	4	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	4	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	4	2	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	3	WB	4	3	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	3	WB	4	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	5	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	6	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	7	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	CS	24	8	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	24	1	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	3	WB	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	3	WB	24	3	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	3	WB	24	4	4	0.0	75.0	25.0	0.0	0.0	0.0
Fatmucket	7	CS	24	1	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	CS	24	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	CS	24	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	7	CS	24	4	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	7	SR	24	1	4	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	7	SR	24	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	7	SR	24	3	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	7	SR	24	4	4	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	20.0	40.0	40.0	0.0	0.0
Fatmucket	10	WB	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	WB	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	WB	4	3	5	0.0	20.0	40.0	40.0	0.0	0.0
Fatmucket	10	CS	4	1	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	CS	4	2	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	CS	4	3	5	0.0	40.0	40.0	0.0	20.0	0.0
Fatmucket	10	CS	4	4	5	0.0	0.0	20.0	80.0	0.0	0.0
Fatmucket	10	CS	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	CS	4	2	5	0.0	40.0	60.0	0.0	0.0	0.0
Fatmucket	10	CS	4	3	5	0.0	20.0	80.0	0.0	0.0	0.0
Fatmucket	10	SR	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	SR	4	2	5	0.0	40.0	20.0	40.0	0.0	0.0
Fatmucket	10	SR	4	3	5	0.0	0.0	40.0	0.0	60.0	0.0
Fatmucket	10	SR	4	4	5	0.0	80.0	0.0	20.0	0.0	0.0
Fatmucket	10	SR	4	1	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	SR	4	2	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	SR	4	3	5	0.0	20.0	60.0	20.0	0.0	0.0
Fatmucket	10	WB	24	4	5	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	10	WB	24	5	5	0.0	0.0	60.0	20.0	20.0	0.0
Fatmucket	10	WB	24	6	2	0.0	0.0	100.0	0.0	0.0	0.0

Appendix 3, continued.

Species	Age (wk)	Sediment	Exposure Time (h)	Rep	<i>N</i>	Percentage of Mussels at Burrowing Depths (mm)					
						Surface	0–1.7	1.7–3.4	3.4–5.1	5.1–6.8	6.8–8.5
Fatmucket	10	CS	24	4	5	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	10	CS	24	5	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	10	CS	24	6	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	SR	24	4	5	0.0	80.0	20.0	0.0	0.0	0.0
Fatmucket	10	SR	24	5	5	0.0	60.0	40.0	0.0	0.0	0.0
Fatmucket	10	SR	24	6	5	0.0	40.0	40.0	20.0	0.0	0.0
Fatmucket	20	SR	4	1	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	2	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	3	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	SR	4	4	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	1	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	2	1	0.0	100.0	0.0	20.0	0.0	0.0
Fatmucket	20	CS	4	3	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	CS	4	4	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	WB	4	1	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	4	2	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	4	3	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	4	4	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	4	5	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	1	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	2	1	0.0	100.0	0.0	0.0	0.0	0.0
Fatmucket	20	WB	24	3	1	0.0	0.0	100.0	0.0	0.0	0.0
Fatmucket	20	WB	24	4	1	0.0	0.0	0.0	100.0	0.0	0.0
Fatmucket	20	WB	24	5	1	0.0	0.0	100.0	0.0	0.0	0.0