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[Article]

Determining a Suitable Substrate Size and Sampling Frequency for Rearing Juvenile Rainbow Mussels *Villosa iris*

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Abstract.—The effects of sampling frequency and substrate size on the overall size and survival of juvenile rainbow mussels *Villosa iris* were investigated in 4-L round flow-through tanks. All tanks contained either fine sediment (<50 μm), fine limestone sand (500–850 μm), or coarse limestone sand (1,000–2,500 μm) and were sampled every 2 weeks or once at the end of the 10-week experiment. Tanks left unsampled for 10 weeks had 12.8% higher survival regardless of substrate treatment. Juveniles in unsampled tanks also had greater shell length than those in sampled tanks. Juveniles cultured in coarse sand had the best survival in the sampled tanks (36.4%), and juveniles reared in fine sand had the best survival in the unsampled tanks (51.9% at 10 weeks). Analyses of gut contents at 10 weeks revealed that juveniles were consuming large amounts of detritus and algal cells 5–10 μm in size, primarily *Coelastrum*, *Chlorococcum*, *Chlorella*, and *Navicula* spp. These results show that frequent disturbance of juveniles by sampling may impede physiological functions, resulting in stress and a decrease in overall survival and shell length. It also appears that fine limestone sand (500–800 μm in size) is the best substrate for juvenile culture purposes in similar rearing systems.

Declines in freshwater mussel fauna (family Unionidae) have initiated efforts to artificially propagate imperiled species to augment wild populations and enhance recovery. Many aspects of culturing freshwater mussels in captivity have been researched, including dietary requirements (Gatenby et al. 1997; Beck and Neves 2003), temperature effects (Stuart et al. 1999; Hanlon 2000), and the role of fine sediment on the overall size and survival of juvenile mussels (Gatenby et al. 1996; Hanlon 2000; Zimmerman 2003). However, compared with other aspects of mussel culture, relatively little research has been conducted on the influence of substrate size and sampling frequency on the overall size and survival of juvenile freshwater mussels.

The size of substrate used in a culture system could affect juvenile mussels in a variety of ways, including their ability to feed and escape predation and high flow. Rogers (1999) reported that juvenile rainbow mussels *Villosa iris* reared in fine substrate (<120 μm) had 7% survival and grew only 0.86 mm in length after 16 weeks, while juveniles in a mixed sediment (>1,400 μm) had 26% survival and grew 1.06 mm in length. Beaty and Neves (2004) reported that neither shell lengths nor survival was affected by rearing rainbow mussel juvenile in two sizes of fine substrate (<120 μm and 120–600 μm).

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Cope and Waller (1995) reported that stress resulting from handling, removal from substrate, and emersion can negatively affect the growth and survival of adult freshwater mussels. One often overlooked, and potentially important, source of stress that could negatively influence environmentally sensitive juvenile mussels is sampling. Sampling juveniles typically involves removing the juveniles from their rearing substrate, handling them to get estimates of shell lengths and survival, exposing them to air for a short period of time, and returning them to their culture systems in an unnatural position. Previously, some studies have attempted to analyze the effects of sampling frequency on juvenile mussels and concluded that sampling may decrease shell lengths and survival rates. O'Beirn et al. (1998) reported that shell lengths decreased as sampling frequency increased with the wavy-rayed lampmussel *Lampsilis fasciola*, and Zimmerman (2003) reported that unsampled oyster mussels *Epioblasma capsaeformis* and wavy-rayed lampmussels had better survival than those that were sampled every 2 weeks. Likewise, Beaty (1997) reported lower survival in juvenile rainbow mussels that were sampled compared with juveniles left unsampled.

Juveniles feed on bacteria and detritus (Yeager et al. 1994; Gatenby et al. 1996), but algae is their primary food source (Gatenby et al. 1996, 1997; Beck and Neves 2003). Beck and Neves (2003) suggest that juvenile mussels selectively feed and do so primarily on the basis of particle size. Diet studies with juvenile mussels typically involve adding various cultured algae species to closed, static systems, such as glass dishes or jars

containing juveniles. Juvenile gut contents are then examined to determine which algal species are selected for consumption (Gatenby et al. 1996, 1997; Beck and Neves 2003). However, locating juveniles in the wild and determining which algal species are preferred in a natural setting is much more difficult (Beck and Neves 2003). Determining which algal genera are being consumed by juveniles in this experiment is important, since the water supplying these culture systems is directly pumped from the South Fork Holston River, Virginia, and is potentially high in algal diversity. Distinguishing which algal genera are preferred by juveniles in natural river water is an important component of helping to develop better captive feeding regimes.

The purpose of this study was to determine the influence of substrate size and sampling frequency on the overall size and survival of juvenile rainbow mussels reared in round flow-through tanks. Shell lengths and survival were evaluated using three substrate particle sizes (<50-μm fine sediment, 500- to 850-μm limestone sand, and 1,000- to 2,500-μm limestone sand) along with a sampling frequency of every 2 weeks or only once at the end of the 10-week experiment as a reference. Additionally, gut contents of juveniles were examined at the end of this experiment to determine which algal genera were being consumed.

Methods

This study was conducted at the Aquatic Wildlife Conservation Center (AWCC), a freshwater propagation facility operated by the Virginia Department of Game and Inland Fisheries. This facility in Marion, Virginia, utilizes river water, pumped from the South Fork Holston River (river kilometer 169), to supply all culture systems. Six gravid rainbow mussels were collected from the North Fork Holston River in Chatham Hill, Smyth County, Virginia, in early June 2004. Sixteen rock bass *Ambloplites rupestris* were collected with a backpack electroshocker from Tom's Creek in Whitethorne, Montgomery County, Virginia, and used as host fish. Glochidia from the six rainbow mussels collected from the North Fork Holston River and glochidia from two rainbow mussels collected from Indian Creek, Cedar Bluff, Tazewell County, Virginia, also collected in early June 2004, were mixed together to ensure an adequate number and gene pool of glochidia were available. Mixing of glochidia from two different river drainages was deemed appropriate since these juveniles were used for research purposes only and not released back into the wild. Infestation of fish and collection of juveniles followed standard infestation techniques (Zale and Neves 1982). All juveniles for this study were cultured at the Freshwater Mollusk Conservation Center (FMCC) located at Virginia Polytechnic Institute and State University in Blacksburg, Virginia.

Newly transformed juveniles were transported from FMCC to AWCC on June 29 in 1-L plastic containers. Juveniles were acclimated to water conditions at AWCC for 1 h by slowly adding river water to the containers. A total of 10,500 juveniles were divided among five tanks for each of the three substrate treatments at 700 juveniles/tank. A total of 4,800 juveniles also were divided into subgroups of 400 juveniles/tank for a total of four reference tanks per substrate treatment that would remain unsampled until the end of the experiment.

The three sizes of substrate used for this experiment were fine sediment at less than 50 μm, which was allowed to accumulate from entering river water in the tanks, and 200 mL of either 500- to 850-μm limestone sand or 1,000- to 2,500-μm limestone sand. Water for this experiment was screened through a 100- and 50-μm-mesh filter before entering a 946-L reservoir, which then gravity fed the round tanks. Water was transported from the reservoir to the tanks via 5.1-cm-diameter polyvinyl chloride (PVC) pipe. A 1.3-cm PVC ball valve was used to regulate the flow from the 5.1-cm PVC pipe into each tank, creating a circular flow. Each tank was a 4-L round plastic container that was 10 cm high and 26 cm in diameter. A 2.5-cm-high piece of PVC was placed into a male adapter in the bottom center of each tank and served as a drain. This resulted in each tank holding 2.7 L of water. A 150-μm-mesh basket was attached beneath the standpipe and filtered all outgoing water to ensure that any juveniles that escaped were caught and returned to the tank.

Sampling occurred every 2 weeks after the initial release date on June 29. All tanks were sampled on each sample date, except for the 12 tanks that were predetermined to be sampled only once at the end of the 10-week experiment. Before any juveniles were removed from the tanks, the contents of the 150-μm-mesh baskets were examined and juveniles were enumerated and measured. At the end of each sample event for the sampled tanks, rainbow mussels captured in the mesh baskets were placed back into their respective round tanks. Juveniles were removed from the substrate in the tanks by swirling 500 mL of water in a counterclockwise direction to sort rainbow mussels from the substrate. This water was emptied into a series of 1,000-, 400-, and 200-μm vertically stacked sieves, rinsed, and repeated eight times (until no further juveniles remained in the tank). Total survival in each tank was recorded and a subsample of 10 individuals/tank had their shell lengths measured. During each sample event, the mesh baskets under the 12

unsampled tanks were emptied back into their respective tanks without being enumerated or measured (except at 10 weeks). This was done to minimize handling and potential stress of these juveniles.

During each sampling event, incoming sediment (<50 μm) that had accumulated in the tanks was washed from the substrate and collected in a 19-L bucket. This sediment-laden water was then diluted to the 18-L mark with sediment-free water. The contents of the bucket were then stirred and a 1-L sample of the slurry was collected to estimate levels of incoming sediment accumulation. A Barnant vacuum-pressure pump (Barnant Company, Barrington, Illinois; Model 400-1901) was used to filter 200 mL of the slurry through preweighed Whatman GF/C fiberglass filters (47 mm; Whatman Worldwide, Middlesex, UK). Filters and slurry were dried in an oven at 60°C for 24 h or until a stable dry weight was achieved. The samples were placed in a desiccator until they had cooled to 20°C and were then weighed.

The gut contents of juveniles at the end of this experiment were analyzed to determine which algal genera were consumed. On the last sampling date, unsampled and sampled tanks were combined according to substrate size, and 12 rainbow mussel juveniles were randomly selected from each treatment for a total of 36 juveniles. All juveniles were rinsed with distilled water before being placed in appropriately labeled 100-mL glass bottles. Each bottle had 50 mL of distilled water and 2 mL of acid Lugol's solution to preserve algae in the guts. Gut analysis was carried out by pouring each treatment into a petri dish and locating the juveniles with a dissecting microscope at 20× magnification. A Pasteur pipette was then used to select approximately half the juveniles and place them on a microscope slide. The juveniles were then crushed with the rear end of a pair of tweezers to expose the guts and ingested algae. Each slide then underwent seven different linear transects to standardize analyses. Algal genera and size ranges of algae were noted for each of the three treatments using an Olympus light microscope (Olympus Corporation, Tokyo, Japan) at 100× magnification. An algal key was used to aid in the identification of genera (Prescott 1978).

On the last sample date, a water sample was taken from the reservoir to allow a comparison between the algal genera present in the water and those consumed by the juveniles. This water sample was stored in a Nalgene bottle (Nalge Nunc International, Rochester, New York) and preserved with 10 mL of acid Lugol's solution (Vollenweider 1969). This sample was then stored in a cool, dark file cabinet until analysis was completed (Vollenweider 1969). During analysis, a 100-mL Utermohl settling chamber was used for 48 h to concentrate the algae. An inverted Olympus light microscope (Olympus Corporation) at 300× magnification was then used along with a dichotomous key (Prescott 1978) to identify algal genera in each sample. A square grid in the eyepiece was placed on a random point, and the genera within the grid were identified and enumerated. This process was continued until a total of approximately 300 individual algal cells were counted. The conversion formula was calibrated to the microscope magnification by having 130 correct for the number of algal cells in one transect of the counting chamber grid system and having 98 correct for the number of algal cells in 100 mL. The following formula was then applied to determine the number of algal cells in 1 mL of the sample:

$$[(130/\text{number of grids}) \times \text{number of algal cells} \times 98]/100.$$

Statistical analysis.—We conducted all statistical tests in SAS (SAS Institute 2005). We compared survival and length for juveniles, in both mesh baskets (escaped) and in tanks, among substrate treatments in tanks sampled at 2-week intervals using a repeated-measures analysis of variance (ANOVA) assuming compound symmetry (PROC MIX; type = cs). We also used repeated-measures ANOVA to compare escapement among substrates and sampling events. Least-squares means tests were used to make pairwise comparisons.

Survival is reported as the percent of the initial number of juveniles placed in each replicate tank recovered at each sampling event from tanks and mesh baskets. Escapement is reported as the percent recovered in mesh baskets as a percent of total recovered from tanks and mesh baskets combined. For analysis, survival and escapement were arcsine-transformed to ensure homogeneity of variance. We compared differences in length and survival after 10 weeks among sampling and substrate treatments using a two-way ANOVA (PROC MIX) followed by least-squares means tests to make pairwise comparisons.

To ensure that the comparison of lengths at 10 weeks was valid, we conducted a two-way ANOVA to test for initial differences among juveniles randomly assigned to the sampling and substrate treatments. We also used two-way ANOVA (PROC MIX) to compare incoming sediment among sampling treatments and substrate treatments.

Results

Survival, Length, and Escapement over Time for Substrate Treatments in Sampled Tanks

In the tanks sampled every 2 weeks, survival differed among substrate treatments (repeated-mea-

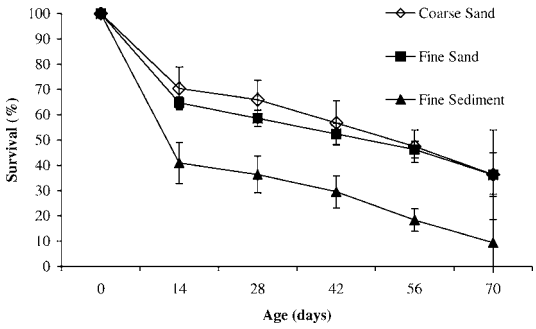

FIGURE 1.—Mean ± SE survival (%) of North Fork Holston River rainbow mussel juveniles reared in three substrate treatments (fine sediment, fine sand, and coarse sand) at the Aquatic Wildlife Conservation Center, 2004. Tanks were sampled once every 2 weeks.

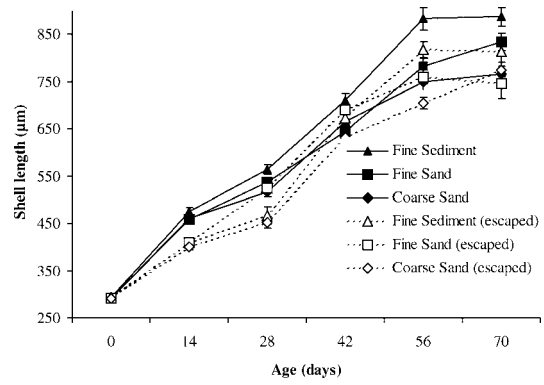

FIGURE 2.—Mean ± SE length of North Fork Holston River rainbow mussel juveniles reared in three substrate treatments (fine sediment, fine sand, and coarse sand) at the Aquatic Wildlife Conservation Center, 2004. Tanks were sampled once every 2 weeks. Lengths of 10 random juveniles in tanks and 10 (at most) juveniles that had escaped from tanks and been recovered in mesh baskets were measured.

sures ANOVA; $P = 0.0067$). Survival also decreased over time ($P < 0.0001$). Differences among substrate treatments observed in the first sampling event (14 d) continued through the duration of the experiment (Figure 1). Juveniles in the fine-sediment treatment had lower survival than juveniles in both fine sand ($P = 0.0084$) and coarse sand ($P = 0.0033$); however, survival between fine and coarse sand was not different ($P = 0.6292$). Juveniles in the coarse sand consistently had the highest survival throughout the experiment. After 10 weeks, juveniles reared in the fine sediment, fine sand, and coarse sand had mean survival rates of 9.4, 36.3, and 36.4%, respectively, and a 27.4% overall survival rate (Figure 1).

During each sampling event, rainbow mussels in the mesh baskets (escaped) had a smaller mean shell length than those in their respective tanks (repeated-measures ANOVA; $P = 0.0002$; Figure 2). Shell lengths of juveniles in tanks and those found in the mesh baskets increased over time ($P < 0.0001$). There is evidence for interaction between escapement and time ($P = 0.0398$): juveniles that escaped were smaller than those in tanks at 2 ($P < 0.001$), 8 ($P < 0.001$), and 10 weeks ($P < 0.001$), but were more similar at 4 ($P = 0.0965$) and 6 weeks ($P = 0.595$). Juveniles in tanks and those found in the mesh baskets were consistently larger from week to week ($P < 0.05$), except between 8 and 10 weeks ($P = 0.1077$).

Length varied among substrate treatments for juveniles in both mesh baskets and in tanks ($P = 0.0141$); little evidence for interaction between escapement and substrate was observed ($P = 0.1499$). Interactions were observed between substrate and time ($P = 0.0006$). Length was greater in fine sediment than in coarse sand ($P = 0.0043$). Evidence for differences between fine sediment and fine sand is weaker ($P = 0.0927$) and may be confounded by variation over time. Length was similar for fine sand and coarse sand ($P = 0.2298$). Length was similar in all substrates at 2 and 4 weeks ($P > 0.1$). Juveniles had greater shell lengths in fine sediment than in coarse sand for 6 ($P = 0.0174$), 8 ($P < 0.0001$), and 10 weeks ($P = 0.0054$). Juveniles in the fine-sediment treatment, although consistently showing the lowest survival in the sampled tanks, had greater shell lengths (Figure 2). There was more deviation from this pattern in escaped juveniles collected from mesh baskets. Juveniles in fine sediment, fine sand, and coarse sand had mean lengths of 887, 834, and 765 µm, respectively, at the end of the experiment. This represented a 562-, 559-, and 564-µm mean increase in shell length for juveniles in fine sediment, fine sand, and coarse sand, respectively, for the duration of the experiment.

A total of 1,566 juveniles escaped the grow-out tanks that were sampled every 2 weeks (Figure 3). Escape rates differed among substrate treatments (repeated-measures ANOVA; $P = 0.0119$) and sampling events ($P < 0.0001$); no interaction between time and substrate was observed ($P = 0.1016$). Escape rates were higher in fine sediment than in fine sand ($P = 0.0102$) or coarse sand ($P = 0.0073$). There was no difference between fine sand and coarse sand ($P = 0.8609$). For the fine-sediment, fine-sand, and coarse-sand treatments, the total escapement was 645, 536, and 398 juveniles out of an initial 3,500 juveniles placed across replications of substrate treatments, respectively. Escapement varied among sample events; the highest escapement was over 94% for a fine-sediment treatment replicate at 8 weeks.

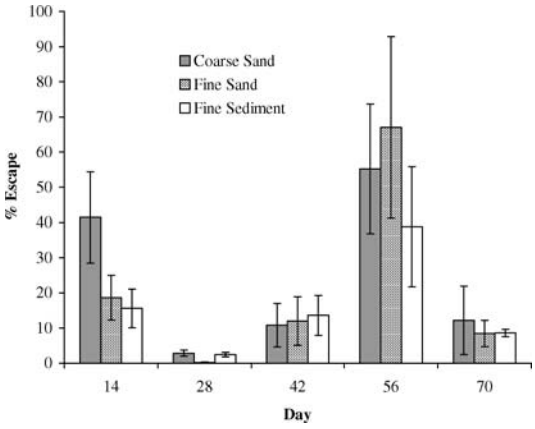

FIGURE 3.—Mean ± SE number of North Fork Holston River rainbow mussel juveniles recovered in mesh baskets as a percent of total recovered from both tanks and mesh baskets (% escape from tanks) at the Aquatic Wildlife Conservation Center, 2004. Tanks and mesh baskets for fine sediment, fine sand, and coarse sand were sampled once every 2 weeks.

Survival and Length in Substrate Treatments for Sampled and Unsampled Tanks

We observed no differences in the initial lengths of juveniles randomly assigned to the sampling (two-way ANOVA; $P = 0.4518$) and substrate ($P = 0.9325$) treatments, so comparisons among treatments without consideration for initial length were valid. Tanks left unsampled for 10 weeks had 12.8% higher survival, regardless of substrate treatment (two-way ANOVA; $P = 0.0018$; Figure 4). In the unsampled tanks, survival was lower in the fine sediment (27.5%) than in fine sand ($P = 0.0037$) or coarse sand ($P = 0.0719$). The fine-sand and coarse-sand treatments in the unsampled tanks had survival rates of 51.9% and 41.1%, respectively, but differed little ($P = 0.1845$).

Juveniles in unsampled tanks had greater shell lengths than those in sampled tanks (two-way ANOVA; $P = 0.0414$). Juveniles differed in length among substrate treatments ($P = 0.0096$); however, interactions between substrate and sampling treatments were observed ($P = 0.0106$). Juveniles in the unsampled tanks had comparable shell lengths among the three treatments at the end of the experiment: the fine-sediment, fine-sand, and coarse-sand treatments had mean lengths of 856, 852, and 857 μm, respectively (Figure 5).

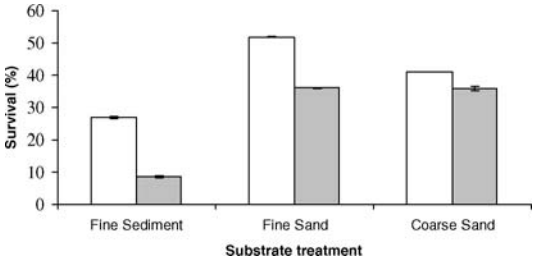

FIGURE 4.—Mean ± SE survival at 10 weeks for unsampled (white bars) and sampled (gray bars) North Fork Holston River rainbow mussel juveniles reared in three substrate treatments at the Aquatic Wildlife Conservation Center, 2004.

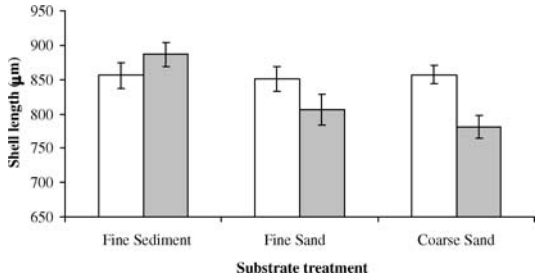

FIGURE 5.—Mean ± SE length at 10 weeks for unsampled (white bars) and sampled (gray bars) North Fork Holston River rainbow mussel juveniles reared in three substrate treatments at the Aquatic Wildlife Conservation Center, 2004.

At 10 weeks, the escapement enumerated in the unsampled tanks was far less than that in the sampled tanks; only 13 juveniles were collected, 5 in both the fine-sediment and fine-sand treatments and 3 in the coarse-sand treatment from an initial 1,600 across replications for each treatment.

Incoming Sediment

More incoming sediment accumulated in the tanks containing substrate than those without substrate (Figure 6). As expected, unsampled tanks accumulated significantly more incoming sediment after 10 weeks ($P < 0.0001$) than those that were sampled every 2 weeks. There was no difference in accumulation among substrate treatments ($P = 0.1931$) after 10 weeks. The unsampled fine-sediment, fine-sand, and coarse-sand treatments contained mean ± SE incoming sediment loads of 1.50 ± 0.13, 2.01 ± 0.29, and 1.77 ± 0.17 mg/100 cm^2, respectively. The average accumulation of incoming sediment over 10 weeks in the sampled fine-sediment, fine-sand, and coarse-sand treatments was 0.32 ± 0.09, 0.42 ± 0.04, and 0.47 ± 0.04 mg/100 cm^2, respectively. Variability was observed in sampled tanks over time (repeated-measures ANOVA; $P < 0.001$) and among substrate treatments ($P = 0.0020$); however, support for interaction between substrate and time was weak ($P = 0.0520$). Fine sediment had lower accumulation than fine sand ($P = 0.0086$) or coarse sand ($P = 0.0008$). No difference in accumulation was observed between fine sand and

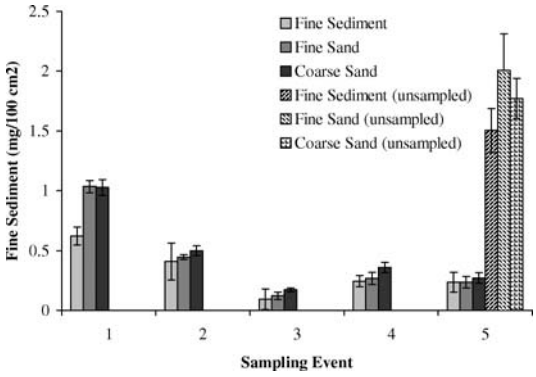

FIGURE 6.—Mean values of incoming sediment in the three substrate treatments for North Fork Holston River juvenile rainbow mussels. The bars for sampling events 1–4 and the first three bars for sampling event 5 pertain to tanks sampled at 2-week intervals; the last three bars for sampling event 5 pertain to tanks sampled only at the end of the 10-week experiment.

coarse sand ($P = 0.4614$). The accumulation of sediment decreased over time.

Gut Content Analysis

Several problems arose with the gut content analysis. First, the rainbow mussel juveniles used for this experiment had extremely strong shells that were possibly calcified before preservation. Calcification made the preferred method of using a cover slip to crush the juveniles inadequate. Second, previous juvenile gut content analyses had been done using an epifluorescent microscope, which illuminates chlorophyll in algae and makes identification easier. Since an epifluorescent microscope was not available, a binocular light microscope was used. Much of the algae observed had their chlorophyll already digested, or were digested seemingly after the algae had died. However, some useful quantifiable and qualitative data were obtained.

The mean lengths of the juveniles used for this analysis from the fine-sediment, fine-sand, and coarse-sand treatments were 926, 798, and 867 μm, respectively, and were not significantly different ($P = 0.1792$). Considerable detritus was found in the gut contents of juveniles raised in the fine-sediment and fine-sand treatments. However, less detritus was observed in the juveniles from the coarse-sand treatment. Four taxonomic groups of algae (blue-green algae [Cyanoprokaryota], cryptophytes [Cryptophyta], diatoms [Bacillariophyta], and green algae [Chlorophyta]) were present in the gut contents of juveniles. Fine sediment and fine sand had very similar results in terms of number of genera present, number of cells present, and relative abundance (Table 1). Juvenile gut contents examined from the fine-sand and fine-sediment treatments yielded 83 and 78 cells, respectively, and contained 11 and 10 genera, respectively. However, juvenile gut contents from the coarse-sand treatment yielded only 11 algal cells and 5 genera (Table 1). In the fine-sand treatment, the three most abundant genera in decreasing order were *Coelastrum*, *Chlorella*, and *Navicula* spp. The fine-sediment treatment had the same results, except that *Navicula* was the second-most abundant genus and *Chlorella* was the third. Lastly, in the coarse-sand treatment, the most abundant genera in decreasing order were *Chlorella*, *Chlorococcum*, and *Navicula*, *Pinnularia*, and *Synedra* spp. in equal abundances. The majority of algal cells consumed in this experiment were approximately 5–10 μm in size.

TABLE 1.—Relative abundance of algae in the gut contents of rainbow mussel juveniles in three substrate treatments after 70 d (June 29 to September 7, 2004).

	Relative abundance (%)		
Algae	Fine sand	Coarse sand	Fine sediment
Blue-green algae (Cyanoprokaryota)			
Oscillatoria	1.2		3.8
Cryptophytes (Cryptophyta)			
Chroomonas	1.2		
Diatoms (Bacillariophyta)			
Cocconeis			1.3
Cyclotella			1.3
Cymbella	4.8		
Gomphonema	3.6		2.6
Navicula	9.6	9.1	30.8
Pinnularia	1.2	9.1	1.3
Synedra		9.1	1.3
Green algae (Chlorophyta)			
Chlorella	30.1	54.5	15.4
Chlorococcum	2.4	18.2	
Coelastrum	38.6		41.0
Oocystis	2.4		
Scenesdesmus	4.8		
Tetraedron			1.3
Total cell density	83.0	11.0	78.0
Number of genera	11	5	10

In total, three phyla and 11 genera were present in the water at AWCC (Table 2) when the gut contents were preserved. The mean algal density was 1,669 cells/mL, the three most dominant algal genera in decreasing order being *Chlamydomona*, *Pandorina*, and *Navicula* spp. (Table 2). Green algae were the most abundant genera, making up 72.7% of the total algae reported, and diatoms were the next most abundant, representing 27.0% of all algae. Two additional phyla (Cyanoprokaryota and Cryptophyta) and 7 additional genera (*Chlorella*, *Chroomonas*, *Coelastrum*, *Oocystis*,

TABLE 2.—Algae cell counts from water entering grow-out systems at the Aquatic Wildlife Conservation Center on September 7.

Algae	Characteristic			
	Form[a]	Habitat[b]	Ingestibility[c]	Number of cells
Diatoms (Bacillariophyta)				
Cocconeis	U	B, P	I	8
Cyclotella	U	P	I	3
Fragilaria	C	P	X	18
Gomphonema	U	B, P	I	45
Navicula	U	B	I	65
Pinnularia	U	B	I	2
Euglenolds (Euglenophyta)				
Phacus	U	P		2
Green algae (Chlorophyta)				
Ankistrodesmus	U	P		3
Chlamydomonas	U	P		182
Chlorococcum	U	B, P	I	60
Pandorina	C	P		135
Total				523
Number of Genera				11
Number of grids counted				40
Cells/mL				1,668.7

[a] Unicellular (U), colonial (C), or filamentous (F).
[b] Planktonic (P) or benthic (B).
[c] Ingestible (I) or not likely to be ingested by juvenile mussels.

Oscillatoria, *Scenesdesmus*, and *Tetraedron* spp.) were found in gut contents of juveniles that were not found during analysis of water samples (Table 1).

Discussion

Mean survival of juveniles in sampled and unsampled tanks combined was 33.8%after 10 weeks. If the fine-sediment treatment is excluded, overall survival was 41.8%, which is comparable or better than many previous experiments with rainbow mussel juveniles (Beaty 1997; Mummert 2001; Zimmerman 2003). Mean length of juveniles after 10 weeks (840 μm) was considered average, since other studies at AWCC have had similar or greater lengths at 90 d (Hanlon 2000; Zimmerman 2003).

The results of this study show that juveniles sampled every 2 weeks and reared in fine or coarse sand have better overall survival than those reared solely in fine sediment without a more stable substrate (Figure 1). In the case of unsampled tanks, survival was lower in the fine sediment (27.5%) than in fine sand ($P = 0.0037$) or coarse sand ($P = 0.0719$). Unsampled juveniles reared in the fine-sand treatment had the greatest survival at 51.9%. These results agree with those of Rogers (1999), who reported that juveniles reared in fine sand (500–800 μm) had significantly higher survival (23.1%) than those in fine sediment (<120 μm) after 16 weeks. It has been suggested that the presence of substrate allows juveniles to feed interstitially and to attach to the substrate with byssal threads (Yeager et al. 1994). In our experiment, juveniles without sufficient substrate were possibly unable to burrow or attach to anything, resulting in poorer survival. O'Beirn et al. (1998) also reported that juveniles held without substrate did not feed and often closed in flowing water. Poor survival in the fine-sediment treatments and the decreased shell lengths in juveniles in the mesh baskets could have resulted from juveniles remaining closed much of the time.

Interstitial feeding could also explain why juveniles in the unsampled tanks had higher survival in the fine sand than those in the coarse sand. The much larger grain size in the coarse-sand treatment may have allowed more of the incoming sediment to settle through the sand, packing the interstitial spaces with incoming sediment to a greater extent and inhibiting feeding. With most of the incoming sediment accumulating on the surface of the tighter-packed fine sand, juveniles may have been able to escape the incoming sediment by moving deeper in the fine sand to feed.

Juveniles reared in the fine-sediment treatment in the sampled tanks exhibited the poorest survival. This treatment also contained the least amount of incoming sediment, but juveniles in these tanks exhibited the largest shell lengths. Expectation was that treatments with the poorest survival would also exhibit the smallest shell lengths, but this was not the case. Beaty and Neves (2004) had similar results in which juveniles cultured in fine sediment (<120 μm) grew slightly larger than those in coarse sediment (120–600 μm). One possible reason for these results is that incoming sediment in the substrate-free tanks was constantly agitated with circular flow, much more than the incoming sediment in either the fine-sand or coarse-sand treatment, which tended to settle in the substrate. Rogers (1999) hypothesized that fine sediment, which is more loosely packed, such as in the fine-sediment treatments, could facilitate feeding of juvenile mussels. However, the greater overall size of juveniles in the unsampled tanks and comparable size, regardless of substrate treatment in the unsampled tanks, is more likely related to survival of the most robust juveniles. Under stressful conditions and with no way to secure themselves, the least-fit mussels probably died, leaving only the largest and healthiest mussels to colonize the sediments.

At AWCC, Zimmerman (2003) concluded that incoming sediment levels of up to 3.33 mg/100 cm^2 were not lethal to mussels but did reduce shell lengths. In our study, the best survival (51.9%) occurred in the fine sand of unsampled tanks, which also had the highest mean incoming sediment load (2.01 mg/100

cm^2). High accumulations of incoming sediment of up to 2.01 mg/100 cm^2 did not appear to affect overall size. Mean length of juveniles reared in the unsampled tanks was significantly greater (855 μm) with mean incoming sediment levels of 1.76 mg/100 cm^2 than juveniles in the sampled tanks (829 μm) with mean incoming sediment levels of 0.40 mg/100 cm^2.

Historically, research at AWCC has tried to strike a balance between juvenile sampling, which may cause stress and potential loss of juveniles, and no sampling, which results in excessive sediment deposition that can negatively affect juveniles. Sampling the rainbow mussels in this experiment every 2 weeks appeared to negatively affect survival and overall size during the course of 10 weeks, as overall survival was 12.8% greater and mean lengths were 26 μm larger in the unsampled tanks. O'Beirn et al. (1998) reported similar results; sampled wavy-rayed lampmussel juveniles had smaller shell lengths than those that were unsampled. These findings are contrary to those reported by Hanlon (2000), where rainbow mussels left unsampled had sizes smaller than those that were sampled. Although not measured in that experiment, it is likely that since the water was not filtered, incoming sediment loads were much higher in the unsampled treatment and, possibly, could have negatively affected overall size and survival.

Juvenile mussels in the fine-sand and fine-sediment treatments ingested algal taxa in similar amounts; *Coelastrum* cells were most abundant and *Chlorella* and *Navicula* were either second or third in abundance (Table 1). However, gut contents from juveniles reared in the coarse-sand treatment were quite different; *Chlorella* was most abundant, *Chlorococcum* was second, and three other genera (*Navicula*, *Pinnularia*, and *Synedra*) tied for third (Table 1). In this experiment, gut contents of the three substrate treatments combined contained green algae, diatoms, cryptophytes, and blue-green algae at 69.6, 28.4, 1.7, and 0.4%, respectively. Little is known of the dietary requirements of freshwater mussels (Parker et al. 1998). However, it has been reported that diatoms are a very important food source since they are composed of a large percentage of polyunsaturated fatty acids and oils, two components crucial to juvenile development (Gatenby et al. 1997). In addition, green algae, although typically considered poorer in nutritional value than diatoms, are also an important part of a juvenile mussel's diet since they are high in total lipid content (Gatenby et al. 1997). Gatenby et al. (2003) also reported that diets consisting of greater algal variety are better than diets of low variety in terms of juvenile survival and growth. Therefore, the high survival and good overall size of juveniles observed in this study could be partially attributed to the large diversity of algae available in the water and subsequent consumption indicated through analyses of the gut contents.

Gut content analysis of the juveniles from the coarse-sand treatment indicated that these juveniles were feeding less than juveniles in the other treatments, since few cells of various genera were consumed. In addition, it was apparent that detritus amounts were far less in the juveniles from the coarse-sand treatment, suggesting these juveniles were not feeding at the same rates found in the other treatments. These results may partially explain why juveniles in this treatment were smaller (755 μm) than juveniles in either the fine-sediment (834 μm) or fine-sand (887 μm) treatments.

The round flow-through tank systems used in this experiment were deemed successful for rearing juvenile freshwater mussels. These round tanks required little space, contained minimal amounts of substrate, and were low maintenance. Also, water moving in a circular motion in these tanks probably allows juveniles more time to consume suspended food particles since the water is retained longer in this system than in linear flow-through systems. Overall, rearing juveniles in fine-sand limestone substrate (500–850 μm) and infrequent sampling were shown to be viable options for culturing purposes. More experimentation is needed with other species of unionids to determine whether these results are applicable across a wide array of species.

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