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Influence of sediment and algal food on cultured juvenile freshwater mussels

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Abstract. The influence of river sediment and the suitability of algae as food for juvenile rainbow mussels (*Villosa iris*) and giant floaters (*Pyganodon grandis*) were investigated by rearing mussels on various combinations of green algae (*Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii*), a commercial suspension of bacteria, and fine sediment (including autoclave-sterilized sediment). After 45 d, juvenile *V. iris* reared on sediment and algae had significantly better growth in shell length (552 μm) and survival (66.5%) than growth (437 μm) and survival (22.2%) of *V. iris* reared without sediment and fed only algae. A commercial suspension of bacteria added to a diet of algae also did not improve growth (462 μm) or survival (2.7%) of juvenile *V. iris*. Shell lengths of *P. grandis* juveniles fed algae and reared on various substrates (fine sediment, autoclaved sediment, and autoclaved sediment and bacteria) were similar among treatments. After 45 d, juvenile *P. grandis* reared on algae and fine river sediment achieved a mean length of 789 μm, representing a 2-fold increase in shell length, and 58.8% survival.

Resident bacteria in riverine sediments seemingly were not essential to growth and survival of juvenile mussels. Mussels were observed pedal-feeding for 120 ± 30 (±SD) d; thus, sediment may serve as a substratum for pedal-feeding juveniles to collect food particles. After 272 d post-metamorphosis, *V. iris* juveniles reared on sediment and algae achieved a maximum shell length of 4520 μm (mean length of 2968 μm), representing a 17-fold increase in length, and 5% survival. After 195 d, juvenile *P. grandis* had a maximum shell length of 7846 μm (mean length of 4877 μm), representing a 22-fold increase in length, and 13% survival.

Key words: juvenile freshwater mussels, pedal-feeding, growth, survival, fine sediment, algae, bacteria.

The US Bureau of Fisheries began propagation and culture of freshwater mussels (Unionidae) in the early 1900s to replenish beds depleted of shells taken for the manufacture of buttons (Lefevre and Curtis 1910a, 1912, Surber 1912, Reuling 1919, Coker 1921, Coker et al. 1921, Howard 1922). Survival and growth of cultured juvenile mussels was poor (Howard 1922), and research was discontinued with the introduction of plastic buttons and subsequent decreased demand for pearl-buttons. Over the last 20 y, drastic declines in the Unionidae have been documented, and more than 100 species now are at risk of extinction in the US (Williams et al. 1993). Factors implicated as the cause of declining mussel populations include: dam construction which inhibits dispersal of fish hosts and alters mussel habitat; dredging associated with maintaining navigation channels which destroys mussel beds; barge traffic which disturbs and can displace mussels into unsuitable habitats; overharvest; and chemical pollution from agricultural and industrial effluents. In addition, the recent invasion of the zebra mussel (*Dreissena polymorpha*) has the potential to eradicate many mussel populations. Thus, aquaculture of freshwater mussels has been suggested as a means of restoring, rehabilitating, and preventing extinctions of our native mussel fauna.

Phytoplankton is the principal food of marine bivalves (Ukeles 1971, Webb and Chu 1983), but the relative importance of bacteria, organic detritus, and protozoans as food, and the possible requirement of certain environmental bacteria for digestion, remain unknown (Urban and Langdon 1984, Crosby et al. 1989, 1990, Baldwin and Newell 1991). Previous studies on juvenile

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freshwater mussels have identified algae as suitable food and have shown that the addition of sediment to the culture chamber enhanced growth and survival (Hudson and Isom 1984, Lasee 1991). Several species of bivalves, marine and freshwater, are reported to feed with foot ciliation (Allen 1961, Morton 1976, Allen 1985) until filter-feeding organs have fully developed. Lasee (1991) showed that recently metamorphosed pocketbook mussels (*Lampsilis ventricosa*) possess a foot, rudimentary gills, and immature digestive and circulatory organs, suggesting that freshwater mussels are not capable of filter-feeding immediately post-metamorphosis to their sedentary life stage. Yeager et al. (1994) observed pedal-sweeping and pedal-locomotory behaviors in juvenile *Villosa iris*. Thus, many species of freshwater mussels may employ pedal-feeding during their early juvenile life-stage until further development occurs.

To assess the feeding ecology and nutritional requirements of juvenile freshwater mussels, the specific objectives of this study were to determine the influence of fine sediment, algal food, and bacteria on growth and survival of 2 mussel species reared in the laboratory. The rainbow mussel (*Villosa iris*) and giant floater (*Pyganodon* (*Anodonta*) *grandis*) were selected for this study, because they are common in Virginia and inhabit different environments. *Villosa iris* is a small species which, like many endangered freshwater mussels, is found in fast-moving riverine environments; *P. grandis* is a large species that inhabits slow moving or lacustrine environments.

Methods

Recently metamorphosed *V. iris* and *P. grandis* were reared on various combinations of algae, bacteria, and riverine sediments. Two experiments were conducted for 45 d to determine the influence of sediment and algae on growth (shell length) and survival of laboratory-reared juvenile mussels. Experiment 1 was conducted using *V. iris*, and Experiment 2 was conducted using *P. grandis* (Table 1). Gut contents of juveniles and river sediment were examined for algae and bacteria. During the course of this study, it became apparent that juvenile mussels were using their foot to capture food. Thus pedal-feeding behaviors were described and the number of days post-metamorphosis that juveniles employed pedal-feeding was estimated. In addition, we were able to maintain juvenile mussels in laboratory culture longer than expected. Thus growth and survival of juvenile mussels were monitored a total of 272 d for *V. iris* and 195 d for *P. grandis*.

Host-fish infestation

Newly metamorphosed mussels were obtained from host-fish infestations in the laboratory. Gravid *V. iris* were collected from Copper Creek, Scott County, Virginia. Gravid *P. grandis* were collected from Claytor Lake, Montgomery County, Virginia. Glochidia were flushed from the gills of gravid females by puncturing the ventral margin of the marsupium with a hypodermic syringe and needle. Viability of glochidia was determined by placing a sample into a saline solution to observe the closure of valves (Zale and Neves 1982). Host fish (Table 1) 15–30 cm long were collected from Tom's Creek, Montgomery County, Virginia or purchased from a local pet-store, treated for parasites, and acclimated in the laboratory prior to infestation with glochidia (Zale and Neves 1982, Neves et al. 1985).

Infestation involved placing 3–5 fish in an 8-L tank with enough water to cover the dorsal fin. Large airstones on the bottom of the tank provided vigorous aeration. Glochidia were then added to the tank with the fish; after 25–30 min exposure to glochidia, the fish were removed and placed individually in static 38-L or 76-L aquaria filled with dechlorinated tap water at 20–25°C. At this temperature, 10–21 d are required for metamorphosis of glochidia of *V. iris* (Zale and Neves 1982, Neves et al. 1985). Therefore, beginning 13 d until about 23 d post-infestation with *V. iris*, fish tanks were siphoned daily and their contents filtered through a graduated series of sieves: 306, 220, 150, and 130 μm. Metamorphosis of juveniles of anodontine species requires 7–14 d post-infestation (Lefevre and Curtis 1910b). From 7 to 21 d post-infestation with *P. grandis*, tanks were siphoned daily and the contents filtered through sieves. A dissecting microscope and a capillary pipette were used to collect juveniles from the filtered contents. By using 3–4 adult female mussels and 7–10 host fish, we obtained approximately 2000–3000 juvenile mussels. After host-fish infestation, *V. iris* females were returned to the creek,

and *P. grandis* individuals were used in further studies.

Juvenile mussel culture

River water and sediment were collected from the New River, Montgomery County, Virginia. Water hardness ($CaCO_3$) was 55.0 mg/L, and pH was 7.6. River water was filtered using a 4.25-cm diameter Whatman Glass Microfiber Filter to remove particles >0.45 μm. Sediment was maintained in the laboratory under aeration and, when needed, was sieved through a 130-μm mesh screen before being added to the juvenile culture chamber.

Newly metamorphosed juveniles were transferred to 5 replicate glass culture dishes, 8 cm in diameter, 5 cm high, and filled with 175 mL of filtered river water. A slow stream of air was introduced into each of the culture dishes by fixing an Eppendorf pipette to the end of vinyl air tubing. Approximately 25–40 mL (4 g dry wt) of fine sediment was added to the culture dishes receiving the substratum treatments. Fine sediment covered an area of 50 cm^2 and was 1.0–0.5 mm deep and loosely packed in the culture dish. Sediment particles we term "fine" are defined as "fine sand to clay" by Wentworth (1922). Water temperature was monitored daily, water was changed weekly, and fine sediment was replenished weekly. About two-thirds of the sediment in any given culture dish was discarded and replaced with fresh, fine sediment. Juveniles were fed a daily tri-algal mixture of 3.0×10^5 to 5.0×10^5 cells/mL. All experiments were conducted on a 12:12 h light:dark cycle.

Algal culture

Unialgal cultures of *Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii* were grown in Bold's Basal Medium (Appendix 1, Nichols 1973) under continuous cool white fluorescent light (photon flux: 60–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20 ± 1°C. At near-maximum stationary phase, algal cells were counted in a hemacytometer. Cultures were then centrifuged at 7000–10,000 rpm for 25 min and the medium was decanted. Algae were resuspended in tap water to achieve densities that would yield final concentrations of 3.0×10^5 to 5.0×10^5 cells/mL in the juvenile culture dishes. These concentrated algal feeds were kept under dark refrigeration for up to several weeks.

Experiment 1 with Villosa iris

Four treatments, 5 replicates each, tested whether a substrate (fine sediment, <130 μm) influenced growth or survival of recently metamorphosed mussels, whether algae were a suitable food source, and whether bacteria were essential to the diet (Table 1). Treatments included a tri-algal mixture of *Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii* (CAC); CAC plus Aqua Bacta-Aid (ABA), a bacterial suspension marketed for the aquaculture industry (Appendix 2) (CAC/ABA); CAC plus fine sediment (CAC/Sed); and an Unfed control. The green algae in CAC are commonly used in freshwater invertebrate culture (Foe and Knight 1985, Lasee 1991). ABA was added once a week to the chambers receiving the treatment CAC/ABA, at 3.25 mg/L, to replenish the bacteria according to manufacturer's specifications. Each dish contained 55–57 juveniles.

Growth and survival analyses.—We examined growth and survival over 45 d, because juvenile mussels have food reserves from their glochidial stage that allow them to live for at least 2 wk post-metamorphosis (Lasee 1991). Shell lengths of a random sample of 15 to 25 juveniles in each dish were measured weekly using a calibrated ocular micrometer on a dissecting microscope; sometimes less than 15 survivors remained in a culture dish within a treatment. Survival also was assessed weekly. All juveniles in a culture dish were sieved-out and removed to a Petri dish. Randomness was achieved by moving the Petri dish on the dissecting stage and measuring the 1st animal that came into view. Juveniles were measured, counted, and then returned to their culture dish. Thus no juvenile could be measured twice. Shell lengths and percent survival among feeding treatments were compared using 1-way ANOVA followed by Tukey-Kramer multiple comparisons (Sokal and Rohlf 1981). Arcsine transformation was applied to percent survival data to satisfy the normality assumption. Multiple comparisons were evaluated at $\alpha < 0.01$ to control the overall experimentwise error of $\alpha = 0.05$ (Zar 1974). All statistical tests were calculated using JMP 2.0 (SAS Institute, Inc., Cary, North Carolina).

Gut content analyses.—Fluorescence microsco-

TABLE 1. Experiments to test the influence of fine sediment on juvenile freshwater mussels, and the long-term survival of mussels reared in the laboratory. CAC = *Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii,* Sed = fine sediment, StSed = autoclave-sterilized fine sediment, ABA = Aqua Bacta-Aid (Appendix 1).

Mussel species	Host fish	Treatment (replicates)	Duration of experiments (d)	No. of juveniles per replicate
Experiment 1				
Villosa iris (rainbow mussel)	*Ambloplites rupestris* (rock bass), and *Micropterus salmoides* (largemouth bass)	CAC (5)	45	55–57
		CAC/ABA (5)	45	55–57
		CAC/Sed (5)	45	55–57
		Unfed (5)	45	55–57
Experiment 2				
Pyganodon grandis (giant floater)	*Ambloplites rupestris* (rock bass), *Lepomis macrochirus* (bluegill), *Micropterus salmoides* (largemouth bass), and *Carassius auratus* (goldfish)	CAC/Sed (3)	45	200–205
		CAC/StSed (3)	45	200–205
		CAC/StSed/ABA (3)	45	200–206

Mussel species	Host fish	Long-term diet (cultures)	Duration of culture (d)	No. of juveniles per culture
Long-term cultures				
V. iris	As in Experiment 1	CAC/Sed (3)	272	200
P. grandis	As in Experiment 2	CAC/Sed (5)	195	200–205

py, which induces a distinctive red fluorescence of chlorophyll under blue light, was used to verify that juveniles were ingesting algae, and that the algae were well distributed throughout the substratum. Juveniles were placed on microscope slides and squashed under cover slips for examination.

Pedal-feeding observations.—Locomotory and feeding behaviors of *V. iris* were observed using a dissecting microscope at the time of shell measurements and survival assessment. Approximately 20 juveniles from each culture dish were observed for about 25 min each week.

Experiment 2 with Pyganodon grandis

Observations during the experiment with *V. iris* suggested that mussels were using their feet to capture food, i.e., pedal-feeding (Reid et al. 1992, Yeager et al. 1994). Therefore, Experiment 2 focused on whether bacteria in river sediment were important to juvenile growth and survival, or whether fine sediment merely provided a physical substratum for pedal-feeding. Growth of *P. grandis* fed only algae in preliminary trials[1] was poor, and availability of juvenile mussels from host-fish infestation was limited. Thus, we did not test a treatment of algae without sediment in Experiment 2. The treatments tested in triplicate were a tri-algal diet of *Chlorella vulgaris, Ankistrodesmus falcatus, and Chlamydomonas*

[1] In preliminary tests, juvenile *P. grandis* fed the green algae (CAC) without sediment grew minimally ($\bar{x} \pm 1$ SD = 412 ± 43 μm, n = 60) after 45 d. Juveniles were reared in 3 replicate culture dishes, which were modified versions of those used to rear oyster bivalve larvae, and characterized by a downward flow of water ("downwellers") (Wikfors et al. 1992). Juvenile mussels rested on 150-μm mesh screen which lined one end of a cylinder; the cylinder was placed in a larger chamber containing 500 mL of water. The dimensions of the cylinder were the same as the static culture dishes. Water circulated in the entire culture dish at approximately 200 mL/min. In other preliminary tests using *V. iris* fed only green algae, we compared growth and survival between juveniles reared in static culture dishes and downwellers after 30 d. In these tests, growth of *V. iris* was negligible in static systems (357 ± 52 μm, n = 3) and in downwellers (324 ± 34 μm, n = 9).

reinhardtii with colonized fine sediment (CAC/Sed); CAC with autoclave-sterilized fine sediment (CAC/StSed); and CAC with autoclave-sterilized fine sediment plus ABA(CAC/StSed/ABA) (Table 1).

River water and sediment were collected from the New River, Montgomery County, Virginia. Colonized fine sediment referred to the <130-μm sieved sediment with its associated bacterial flora and organic material. Sterile sediment was obtained by autoclaving river mud for >15–20 min at >121°C and >15 psi, then sieving through a 130-μm mesh screen before adding to the culture dishes. Juveniles were reared in static dishes containing filtered river water and one of the above treatments. Juveniles were fed tri-algal diets daily at a concentration of 3.0×10^5 to 5.0×10^5 cells/mL. Fine sediment and ABA (added weekly at 3.25 mg/L) were not included in particle concentration calculations. Algae were cultured, and tri-algal mixtures were prepared as described previously.

Growth and survival analyses.—Surviving juveniles were counted, and shell lengths of a random sample of at least 15 juveniles in each chamber were measured using a calibrated ocular micrometer on a dissecting microscope. Juveniles were replaced after measuring. Feeding treatments were compared after 45 d, using 1-way ANOVA, followed by Tukey-Kramer multiple comparisons (Sokal and Rohlf 1981). Arcsine transformation was applied to the percent survival data to satisfy the normality assumption. Multiple comparisons were evaluated at $\alpha < 0.02$ to control the overall experiment-wise error of $\alpha = 0.05$ (Zar 1974). All statistical tests were calculated using JMP 2.0 (SAS Institute, Inc., Cary, North Carolina).

Gut content analyses.—Fluorescence microscopy also was used to verify that juveniles were ingesting algae, and that the algae were well distributed throughout the substratum. In addition, DAPI (4′,6′-diamidino-2-phenylindole; Sigma Chemical Co., St. Louis, Missouri), a flourochrome stain specific for DNA, was used to determine presence of bacteria in the tri-algal diet, fine sediment, and in the guts of juvenile mussels (King and Parker 1988). Juveniles were placed on microscope slides, squashed under cover slips, and stained for examination. DAPI-stained samples fluoresced blue under ultraviolet light.

Pedal-feeding observations.—Locomotory and feeding behaviors of juvenile mussels were observed using a dissecting microscope. Approximately 20 juveniles from each culture dish were observed for about 25 min each week.

Villosa iris reared longer than 45 d post-metamorphosis

Recently metamorphosed juveniles were cultured in 3 replicate static dishes with fine sediment. Each dish was stocked with 200 juveniles. At 165 d post-metamorphosis, *V. iris* juveniles were transferred to a round 4-L tank containing filtered river water and fine sediment to a depth of about 1 cm. Water was aerated and a circular flow was created within the tank. Juveniles were fed a daily concentration of 3.0×10^5 to 5.0×10^5 cells/mL of CAC; water was changed and sediment replenished weekly. Growth in shell length from a random sample of 20 juveniles from each culture dish, and then from the 4-L tank, was measured and survival was assessed bimonthly to 272 d post-metamorphosis.

Pyganodon grandis reared longer than 45 d post-metamorphosis

Survivors from Experiment 2, reared on treatments CAC/Sed, CAC/StSed, and CAC/StSed/ABA, were cultured in 5 replicate culture dishes to 126 d post-metamorphosis; sediment was not autoclaved after the initial 45 d experimental period. At 126 d post-metamorphosis, *P. grandis* juveniles were placed in a round 4-L tank, as already described. Juveniles were fed a daily concentration of 3.0×10^5 to 5.0×10^5 cells/mL of CAC; water was changed and sediment replenished weekly. Shell lengths from a random sample of 15 juveniles in each culture dish, and then from the 4-L tank, were measured and survival was assessed bimonthly to 195 d post-metamorphosis.

Results

Experiment 1 with Villosa iris

Juveniles of *V. iris* reared on the various treatments showed highly significant differences in mean shell lengths ($p < 0.0001$) (Table 2). The CAC/Sed treatment was significantly different from all other treatments ($p < 0.009$), and final mean lengths of juveniles in the CAC and CAC/

TABLE 2. Mean, standard deviation, and range of shell lengths (μm), and percent survival of *Villosa iris* juveniles reared on various treatments for 45 d post-metamorphosis (Experiment 1). Means with same superscripts (a, b) are similar according to Tukey-Kramer test ($\alpha < 0.009$). *p*-values are given for the ANOVA test for treatment effect ($\alpha = 0.05$).

Treatment	Length (μm)*		Total juveniles measured	Survival (%)
	Mean ±1 SD	Range		
CAC	$437^{b} \pm 41.7$	333–589	9	22.2^{b}
CAC/ABA	$462^{b} \pm 37.0$	384–462	21	2.7^{b}
CAC/Sed	$552^{a} \pm 28.6$	462–692	60	66.5^{a}
Unfed	$386^{b} \pm 19.9$	256–513	7	$51.3^{a,b}$
	$p < 0.0001$			$p = 0.0128$

* Initial length of juveniles was 250 ± 37 (200–385) μm

ABA treatments were similar to those of the juveniles in the Unfed treatment (Table 2). Mean (±1 SD) shell length of juveniles reared on CAC/Sed after 45 d was 552 ± 28.6 μm, a 2-fold increase in shell length. Survival was also significantly different among treatments ($p = 0.0128$) (Table 2). Tukey-Kramer multiple comparison tests showed that the treatments CAC and CAC/ABA were statistically similar to the Unfed control, but significantly less than CAC/Sed; however, survival for CAC/Sed also was similar to the Unfed control. Variability within a treatment was high. For example, survival varied from 11% to 100% for CAC/Sed, and from 3.6% to 45.4% for CAC. Thus, the Tukey-Kramer multiple comparison was of insufficient robustness to detect differences between a mean survival of 2.7% for CAC/ABA and 51.3% for the Unfed control.

Gut content analyses and pedal-feeding observations.—Fluorescence microscopy showed that juveniles reared with a substratum ingested algae. Subsequent gut squashes showed partially digested algal cells or ghost cells lacking chloroplasts as well as colloidal particles. Juveniles were very mobile, using foot movements to pull themselves along while exhibiting foot-sweeping behavior. When fine sediment was present, foot movements drew particles toward the pedal gape. The foot was ciliated, and some particles adhered to the foot, while larger particles were lost as the juvenile retracted its foot inside the valves. These behaviors have been called pedal-sweep and pedal locomotory-feeding or "pedal-feeding" for all food-collecting behaviors in which bivalves use their feet (Reid et al. 1992, Yeager et al. 1994). Gut squashes of juveniles reared on algae only and algae and bacteria showed red-fluorescing chlorophyll, indicating these juveniles had also ingested algae; however, we observed about 75% fewer particles swept into the pedal-gape by juveniles in treatments without a substratum. *V. iris* exhibited locomotory and pedal-sweep movements for approximately 140 d. After 272 d, *V. iris* were observed positioned anterior-end in the sediment; apertures were visible and siphoning.

Experiment 2 with Pyganodon grandis

Mean shell lengths of *P. grandis* juveniles in all treatments were similar after 45 d ($p = 0.3486$) (Table 3). The addition of ABA (bacteria) to autoclave-sterilized sediment did not enhance growth when compared to other treatments. Survival was similar for all treatments ($p = 0.3073$); juveniles fed CAC/Sed exhibited the highest survival of 58.8%, though not significantly different from the others (Table 3).

Gut content analyses and pedal-feeding observations.—Fluorescence microscopy confirmed that juveniles were ingesting algae, and that the algae were well distributed throughout all substratum-types. The DAPI stain for DNA showed rod-shaped bacteria in the tri-algal diets and in the guts of juvenile mussels. It is not known whether the bacteria in the mussels were acquired from algal diets, river sediment, or were a natural gut flora. Juveniles were mobile, exhibiting pedal-sweep and pedal-locomotory behaviors (Reid et al. 1992) for at least 120 d post-metamorphosis. Particles adhering to the ciliated foot entered through the pedal gape, and sometimes entered through a current generated by the sweeping of the foot. At 9 mo post-metamorphosis, *P. grandis* were observed positioned

TABLE 3. Mean, standard deviation, and range of shell lengths (μm), and percent survival of *Pyganodon grandis* juveniles reared on various treatments for 45 d post-metamorphosis (Experiment 2). Means with same superscripts (a, b) are similar according to Tukey-Kramer test ($\alpha < 0.0167$). *p*-values are given for the ANOVA test for treatment effect ($\alpha = 0.05$).

Treatment	Length (μm)*		Total juveniles measured	Survival (%)
	Mean ±1 SD	Range		
CAC/Sed	789[a] ± 43.3	600–1000	45	58.8[a]
CAC/StSed	862[a] ± 84.3	529–1114	30	36.9[a]
CAC/StSed/ABA	827[a] ± 26.1	586–1000	30	44.0[a]
	$p = 0.3486$			$p = 0.3073$

* Initial length of juveniles was 367 ± 20 μm

anterior end in the sediment; apertures were visible and siphoning.

Villosa iris reared longer than 45 d post-metamorphosis

Juveniles fed CAC/Sed in 2 replicate culture dishes died at about 90 d post-metamorphosis (Table 4). We believe mortality in these dishes was due to mechanical problems and not related to the treatment; therefore, we calculated percent survival for the culture dish with survivors. At 165 d post-metamorphosis, survival was 8.0% and at 272 d post-metamorphosis, survival was 5%. Mean length at 165 d post-metamorphosis was 1146 ± 248 μm, and ranged between 718 and 1590 μm. After 272 d post-metamorphosis, mean length was 2968 ± 405 μm and ranged between 2000 and 4520 μm (Table 4). The maximum length at 272 d was 17 times greater than that of a 1-d-old juvenile.

Pyganodon grandis reared longer than 45 d post-metamorphosis

Mean shell length of *P. grandis* juveniles at 126 d was 2449 ± 501 μm and ranged between 1410 and 3846 μm. Mean shell length at 195 d was 4319 ± 831 μm and ranged between 2460 and 7846 μm. The maximum length was 22 times greater than that of a 1-d-old juvenile, and percent survival after 126 d and 195 d was 27.5% and 12.9%, respectively (Table 5).

Discussion

Laboratory cultures with Villosa iris

Many researchers have suggested that the benefit of silt or fine sediment to juvenile bivalves is that resident bacteria in aquatic sediments enhance enzymatic activity or aid in digestion (Urban and Langdon 1984, Crosby et al. 1990). If true, then the addition of certain bacteria to culture chambers (without sediment), along with a food source such as algae, should satisfy dietary requirements of juvenile mussels. The addition of ABA, which contains several species of bacteria common to riverine systems, did not enhance growth or improve survival. In fact, juveniles fed algae with ABA grew no more and survived no better than unfed juveniles and juveniles fed only algae. A diet of algae in sed-

TABLE 4. Mean, standard deviation, and range of shell lengths (μm) and percent survival of *Villosa iris* juveniles reared on CAC/Sed for 272 d post-metamorphosis.

Time (d)	Length (μm)*		Total juveniles measured	Survival (%)
	Mean ±1 SD	Range		
60	658 ± 83	500–886	60	51.6
100	874 ± 137	643–1143	16	36.0
165	1146 ± 248	718–1590	16	8.0
100	1245 ± 258	769–1667	16	8.0
272	2968 ± 405	2000–4520	10	5.0

* Initial length of juveniles was 263 ± 37 μm

TABLE 5. Mean, standard deviation, and range of shell lengths (μm), and percent survival of *Pyganodon grandis* reared on CAC/Sed* for 195 d post-metamorphosis.

Time (d)	Length (μm)** Mean ±1 SD	Range	Total juveniles measured	Survival (%)
60	1281 ± 201	786–2229	75	48.4
92	1994 ± 336	799–3404	75	34.6
126	2449 ± 501	1410–3846	75	27.5
195	4319 ± 831	2460–7846	43	12.9

* Juveniles in Experiment 2 were reared on 3 different treatments immediately post-metamorphosis to 45 d (Table 1); survivors were transferred to 5 culture chambers and reared on CAC/Sed from 45 d post-metamorphosis to 195 d

** Initial length of juveniles was 367 ± 20 μm

iment, however, supported juveniles well beyond 45 d, suggesting that the importance of sediment to juvenile growth and survival is more than the accompanying bacteria.

In the few other feeding studies of freshwater bivalves, conflicting results on the importance of sediment have been presented. Foe and Knight (1985) showed that growth of asiatic clams (*Corbicula fluminea*) was independent of suspended sediment. Similarly, Lasee (1991) showed that 50–100 g/L of sediment added to aquaria did not greatly improve growth or survival of juvenile pocketbook mussels (*Lampsilis ventricosa*) after 56 d. In contrast, Hudson and Isom (1984) achieved greater survival (91.9%) and enhanced growth (610 μm) for 30-d-old *Utterbackia* (*Anodonta*) *imbecillis* juveniles with the addition of sediment to the culture chamber. They added 700 mg/L of "cloudy silt suspension" in one treatment and in another treatment, enough sediment to produce a thin layer (~1 cm) on the bottom of the culture chamber. No significant difference in growth occurred after 30 d between the 1-cm-layer-of-sediment treatment and the "cloudy silt suspension". They suggested that sediment provided nutrition in the form of organic detritus.

Our results for *V. iris* concur with those of Hudson and Isom (1984): namely, that growth in shell length and survival of juveniles are enhanced by the presence of fine sediment. However, our observations of juveniles confirmed that they pedal-feed post-metamorphosis (Yeager et al. 1994). The guts of juveniles were packed with chlorophyll and colloidal particles, indicating that they had ingested algae associated with the fine sediment. Cultures without sediment clearly contained fewer particles/cm^2. However, the amount of algae given daily to these cultures was more than enough food to achieve maximum growth in several species of bivalves (D. Kreeger, Academy of Natural Sciences, personal communication). Thus, the primary role of sediment may be to provide a substratum or a place for pedal-feeding juveniles to collect food. If early juvenile mussels are unable to effectively filter-feed, then pedal-feeding in the sediment would be necessary to obtain organic nutrients and inorganic minerals. This is an area of research that needs further testing.

Juvenile mussels may adopt filter-feeding in conjunction with pedal-feeding before completely converting to a filter-feeding life style, as formation of the ctenidia continue (Lasee 1991). Lopez and Holopainen (1987) have shown that very small bivalves, such as fingernail clams (*Pisidium* spp), suspension-feed in the interstitial spaces of muds. Yeager et al. (1994) found newly metamorphosed juvenile mussels burrowed in sediment to a depth of 1 cm, and observed pedal-sweeping behaviors—which they termed deposit-feeding (Reid et al. 1992)—and suspension-feeding. However, Lopez and Holopainen (1987) applied the term interstitial suspension-feeding to those bivalves using siphon or aperture action to draw in water from the interstitial spaces of sediment and using gills to filter and transfer food particles to the mouth. Thus, it is plausible that juvenile freshwater mussels filter-feed or suspension-feed as gill development continues, and that unconsolidated sediment of varying particle sizes will facilitate pedal-feeding or deposit-feeding.

Hudson and Isom (1984) did not report whether all 700 mg/L of silt remained in suspension for the duration of their experiment. It

is possible that some of this silt settled to the bottom of the aquaria and provided a substratum for pedal-feeding mussels. Fine sediment also may serve as an internal grinding substrate for digesting algal cells. Preliminary experiments by Enright et al. (1986) established that kaolin marginally enhanced growth of the European oyster (*Ostrea edulis*), but Urban and Langdon (1984) reported a marked increase in growth of juvenile American oysters when kaolin was added to a combination of live algae and a non-algal supplement such as rice, whey, or yeast. Urban and Langdon (1984) concluded that kaolin provided a grinding substratum for the crystalline style, thus increasing digestibility of those algae with tough cell walls. Bricelj et al. (1984), however, dispelled this hypothesis for the hard clam (*Mercenaria mercenaria*). According to Lasee (1991), encysted unionid glochidia lack a crystalline style. At 12 h to 2 d post-metamorphosis, juveniles develop a posterior style sac stomach (characterized by a crystalline style and cilia) which would allow them to digest algae. By 56 d post-metamorphosis, the style sac is a separate structure from the stomach, indicating that mussels may be fully capable of digesting algae. The hypotheses that fine sediment may serve as a grinding substrate to enhance digestion of algae in freshwater mussels is untested.

Laboratory cultures with Pyganodon grandis

Growth and survival of *P. grandis* juveniles seemed facilitated by a substratum, presumably for pedal-feeding juveniles to collect food particles such as algae. The addition of ABA to autoclave-sterilized sediment did not enhance growth or improve survival, nor did sterilized sediment hinder growth or survival. Thus, the role of sediment to provide specific bacteria essential to juvenile mussel digestion is unconfirmed. Sediment may provide some nutritional value, as it contains organic material with or without sterilization. Crosby et al. (1990) showed that the American oyster (*C. virginica*) was able to ingest and assimilate organic detritus as the result of bacterial action on detrital complexes. It is well known in agriculture that soil clays possess the property of base-exchange; potassium, calcium, and magnesium are adsorbed by clay creating a storehouse of certain elements and nutrients (Kelley 1942). In addition, Grimm (1942) reported that the capacity of certain clay minerals (kaolinite, illite) for base-exchange increased with decreasing particle size. The structural organization of fine soil particles allows incorporation of organic compounds. Compounds such as amino acids and sugars can adsorb onto the surface of soil particles (Weiss 1969). Pedal-feeding juveniles that ingest fine sediments associated with algae could, therefore, acquire minerals and nutrients essential for good growth and survival.

Growth and survival of mussels in laboratory culture and outdoors

The difference in shell lengths of the 2 species cultured in our experiments was anticipated given the documented difference in growth rates of adults (Table 6, Grier 1922). We expected greater differences in survival for the 2 species, given the *V. iris* requirement for a riverine habitat and *P. grandis* preference for standing water. Interestingly, little difference in survival over time was apparent (Table 6), which we attribute to the conditions of the culture environment. Howard (1922) hypothesized that a controlled environment, where predation and siltation were minimized, enhanced survival of juvenile freshwater mussels. Aldridge and Payne (1987) reported that unionids exposed to suspended solids reduced their metabolic rate and experienced lower filter clearance rates of food particles. Such reductions in food clearance rates have been correlated with reductions in growth rates of the hard clam (*Mercenaria mercenaria*) (Bricelj et al. 1984).

Comparison of percent survival of mussels in this study with survival reported in other studies was somewhat difficult, as most studies failed to report the number of survivors or reported only limited results (Table 6). *Lampsilis ventricosa* cultured in aquaria for 56 d without a substratum and outdoors for 80 d showed much poorer survival (7% and 2.5%, respectively) than *V. iris* reared on algae and fine sediment (CAC/Sed) for 60 d (51.6%) or *P. grandis* cultured in aquaria for 92 d (34.6%) (Table 6). These contrasts suggest that the lower incidence of predation and reduced turbidity in the laboratory may enhance survival (Howard 1922, Aldridge et al. 1987). In addition, pedal-feeding juvenile mussels may require a substratum such as fine sediment to acquire necessary food ma-

TABLE 6. Summary of maximum shell lengths and % survival of juvenile mussel species. nd = no data.

Species	Length (mm)	Age (d)	Survival (%)	Reference
Villosa iris	0.89	60	51.6	This study
Lampsilis ventricosa	1.1	56	~6.5	Lasee (1991)
Anodonta cellensis	3.1	60	nd	Herbers (in Howard 1922)
Pyganodon grandis	2.2	60	48.4	This study
Velesunio angasi	3.7[a]	63	93.0	Humphrey (1987)
Lampsilis siliquoidea[b]	7.0	67	nd	Howard (1922)
Lampsilis siliquoidea[c]	13.0	67	nd	Howard (1922)
Utterbackia imbecillis	5.1	74	1.0	Hudson and Isom (1984)
Lampsilis ventricosa[d]	2.0	80	2.5	S. Ziegler, National Biological Service (personal communication)
Pyganodon grandis	3.4	92	34.6	This study
Pyganodon grandis	7.9	195	12.9	This study
Villosa iris	1.7	200	8.0	This study
Villosa iris	4.5	272	5.0	This study
Villosa iris	2.7	365	nd	Neves and Widlak (1987)
Pyganodon grandis	32.0	365	nd	Bright et al. (1990)

[a] Reported 14-fold increase multiplied by assumed initial length of 260 μm
[b] Juveniles reared in aquaria
[c] Juveniles reared in floating cages in the Mississippi River
[d] Juveniles reared outdoors in the Black River, Wisconsin

terials. That juvenile *V. iris* from the Unfed treatment in Experiment 1 exhibited similar survival at 45 d to juvenile *V. iris* reared on fine sediment and fed algae may indicate inherent survival capabilities of juvenile mussels in environments free of predation (Table 2). However, in preliminary work and other experiments testing an unfed treatment, survival was much lower (6% and 15.5%) than survival in Experiment 1. Juveniles with closed valves could have been mistakenly counted as live when in fact they were dead. It is difficult to determine moribund juveniles if the valves are not gaping open.

P. grandis is a large mussel species, as are most species in prior studies. Shell length of *P. grandis* in our laboratory was similar to that of other species reared indoors, indicating that the culture environments or diets were nutritionally similar (Table 6). However, shell lengths of *P. grandis* in the Minnesota River (Bright et al. 1990) and from Howard's (1922) cultures of *L. siliquoidea* in the Mississippi river culture and in aquaria were strikingly different from *P. grandis* reared in our laboratory, probably the result of differences in diet and culture environment. For example, the natural environment may provide additional nutrients, vitamins, or food abundance required by freshwater mussels, but not present in laboratory experiments. Howard (1922) concluded that juveniles grown outdoors were larger than laboratory-cultured juveniles of the same age. He reported that 30-d-old, river-cultured juveniles of *L. siliquoidea* placed in aquaria for 37 d grew only 1 mm, compared to the same age juveniles remaining in the river which grew an additional 7 mm. However, wild juvenile *V. iris* in Big Moccasin Creek, Virginia, at <1 yr old (Neves and Widlak 1987) had mean shell lengths (2.7 mm) and size ranges (0.8–5.0 mm) similar to those of our 272-d-old laboratory-cultured *V. iris*. That differences in shell length between wild and laboratory reared *V. iris* juveniles were neglible after 1 y may be attributable to slow growth rates of small-sized species (Neves and Widlak 1987).

In this study, we conclude that sediment facilitates collection of food materials by pedal-feeding freshwater mussels, that bacteria associated with river sediments are not directly essential to juvenile digestion or growth, and that live algae are suitable food for juvenile *V. iris* and *P. grandis*. In addition, we believe that a properly controlled laboratory environment, with adequate water quality and no predation, can enhance survival and growth of juvenile freshwater mussels. Information on life history requirements is limited, with nutritional requirements probably the least understood. Re-

search in the culture of finfish and shellfish shows that various life stages have different requirements for protein, carbohydrates, and polyunsaturated fatty acids (Ukeles 1971, Takeuchi et al. 1978); therefore, it is likely that juvenile mussels also will have specific nutritional requirements. Future research in the culture of freshwater mussels should focus on diet development and environmental requirements of the juvenile life stage.

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APPENDIX 1. Algal culture medium (Bold's Basal Medium (Nichols 1973) or Modified Bristol's solution).

Chemical	Stock concentration	Amount of stock/L
$NaNO_3$	10 g/400 mL	10 mL
$CaCl_2 \cdot 2H_2O$	1 g/400 mL	10 mL
$MgSO_4 \cdot 7H_2O$	3 g/400 mL	10 mL
K_2HPO_4	3 g/400 mL	10 mL
KH_2PO_4	7 g/400 mL	10 mL
NaCl	1 g/400 mL	10 mL
$Na_2EDTA \cdot 2H_2O$	50 g/L	1 mL
KOH	31 g/L	1 mL
$FeSO_4 \cdot 7H_2O$	5.0 g/L	1 mL
H_2SO_4	1.0 ml/L	1 mL
H_3BO_3	11.42 g/L	1 mL
$ZnSO_4 \cdot 7H_2O$	8.82 g/L	1 mL
$MnCl_2 \cdot 4H_2O$	1.44 g/L	1 mL
MoO_3	0.71 g/L	1 mL
$CuSo_4 \cdot 5H_2O$	1.57 g/L	1 mL
$Co(NO_3)_2 \cdot 6H_2O$	0.49 g/L	1 mL
Distilled water		~930 mL

APPENDIX 2. Composition of Aqua Bacta-Aid R1 (ABA) (Water Quality Science, Inc. Bolivar, Missouri). According to the manufacturer, the product is non-pathogenic, non-toxic, and non-irritating.

Bacterial composition[a]	*Aerobacter, Bacillus* (2 species), *Cellulomonas, Nitrobacter, Nitrosomonas, Pseudomonas, Rhodopseudomonas*
Growth medium (% vol)	NH_4Cl (0.10), K_2HPO_4 (0.10), $MgSO_4 \cdot 7H_2O$ (0.05), Na Acetate (0.10), Yeast extract (0.10), Na_2S (0.5)[b]

[a] Total cells/L = 180 billion bacteria
[b] Keeps the bacteria alive, but not growing