Development of an Algal Diet for Rearing Juvenile Freshwater Mussels (Unionidae)

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(ABSTRACT)

Feeding selectivity by the rainbow mussel (*Villosa iris*) was examined for three age groups; 2-3 days old, 50-53 days old, and 3-6 years old. The mussels were fed an algal diet consisting of *Scenedesmus quadricauda* (22.3 - 44.5 μm), *Nannochloropsis oculata* (2.8 – 8.1 μm), and *Selenastrum capricornutum* (3.6 – 8.5 μm) in equal cell densities. The change in relative abundance of each algal species within feeding chambers over a 5 hr feeding trial was used to discern selectivity. At the conclusion of the feeding trials, the gut contents of mussels were analyzed for preferential ingestion. The mussels selected for *N. oculata* and *S. capricornutum* over *S. quadricauda* ($p < 0.05$). This may be an indication of particle size-dependent selection. Feeding trials also suggest that selectivity by the rainbow mussel does not change with age. Gut content analyses showed a preferential ingestion of algae, in the sequence *N. oculata*, *S. capricornutum*, then *S. quadricauda*.

The suitability of two algal diets, *S. quadricauda* and *N. oculata*, for rearing captive juveniles of *V. iris* in 145-L recirculating culture systems was compared. Juveniles were fed their assigned diet at a density rate of approximately 30,000 cells/ml for 42 days, and sampled weekly for percent survival and shell length. Regardless of diet, juvenile survival decreased rapidly after 21 days, and growth did not exceed approximately 450 μm. High mortality rates and slow growth of juveniles was likely due to inadequate diets. Juveniles that were fed *S. quadricauda* lacked chlorophyll coloration in their guts, indicating that the juveniles did not ingest this species of algae. Colonies of *S. quadricauda* were likely too large for the juveniles to ingest. The gut content of juveniles fed *N. oculata* showed chlorophyll coloration, indicating that the juveniles

ingested this species, but *N. oculata* may have been difficult for the juveniles to assimilate. Under the culture conditions provided, survival and growth did not compare favorably to those of other studies with *V. iris*.

Newly metamorphosed juveniles of *V. iris* were reared in 145-L recirculating culture systems containing sediment (< 600 μm) of two depths, 5 mm and 15 mm. Mussels were fed a bi-algal diet of *Nannochloropsis oculata* and *Neochloris oleoabundans*. Survival differed significantly between treatments ($p=0.04$), and was higher for juveniles reared in 5 mm of sediment over a 40-day period. Growth was not significantly different between treatments. After 40 days, juveniles achieved a mean length of approximately 578 μm in both treatments. Survival and growth of juveniles compared favorably to those of other culture studies using juveniles of *V. iris.* A shallow layer of sediment is recommended for the culture of juvenile mussels.

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Introduction

In North America, native freshwater mussel populations have experienced severe declines over the past three decades (Williams *et al.* 1993), and captive propagation of juveniles has become one of the primary strategies for the conservation of these species. However, standard protocols for cultivating unionids are not completely developed, even though many populations of threatened and endangered mussel species would benefit from release of cultured juveniles. The speed with which culture protocols are developed and employed is crucial for preserving rare species from extinction.

Culture protocols for many marine bivalves are well developed, and there needs to be greater application of the marine literature by those developing culture protocols for freshwater mussel species. By adapting procedures for rearing the marine hard shell clam (*Mercenaria mercenaria*), Hudson and Isom (1984) were able to culture *Utterbackia imbecillis* and *Epioblasma triquetra* well past 30 days. Prior to using the adapted methods, they were unable to culture the juveniles beyond 2 or 3 wk. More recently, Gatenby *et al.* (1997) and Gatenby (2000) have used protocols from the marine literature to develop a nutritious algal diet suitable for rearing captive freshwater mussels.

The first objective of my study was to evaluate selective feeding by juvenile freshwater mussels, to determine whether they sort particles prior to ingestion. In addition to size of suspended particles (Baldwin 1995, Defossez and Hawkins 1997, Raby *et al.* 1997), a variety of other particle characteristics have been shown to affect feeding behavior of marine bivalves. Chemical cues (Ward and Targett 1989, Ward *et al.* 1992, Baldwin 1995) and electrostatic charge (Solow and Gallager 1990) have been shown to influence capture efficiency and selection by marine bivalves. This knowledge gained from the first objective could then be used in conjunction with the findings of other researchers to develop a suitable algal diet for rearing newly metamorphosed juvenile mussels. Methods used for culturing marine algae were then modified to culture batch quantities of desirable freshwater algae free of contaminants. The algae were used in the second objective of this study, to test the suitability of two uni-algal diets for rearing juvenile mussels in large volume recirculating culture systems.

Some aspects of the life history of unionids are unique, and methods for propagation must be developed distinct from marine species. The most notable is the host fish relationship, and although more research is needed in this area, several methods for transforming glochidia are well-documented (Isom and Hudson 1982, Zale and Neves 1982). Unlike many marine bivalves, juvenile freshwater mussels appear to have substrate requirements (Hudson and Isom 1984, Gatenby *et al.* 1996, O'Beirn *et al.* 1998). There are several hypotheses describing this substrate requirement, although none of them have been strictly supported (Hudson and Isom 1984, Yeager *et al.* 1994, Gatenby *et al.* 1996, O'Beirn *et al.* 1998). Substrate size has been shown to affect growth of juvenile *Lampsilis fasciola* (Rogers 1999). However, the optimum substrate depth for rearing juvenile mussels has yet to be tested. Therefore, the third objective of my study was to compare growth and survival of juveniles reared in two different sediment depths.

It is not always possible to collect the desired quantities of endangered or threatened species for experimental purposes, and in most cases a more common conspecific species has to be used as a surrogate. For my research, *Villosa iris* was chosen as a surrogate for a number of reasons. First, adequate populations are available within 2 hr of Blacksburg, Virginia. Second, large quantities of juveniles can be produced in a laboratory setting (Gatenby *et al.* 1996, Gatenby *et al.* 1997, O'Beirn 1998, Beaty 1999). Third, the reproductive biology (long term-brooder) and habitat are similar to many endangered and threatened species. Fourth, *V. iris* has been a well-studied species, so results can be compared readily to other studies (Gatenby *et al.* 1996, Gatenby *et al.* 1997, O'Beirn 1998, Beaty 1999).

Researchers have also noted that juvenile freshwater mussels should be released in the late spring, when temperatures exceed 15^{o}C (Hanlon 2000). Since the ultimate goal of conservation aquaculture is to produce as many physically fit juveniles as possible for release, I chose to have my experiments last approximately 40-42 days. The rationale behind this decision was that gravid females of long-term brooders would be readily available in late February and early March. It would then take approximately an entire month, depending on water temperature, for larvae to transform in the laboratory. Juveniles would then be cultured for roughly 6 weeks to ensure that survivorship was high, and that the juveniles were physically fit to be released in early June.

Consequently, the juveniles would have a jump-start on the growing season, which is important to ensure optimum survival and growth once released (Hanlon 2000). Additionally, it is not desirable to hold juveniles of endangered species in the laboratory for extended periods of time, as mortality rates can increase (Rogers 1999).

Chapter 1: An Evaluation of Selective Feeding by Three Age Groups of the Freshwater Rainbow Mussel (*Villosa iris*)

INTRODUCTION

Propagation studies of native freshwater mussels (Bivalvia: Unionidae) have been used to describe much of their basic life history, including host fish relationships (Neves *et al.* 1985, O'Connell and Neves 1999), environmental stress thresholds (Dimoch and Wright 1993), and habitat and substrate use (Michaelson and Neves 1995). Dietary studies have not been common, and the nutritional requirements for captive unionids, specifically juveniles, remain undetermined. Algae have been the most popular food source for captive juvenile mussels. Various combinations of algal species have been used to culture juveniles (Hudson and Isom 1984, Gatenby *et al.* 1996, Gatenby *et al.* 1997), and due to their apparent importance in the diet of unionids, algal species have been incorporated into the design of recirculating culture systems as the primary food source (O'Beirn *et al.* 1998, Tankersley and Butz 2000, Henley *et al.* 2001).

The importance of algal species as a food source for captive juvenile mussels is likely a compromise between their physical characteristics and nutritional properties. The lipid content of an algae diet has been directly correlated with juvenile growth (Gatenby *et al.* 1997). In addition, adult unionids have displayed an ability to sort algae based on the cellular characteristics prior to ingestion (Paterson 1984, Miura and Yamahiro 1990, Baker and Levinton 2000). It is possible, therefore, that juveniles also exhibit a selective response for the cellular characteristics of algae.

Selective feeding may advance during the early developmental stages of juvenile mussels. The primary food source for juveniles can change within the first 2 wk post-metamorphosis (Yeager *et al.* 1994), and may correspond with the transition from pedal-feeding to suspension-feeding. This transition is common among bivalves, and it is believed that a pedal-feeding stage takes place in almost all juvenile bivalves (Reid *et al.* 1992). Pedal-feeding by juvenile mussels may extend to 140 days post-metamorphosis (Gatenby *et al.* 1997). Henley *et al.* (2001) suggest that accounting for pedal feeding is so important for successful culture that separate culturing strategies should be

incorporated for individual stages of grow-out. It has not been determined whether different food sources should be used at individual stages of juvenile development.

The purpose of this study was to determine whether selective feeding occurs during early development of captive unionids. This knowledge would then be used to aid in the preparation of a suitable algal diet for captive grow-out of juvenile mussels. Particle selection by *Villosa iris* was examined at three different ages; 2-3 days old, 50-53 days old, and 3-6 years old. Changes in relative abundance were analyzed through time of *Scenedesmus quadricauda*, *Nannochloropsis oculata*, and *Selenastrum capricornutum* in feeding chambers. Gut content analysis was used then to verify selective ingestion by the test mussels. Relative abundance of algae within pseudofeces for rejection and in feces for selection was not analyzed, because insufficient quantities were produced for analyses.

METHODS

Specimens of *V. iris* representing three age groups: age group I of 2-3 days old (shell length: 280-333 μm), age group II of 50-53 days old (385-640 μm) and age group III of 3-6 years old (21-40 mm), were tested. These age groups were chosen based on previously documented transitions from pedal-feeding to suspension-feeding, where age group I feeds solely by pedal-sweeping motions (Yeager *et al* 1994), age group III is an obligate suspension-feeder, and age group II is in a transitional state. Age groups I and II were obtained from propagated individuals at the Virginia Tech Aquaculture Center. Age group III was collected from the Little River, Tazewell County, Virginia. Specimens were rinsed with a 50:50 mixture of water from municipal and ground water sources (hardness of ca. 150 mg/L) over a 200 um sieve, and scrubbed as necessary to remove all epiphytes. Mussels were held in water-filled Petri dishes and fed an algal diet of *N. oculata* prior to use in this study. Immediately preceding the study, all mussels were purged in water-filled Petri dishes without food for 30 hr. Water was changed twice during this period.

The feeding chambers used for this experiment were modeled after jars used for testing selective feeding behaviors in other bivalve mollusks (Shumway *et al.* 1985, Baker *et al.* 1998). The 120 ml jars (diameter=6 cm) were filled with 30 ml of a tri-algal mix (see below). I did not expect individuals of age groups I and II to clear sufficient

algae from the medium to detect individual selectivity, so I relied on a collective removal of algae within the treatment. Therefore, jars of age groups I and II contained 200 individuals, whereas those of age group III required only 1 individual. No aeration was supplied to age groups I and II to allow algae to settle in the jar and to prevent juveniles from being suspended in the water column. Slight aeration was added to the jars for age group III to maintain algal suspension. There were six replicate jars for age groups I and II, and ten for age group III. To account for different rates of cellular division among algal species during the experiment, six replicates of both aerated and non-aerated control jars containing the algal mix were maintained without individuals. All experiments were conducted simultaneously at 24^{o}C for 5 hr in an environmental chamber under normal florescent lighting. Dissolved oxygen was monitored using YSI Model 58 Dissolved Oxygen Meter to insure that feeding behavior was not affected by declining oxygen levels.

Algae culture

Scenedesmus quadricauda, Selenastrum capricornutum, and *Nannochloropsis oculata* were grown at the Virginia Tech Aquaculture Center. Monocultures were diluted, and then combined to obtain an equal abundance of each species within the algal mix, and a density of approximately 15,000 cells / ml for each algal species. *Scenedesmus quadricauda* is a colonial species, and for this experiment, a colony of *S. quadricauda* was treated as a single cell, and the following cell dimensions include the colony plus spines. The range of sizes for each species of algae is as follows: *S. quadricauda*, 22.3 - 44.5 μm; *S. capricornutum*, 3.6 – 8.5 μm; and *N. oculata*, 2.8 – 8.1 μm. These sizes were calculated using a calibrated ocular micrometer from measurements of 100 cells.

Relative Abundance in the Medium

At the beginning and end of the experiment, the jars were shaken to resuspend the cells and to break up any pseudofeces and feces present; then a water sample of 1.5 ml was withdrawn from each jar. These samples were preserved with acid Lugol's solution (Vollenweider 1969), to analyze the change in relative abundance of algal species at a later date. At that time, I used the mean of 12 hemacytometer counts (using a light microscope) to represent the relative abundance of algae per sample. The change in

relative abundance for each replicate then was calculated from the difference between relative abundance in the initial and final samples. Arc-sine transformations were performed on proportion data prior to statistical analysis to satisfy normality assumptions of parametric statistical analyses (MacDonald and Ward 1994, Sokal and Rohlf 1995, Gatenby *et al*. 1997).

To determine whether juvenile mussels sort particles prior to ingestion, the change in relative abundance of each algal species from 0 to 5 hr in the feeding chamber was compared by an index. The index was calculated by subtracting the initial relative abundance from the final relative abundance. The index then was tested for each age group using a one-sample t-test, with the level of significance at $p=0.05$. If no significant change was found for all species, then algal species were not selectively consumed. A decline in the relative abundance of an algal species would have been noted if it was removed from the medium more often than the other species. Therefore, a significant negative value for the change in relative abundance indicated that an algal species was selectively consumed. An apparent increase in the relative abundance of an algal species would have been recorded if the other species were removed from the medium more often; therefore, a significantly positive value indicated that a species was rejected.

If a significant change in relative abundance was found for any of the species, then the change in relative abundance among species was compared to determine whether one algal species was selected in favor of another. To do this, the index of one algal species within a feeding chamber was subtracted from the index of another species within the same feeding chamber. This was done for each replicate, and the difference was tested using a one-sample t-test. If there was a significant difference, then one species was positively selected over the other. This test was repeated until the three algal species were compared against each other for each age group. For age groups I and II, sample sizes were $n=6$, and for age group III, $n=10$.

These same tests were used to analyze for different rates of algal reproduction in the control chambers. No significant change was found for all species; therefore, rates of increase did not differ among the species, and I did not have to correct for different rates of algal reproduction. Sample sizes were $n=6$ for both aerated and non-aerated controls.

To determine whether selective feeding changes between early developmental stages of juvenile mussels, the change in relative abundance of one algal species was tested between two age groups using a two-sample t-test. This test was repeated until all three algal species were compared between all three age groups. If no significant difference was found in any of those tests, then age did not affect feeding selectivity.

Gut Content Analysis

At the end of the experiment, six randomly selected mussels from each age group were preserved with acid Lugol's solution for gut content analysis. Mussels were rinsed with distilled water over a 200 *u*m sieve to remove any uningested particles. The gut contents of age groups I and II were exposed by crushing them with a fire-blunted pipette in 0.25 ml of distilled water. Gut dissections were performed on age group III, and a 0.05 ml sample of the ingested particles was taken and diluted in 0.25 ml of distilled water. I used the mean of 18 hemacytometer counts to quantify the relative abundance of ingested algal species from the diluted samples. Arc-sine transformations were performed on the proportion data prior to statistical analysis.

To detect selective ingestion, the difference between the relative abundance of two ingested algal species in the guts of 6 specimens per age group was calculated, and then tested using a one-sample t-test. If there was a significant difference at p=0.05, then one algal species was selected in favor of another. This test was repeated until all three algal species were compared.

RESULTS AND DISCUSSION

Relative Abundance in the Medium

Results indicated that juveniles of *Villosa iris* possess the ability to feed selectively and presumably sort particles prior to ingestion. The relative abundance of *N. oculata* within the medium decreased significantly from time 0 to 5 hr for all age groups (age group I, p=0.0400; age group II, p=0.0180; and age group III, p=0.0039). The negative index for the change in relative abundance of *N. oculata* showed that the mussels strongly selected for this species (Table 1.1). The relative abundance of *S. quadricauda* increased significantly from time 0 to 5 hr for all age groups (age group I, p=0.0045; age group II, p=0.0125; and age group III, p=0.0093). The positive index for the change in relative abundance of *S. quadricauda* suggests that mussels rejected this

species (Table 1.1). In the water samples, the indices of *N. oculata* and *S. capricornutum* were significantly lower than that of *S. quadricauda* (Table 1.2). *Nannochloropsis oculata* and *S. capricornutum* were filtered from the medium more frequently than *S. quadricauda*, and therefore were selected in favor of *S. quadricauda*. Age of mussels did not affect feeding selectivity or preference (Figure 1.1).

Recorded evidence of selective feeding may be a function of particle size. The smaller cells of *N. oculata* and *S. capricornutum* (2.8-8.5 μm) were selected over of the larger colonies of *S. quadricauda* (22.3-44.5 μm). No other selective feeding studies for juvenile freshwater mussels have been reported, but Yeager *et al.* (1994) found that gut contents of captive juvenile *V. iris* consisted mainly of small particles in the 2-5 μm range. Several studies have indicated that particle selection by adult freshwater mussels may be size dependent. Paterson (1984) found that adult *Elliptio complanata* selected particles in the 4-5 μm range. Baker and Levinton (2000) found that adult unionids taken from the Hudson River typically rejected large green algae, such as *S. quadricauda*, as do zebra mussels (*Dreissena polymorpha*), in favor of smaller particles (Baker *et al.* 1998). Adult *Anodonta calipygos* has been shown to select for green algae in the 5-10 μm range (Miura and Yamahiro 1990). Thus, I believe that particle size influenced feeding selection, and that when given a choice, juveniles will select for algal species in the 2.8-8.5 μm size range.

Gut Content Analysis

Mussels selectively ingested algae in the following descending order: *N. oculata*, *S. capricornutum*, *S. quadricauda* (Table 1.3). *Scenedesmus quadricauda* was not ingested by mussels in age groups I and II (Figure 1.2). This finding is supported by a light micrograph of the arrangement of the digestive tract of a newly metamorphosed (2 day old) juvenile of *Lampsilis ventricosa* (Lasee 1991). The mouth of the juvenile is approximately 16 μm and the esophagus approximately 6 μm. The smallest colony length of *S. quadricauda* that I measured was 22.3 μm. It is not known whether the mouth and esophagus of newly metamorphosed juvenile mussels are capable of accommodating algae the size of *S. quadricauda*, but it is unlikely considering that no colonies of *S. quadricauda* were ingested by age groups I and II in my study. In addition to size of suspended particles (Baldwin 1995, Defossez and Hawkins 1997, Raby *et al.*

1997), a variety of other particle characteristics have been shown to affect feeding behavior of marine bivalves. Chemical cues (Ward and Targett 1989, Ward *et al.* 1992, Baldwin 1995) and electrostatic charge (Solow and Gallager 1990) have been shown to influence capture efficiency and selection by marine bivalves. In my study, selection by juvenile freshwater mussels may have been influenced by other particle characteristics secondary to size. This may account for the observed differences between ingested quantities of *N. oculata* and *S. capricornutum*, which are of similar size.

Large particles the size of *S. quadricauda* were not actively sorted by age groups I and II. *Scenedesmus quadricauda* may have been rejected by age groups I and II because it exceeded the capacity of their mouth or esophagus. Large particles may also have been brushed out of the ciliary current after touching the edges of the valve and mantle marginal folds when carried through the pedal gap (Reid *et al*. 1992). Personal observations of pedal-feeding activities by juveniles of *V. iris* have confirmed an accumulation of particles at the pedal gap. Similarly, the geometry of the algal cells may have resulted in reactions to the hydrodynamic disturbances caused by the currents of mussels during feeding (Gallager 1993). The non-spherical shape of *S. capricornutum* and *S. quadricauda* may have "steered" the cells out of the weak current generated by the mussels. In addition, the spines of *S. quadricauda* may have discouraged ingestion. Since newly metamorphosed juveniles lack a well-developed buccal cavity (Lasee 1991), particle sorting by age groups I and II is most likely passive, but the labial palps may aid in some active selection. The sorting capacity of age group III is primarily related to the complexity of the gill surface area (cirral structure), which may be similar to other freshwater mussels (Silverman *et al.* 1997). For zebra mussels, capture and preliminary selection of particles takes place on the gills, where large particles such as *S. quadricauda* are moved above the food grooves in a mucus string, while smaller particles move deep within the food groove toward the labial palps (Baker *et al.* 1998).

Selective feeding may not be as defined in natural systems, which contain a variety of algae and detritus particles. Parker *et al.* (1998) reported that gut contents of adult *Amblema plicata* and *Quadrula pustulosa* from the Ohio River revealed ingested particles ranging from 4-70 μm, and that the relative abundance of algal species within the gut was similar to their relative abundance in the external environment. The mussels

in their experiment were much larger than *V. iris*, and it is likely that large mussels are capable of ingesting a wider size range of particles. In my study, gut content analyses showed that *S. quadricauda* was ingested by the sub-adult *V. iris*, but in significantly lower abundance than the smaller algae (Figure 1.2).

Prior to my experiment, the mussels were fed a diet of *N. oculata* only; therefore, conditioning to this food source could have affected results. However, no other studies have addressed the possibility that conditioning should be considered a factor. In addition, it is unlikely that 2-3 day old juveniles could have become conditioned to a particular food source in less than one day. It should also be noted that the gut retention time of the algal species used in this experiment have not been reported, but ghost cells were visible in the gut content analyses. No evidence was found to show that the gut content data were skewed by differences in retention times.

The ability of *V. iris* to feed selectively, and presumably sort particles prior to ingestion, was documented among the 3 species tested. During early development, particle size may influence selectivity, where larger particles (22.8-44.5 μm) are rejected in favor of smaller particles (2.8-8.5 μm), but there may be other factors influencing the feeding behavior and ingestion. Although my data showed that adult *V. iris* may ingest *S. quadricauda* when fed mixed algae, the mussels ingested algal cells of the smaller size range in significantly higher quantities. Age groups I and II of *V. iris* ingested only smaller particles. Therefore, when developing a suitable algal diet for rearing juvenile mussels, different species at each stage of development are probably not necessary if the algae used are in the 2.8 – 8.5 μm size range. In addition to size, the nutritional value of the algae and the concentration at which the algae is fed should be considered when developing a suitable algal diet. Gatenby *et al.* 1997 found that an algal diet high in oils, which may have included polyunsaturated fatty acids, was best for culturing newly metamorphosed juveniles. Algal concentrations of 10^6 cells/ml have been shown to maintain glycogen levels in adult *Amblema plicata* and *Quadrula pustulosa* during controlled feeding in a laboratory setting for 30 days (Patterson 1999). Gatenby *et al.* (1997) fed algae at a rate of $3.0 \times 10^5 – 5.0 \times 10^5$ to achieve excellent survival rates of 66.5% at 45 days post-metamorphosis for juveniles of *V. iris*. These concentrations are

very high and impractical for use in large volume recirculating systems, and a ration of at least 20,000 cells/ml should be sufficient for age groups I and II (Rogers 1999).

Table 1.1. The index for change in relative abundance of *Scenedesmus quadricauda* (Sc), *Nannochloropsis oculata* (Na), and *Selenastrum capricornutum* (Se) in a tri-algal mix fed to *Villosa iris* of three age groups.

Age Group	Index** ± Standard Error					
	Sc	p-value	Na	p-value	Se	p-value
I	+15.85 ± 4.72*	0.0045	-12.09 ± 4.75*	0.0404	-3.76 ± 5.85	0.5823
II	+18.87 ± 5.89*	0.0125	-10.96 ± 3.41*	0.0180	-7.91 ± 4.00	0.0797
III	+15.65 ± 4.60*	0.0093	-10.17 ± 2.92*	0.0039	-5.49 ± 3.03	0.1210

* Significant change in relative abundance
** (-) index = selected; (+) index = rejected

Table 1.2. The absolute value of the difference between the index of the change in relative abundance of *Scenedesmus quadricauda* (Sc), *Nannochloropsis oculata* (Na), and *Selenastrum capricornutum* (Se) in a tri-algal mix fed to *Villosa iris* in three age groups.

Age Group	Difference Between Indices ± Standard Error					
	Na-Sc	p-value	Se-Sc	p-value	Na-Se	p-value
I	27.94 ± 7.46*	0.0040	19.61 ± 4.75*	0.0294	8.3 ± 9.55	0.2104
II	29.83 ± 8.75*	0.0048	26.78 ± 9.47*	0.0137	3.10 ± 4.55	0.3036
III	25.82 ± 7.09*	0.0018	21.14 ± 7.22*	0.0134	4.7 ± 3.76	0.1353

* Significant difference between indices of the algal species

Table 1.3. The relative abundance of ingested *Scenedesmus quadricauda* (Sc), *Nannochloropsis oculata* (Na), and *Selenastrum capricornutum* (Se) in the guts of *Villosa iris* in three age groups, and the p-value from statistical comparisons among ingested concentrations.

Age Group	Relative Abundance of Ingested Algae* (%)					
	Sc	Na	Sc	Na-Sc p-value	Na-Sc p-value	Se-Sc p-value
I	0.00 ± 0.00	86.95 ± 2.88	13.05 ± 2.88	0.0001	0.0002	0.0001
II	0.00 ± 0.00	87.34 ± 3.75	12.66 ± 3.75	0.0001	0.0009	0.0001
III	10.79 ± 1.04	61.72 ± 2.83	27.49 ± 3.12	0.0001	0.0016	0.0031

* Significant differences ($p<0.05$) were found among all algal species for each age group

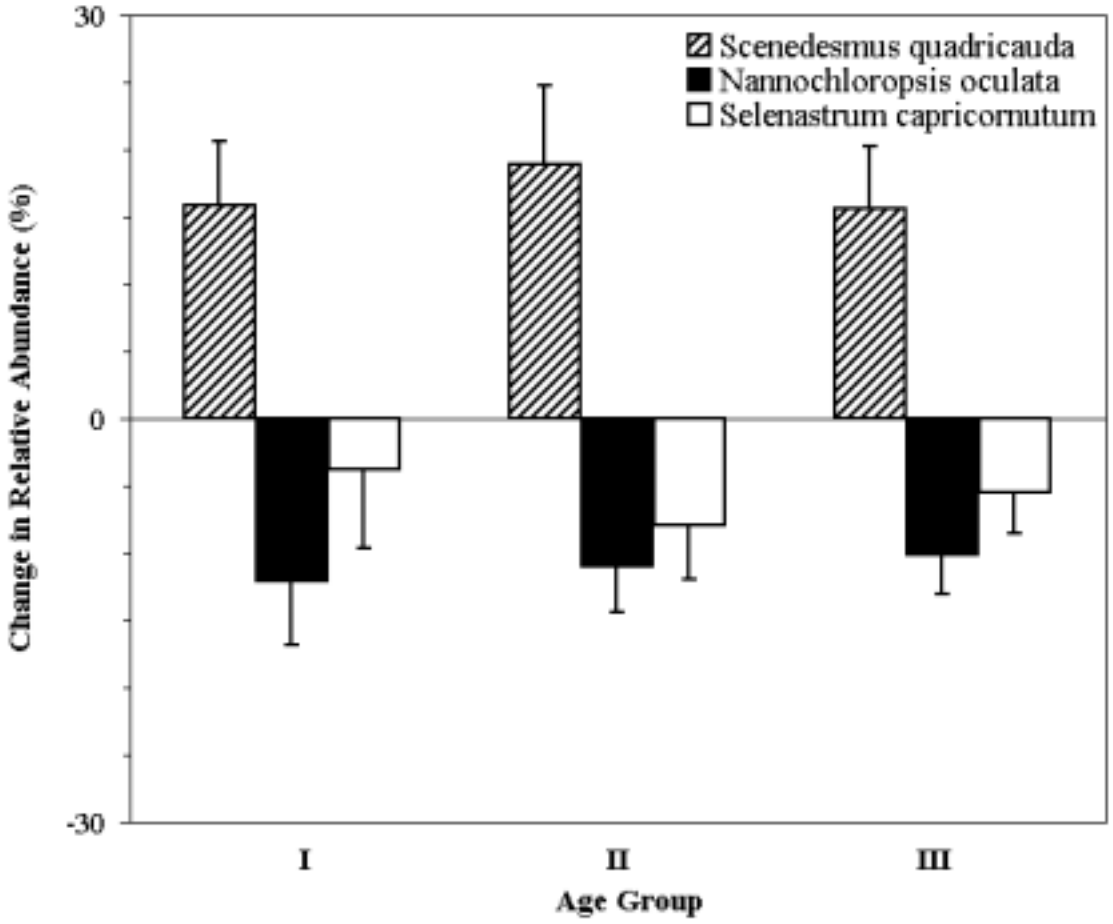


Figure 1.1. Change in relative abundance (± SE) of algal species after 5 hr in feeding chambers with three age groups of *Villosa iris*. Negative values indicate that species were selected. Positive values indicate that species were rejected. Change in relative abundance for *Nannochloropsis oculata* and *Selenastrum capricornutum* was significantly different ($p<0.05$) from that of *Scenedesmus quadricauda*. Changes in relative abundance did not differ significantly ($p>0.05$) among age groups.

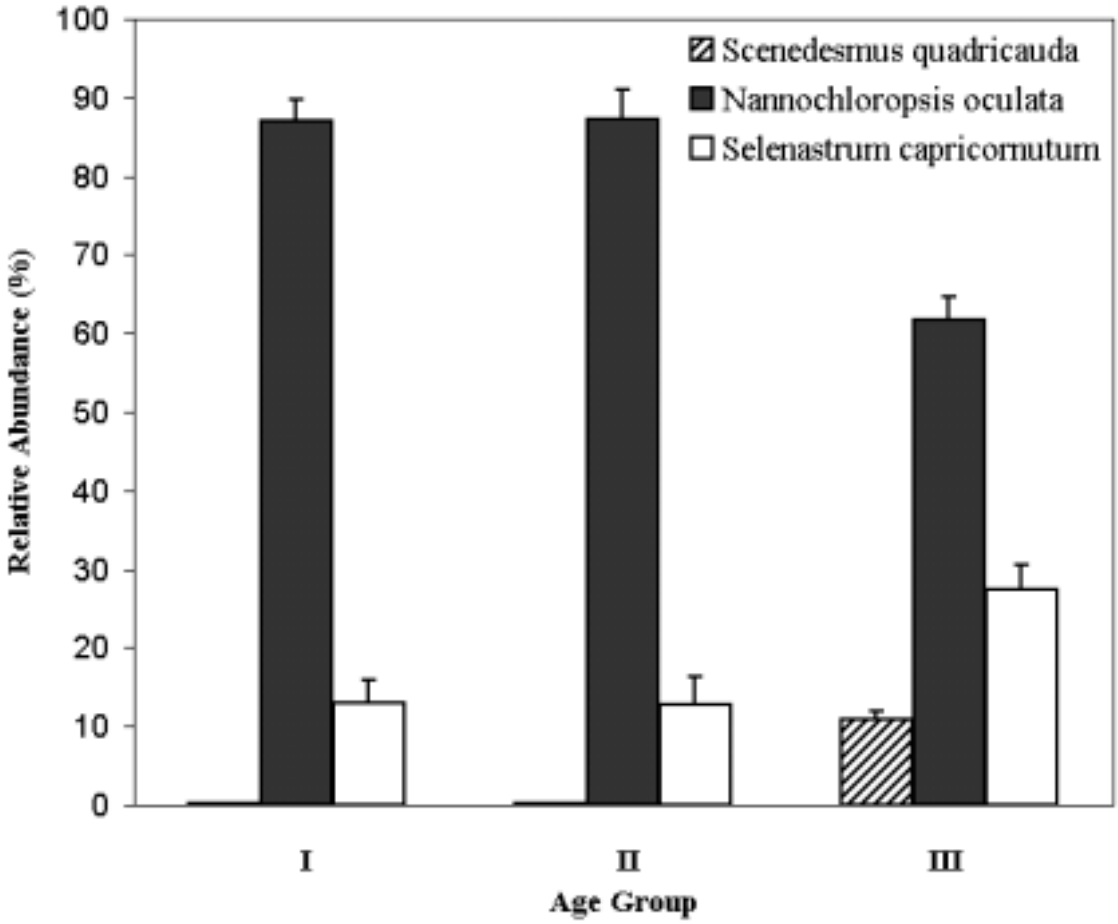


Figure 1.2. Relative abundance (± SE) of three algal species in the guts of *Villosa iris* of three age groups. Significant differences ($p<0.05$) were found among all algal species for each age group. Selective ingestion was not compared among age groups.

Chapter 2: An Evaluation of Two Algal Diets for Juveniles of Villosa iris, and Methods for Batch Culture of Freshwater Algae

INTRODUCTION

With the decline of native North American freshwater mussels, propagation of juveniles for release is being promoted to expedite the recovery of the many threatened and endangered species. To maximize propagation efforts by producing the most individuals of the highest quality for release, the nutritional requirements of captive juveniles must be determined. Proper nutrition in the first month after metamorphosis is vital for juvenile growth and survival (Hudson and Isom 1984, Gatenby *et al*. 1996). The larger the captive juveniles grow before release, the more likely they will have the energy reserves to survive their first winter (Buddensiek 1995, Hanlon 2000).

Currently, only one reported study has compared the suitability of algal diets for rearing captive juveniles (Gatenby *et al.* 1997), but a few algal species have been evaluated by chemical composition to determine their potential as a suitable food (Gatenby 2000). *Scenedesmus quadricauda* was one of the species evaluated by Gatenby (2000), considered of adequate nutritional quality for rearing mussels. *Scenedesmus quadricauda* is often considered a "weed" species by algae culturists due to its invasive, hardy, and low maintenance characteristics in large-scale algal cultures. The combination of good nutritional quality and ease of culture seemingly makes *S. quadricauda* an ideal algal species for raising juvenile mussels in recirculating culture systems. However, both juveniles (See Chapter 1) and adult mussels (Paterson 1984, Miura and Yamashiro 1990, Baker and Levinton 2000) appear to favor algae smaller than *S. quadricauda*.

The suitability of another algal species, *Nannochloropsis oculata*, for rearing juvenile mussels has not been tested. This species is a popular algal food in aquaculture (Duerr *et al.* 1998, Hoff and Snell 1999), used for culturing filter-feeding plankton such as rotifers (Yamasaki *et al* 1989, Hadini *et al.* 1992), and for indirect feeding to cultured fish (Sakamoto *et al*. 1998, Cañavate and Fernández-Díez 2001). *Nannochloropsis oculata* is characterized by high concentrations of lipids (Okauchi 1991, Zou *et al*. 2000), which are likely important for growth and development of juvenile mussels (Gatenby *et*

al. 1997). It is also of the size range that newly metamorphosed juveniles typically ingest (Lasee 1991, Yeager *et al.* 1994, See Chapter 1).

Large recirculating culture systems, typical of those in a freshwater propagation facility (O'Beirn *et al.* 1998, Tankersley and Butz 2000, Henley *et al.* 2001), require large quantities of algae to feed mussels. Starkley *et al*. (2000) suggested that maintaining a continuous supply of mass-cultured food might not be economically practical for state and federal agencies looking to culture freshwater mussels. By modifying methods for culturing marine algae, I have developed inexpensive and simple methods for culturing freshwater algae in bulk quantities, free of weed species and other biotic contaminants. The purpose of this study was to evaluate the suitability of *S. quadricauda* versus *N. oculata* for rearing juvenile freshwater mussels. I cultured and fed large quantities of an uni-algal diet of *S. quadricauda* to newly metamorphosed juvenile *Villosa iris*, to compare survival and growth of the juveniles to those fed an uni-algal diet of *N. oculata*. This study was a follow up to a preliminary experiment comparing growth and survival of juveniles reared on algal and artificial diets (Appendix A).

METHODS

Batch Culture of Algae

My method to batch culture freshwater algae is a modification of those used for culturing large quantities of marine algae (Hoff & Snell 1999), but taking cost and ease of culture into consideration. Extreme sanitation is the key to successful culture. It is important to eliminate contamination through airborne and water sources. Diagnosing contaminant sources and treating them is well described by Hoff & Snell (1999). The most common type of freshwater contamination was in the form of algae-eating protozoans. Once a culture became infested with protozoans, it would crash within 24 hr.

I disinfected my work area, hands, and the external surfaces surrounding the opening of the culture tanks with denatured alcohol prior to working with the cultures. I also reduced airflow by closing doors, and shutting off fans, and wore rubber gloves and a gas mask to protect against the chemicals and fumes. All glassware and stoppers (see Culture Tanks) were stored in bleach water solution when not in use.

Culture tanks were cleaned prior to use by acid washing. The tanks were filled minimally with muriatic acid, and then swirled until acid came in contact with all internal surfaces. The tanks were rinsed three times with water from a municipal source, and then filled for culture and sealed with an appropriate stopper. The chlorine level of the municipal water normally fluctuated around 1.1 mg/L. This level seemed to be sufficient for killing contaminant organisms if left for at least 30 min before dechlorination. I used Pool Breeze Shock Treatment and Super Chlorinator to raise the chlorine level when it was lower than the normal level, and sodium thiosulfate to dechlorinate the water in the culture tanks prior to adding fertilizers or inoculating the tanks. I added only a small quantity of it to minimize the byproduct, sodium chloride. Hoff and Snell (1999) provide other sterilizing options for the use of chlorine.

Culture Tanks

My method to culture algae is derived from the classical batch culture method of Hoff and Snell (1999). Different culture tanks were used at each of three stages of algae grow-out, starting with small volume flasks, and terminating in large fiberglass culture cylinders. For the first stage, I used 1-L flasks, with rubber stoppers to seal the flasks. Two small holes were drilled through the stopper, and a piece of rigid piping (outside diameter: 0.5 cm; Aquatic Eco-Systems, Inc.) was forced through one of the two holes for aeration. The piping was the length of the flask and stopper, with a small section external to the flask so that it could be connected to the airlines. The second hole allowed air to escape.

I used 19-L carboys (diameter: 27 cm; height: 49 cm; Culligan, Inc.) for the second stage of grow-out. The carboys were made of blue transparent plastic, typical of those used for bottled drinking water systems. They were also sealed with rubber stoppers that had two holes drilled in them; rigid piping for these had an outside diameter of 0.8 cm.

For the third stage of grow-out, I used 87-L transparent fiberglass culture cylinders (diameter: 31.2 cm; height: 123 cm; Aquatic Eco-Systems, Inc.). These had a flat PVC lid with styrofoam insulation cut to fit tightly within the tank and then silicon glued to the PVC. The lid had a small hole drilled through the center of it to allow air to escape. I made the lids, but they can be purchased along with the culture tanks. Aeration

was injected through the drain of the cylinder at the base. A vertically positioned plastic male adapter was connected to 1.25-cm PVC piping, at a 90^{o} angle, so the piping could then continue horizontally towards the drain, a ball valve, and finally a reducing tee. The tee was manufactured to accept 1.25-cm PVC on the perpendicular side (air injection), and to accept 2.5-cm PVC on the parallel sides. One side of the tee was a 2.5-cm drain that was controlled with a ball valve. The other side of the tee was connected to the bottom of the cylinder through a 2.5-cm bulkhead fitting. Air stones were never used. Rigid piping was used instead of flexible tubing because it is easier to clean, and reduces the chances for contamination. In addition, flexible tubing tends to rotate by the aeration, while rigid tubing remains firm and consequently the air bubbles cause the water to circulate and maintain cellular suspension in the tank.

For aeration, I used a 0.5-hp Sweetwater air blower (Aquatic Eco-Systems, Inc.). Air was first filtered through a coarse filter, which came with the blower, and then slightly bled to reduce back-pressure prior to entering the airlines. The airlines consisted of 3.75-cm PVC piping that was reduced to 1.25-cm PVC piping once inside the Aquaculture Center. The culture tanks of the first stage were all connected to one ball valve to control airflow, and one male adapter at the end of the PVC airline to which flexible tubing was attached. Every culture tank of the second and third stage had its own ball valve and plastic male adapter. Flexible tubing (inside diameter: 0.8 cm) was connected from the nipple to a disc filter (Gelman Laboratory; Acrovent Device with 0.2 *u*m PTFE Filter PN 4249; Fisher Scientific), which were replaced regularly.

For the first stage of grow-out, culture tanks were connected to a single filter with flexible tubing (inside diameter: 0.5 cm) through a series of plastic tees. Airflow to individual flasks was controlled with clamps. For the second stage of grow-out, the filter was connected directly to the culture tank through flexible tubing (inside diameter: 0.8 cm). Each culture tank of the third stage required two filters on parallel airlines, as only one filter restricted airflow too much to achieve adequate aeration within the cylinders. The two flexible airlines were connected below the filters with a plastic tee before being attached to the culture cylinder.

I used Kent's Pro-Culture F2 Algae Culture Formula (parts A and B; Kent Marine, Inc.). Disposable pipettes and a rubber bulb were used to add the medium to the

culture tanks. I tried to keep both containers for parts A and B off the floor and free from spray that could contain contaminants. It is probably best to store them in a refrigerator. I opened the containers only slightly when withdrawing the fertilizer, and sprayed the pipette and containers with alcohol prior to doing the transfer. I fertilized the culture tanks after dechlorinating them, but prior to inoculation with algae.

I have used both artificial lighting (1.2-m fluorescent shop light) and natural lighting. I typically used artificial lighting. The centers of the culture tanks were placed approximately 30 cm from the light source. A shop light was hung directly above the flasks for the first stage of grow-out. For the second stage of grow-out, 2 shop lights were hung horizontally behind a bank of four 19-L carboys. A shop light was hung vertically behind each culture cylinder for the third stage of grow-out.

Start-up Cultures

To initiate the algal cultures, I obtained algal slants of *S. quadricauda* (University of Texas, Austin, Texas) and agar discs of *N. oculata* (Aquatic Eco-Systems, Inc.) These starter cultures were stored in a refrigerator at 3^{o}C. I used hooked or looped rods to scoop small sections of the starter culture for transfer to 1-L flasks for the first stage of grow-out. Prior to transfer, I flamed the rod using an alcohol burner to sterilize it, and allowed the rod to cool for approximately 15 sec prior to scooping the section. Aeration was kept to a minimum during the first stage of grow-out to prevent excessive loss of water through evaporation before the algae culture reached log phase. Once the culture reached log phase, the entire culture would be transferred to the second phase of grow-out, and then the same from the second to third phase. I checked the cultures for purity prior to transfer. I withdrew a small sample of the culture and look for contaminants using a bright view phase microscope under 400x.

Pure cultures could be preserved in a refrigerator for months before being transferred to the second phase of grow-out. Once I had stockpiled enough pure backup cultures, I would bypass the first stage of grow-out to save time and effort by inoculating 19-L carboys with 1 L of pure culture from the other 19-L carboys. Although I could grow out algae through the third phase, I often harvested algae during the second phase.

Juvenile Culture

Newly metamorphosed juveniles of *V. iris* were obtained from the Virginia Tech Aquaculture Center. The juveniles were less than 4 days old and had a shell length of between 308 and 333 μm. The juveniles were distributed between two algal diet treatments, both of which were randomly assigned six replicate, 145-L recirculating culture systems. The systems were air-driven, and consisted of an interconnected polyethylene feed trough, a polyethylene drum (reservoir), and polyvinyl chloride (PVC) airlift and return piping (Henley *et al.* 2001). The systems were filled with a 50:50 mixture of water from municipal and ground water sources (hardness of ca. 150 mg/L). Prior to use in the systems, water was dechlorinated with sodium thiosulfate as necessary.

To hold juveniles, each system had a 150 μm mesh container (length: 210 mm; width: 140 mm; height: 150 mm) that was framed with PVC "L" shaped brackets (width: 20 mm). The containers were filled with approximately 5 mm of ground dolomitic limestone (450 – 1000 um), obtained from a local quarry. A total of 100 juveniles were placed within each mesh container, for a total of 1200 juveniles in the experiment. A top made of mosquito netting was placed initially on the troughs to prevent chironomids from colonizing the systems, but they were not effective. They were removed within the first few days of the experiment.

Depending on the treatment, juveniles were fed an uni-algal diet of either *S. quadricauda* (22.3 – 44.5 μm) or *N. oculata* (2.8 – 8.1 μm). Algae were added to the systems once daily to raise the overall density within the trough of the system to approximately 30,000 cells / ml. Onset Optic Stow Away temperature loggers monitored water temperatures within the culture systems every 30 min. The pH of the systems was monitored regularly.

Sampling of juveniles occurred at 7-day intervals over a 42-day period. An elutriator, which sorted particles by specific gravity, was used to separate juveniles from sediment. Juveniles were then retained on sieves and placed in a water-filled Petri dish. Percent survival and mean growth of the juveniles were assessed at each sampling interval. Live juveniles were counted using a dissection microscope. The mean shell length was then obtained from shell measurements of 9 randomly selected individuals using a calibrated ocular micrometer on the dissection microscope. If there were less

than 9 live individuals in a sample, then the mean shell length of all juveniles was taken. For day 0, the mean initial shell length of 9 juveniles from the overall batch was used for all replicates. Visual observations of the gut color of juveniles under high back-lighting during the sampling were used to verify that they were ingesting algae. A dense green chlorophyll colored gut signified that algae were being ingested. While the juveniles were being sampled, the mesh container and trough were scrubbed, and approximately one-half of the water volume was replaced. After sampling, juveniles were placed back into their original container with the same sediment.

To determine whether there was a difference between treatments, I compared survival and mean shell length of juveniles fed *N. oculata* to those fed *S. quadricauda*, with a repeated measures analysis of variance for the diet factor at p=0.05. For percent survival, I analyzed arc-sine transformed data. Any samples with zero percent survival were omitted from shell length analyses.

At the conclusion of the experiment, I used a two-sample t-test for unequal variances to determine whether survival at 42 days differed between treatments at p=0.05. To determine whether growth at 42 days differed between treatments, the mean length of those fed *N. oculata* was compared to the mean length of those fed *S. quadricauda*. All of the samples with live juveniles from the *S. quadricauda* treatment had a mean shell length of 444 μm. Since there was no variance among replicates, a 2-sample t-test was not appropriate. Therefore a one-sample t-test was used to compare the two treatments.

RESULTS AND DISCUSSION

Both diets appeared to be inadequate for rearing juvenile mussels to a sufficient release size. Mean shell length of juveniles after 42 days was 446 μm ± 9.79 for those fed *N. oculata*, and 444 μm ± 0.00 for those fed *S. quadricauda* (Table 2.1). Hanlon (2000) reported that over-winter survival for juveniles of *Lampsilis fasciola* was ≤ 8.0 % if mean shell length did not exceed 480 um. Therefore, it is doubtful that my juveniles would have been of adequate size and with sufficient energy stores to survive their first winter. Individual juveniles of *V. iris* are capable of reaching 692 μm after 45 days, under different culture conditions (Gatenby *et al*. 1996).

In this study, juveniles from both treatments showed similar, slow growth. I found no significant difference in growth between treatments (p>0.05); or mean shell

length after 42 days ($p>0.05$) (Table 2.2). Growth of juveniles in both treatments began to plateau after 1 wk post-metamorphosis, as shell length approached approximately 458 μm (Figure 2.1). Gatenby *et al.* (1996) found that the mean shell length for juveniles of *V. iris* ranged only between 386 – 462 μm if they were not fed adequately by 45 days. In another laboratory study, Lasee (1991) observed slow growth for juveniles of *L. ventricosa* followed by a mortality event, which may have been correlated with the loss of stored lipids in the digestive gland. It is likely that juveniles in my study used up their lipid stores in the first few weeks and did not obtain adequate nutrition from the algal diets to advance growth or maintain survival.

High mortality is typical when juveniles are not fed properly or their environmental requirements are not met (Hudson and Isom 1984, Buddensiek 1995, Gatenby *et al.* 1996, Gatenby *et al.* 1997, O'Beirn 1998, Henley *et al.*2001). Survival of juveniles from both of my treatments decreased rapidly after 21 days (Figure 2.2). At the end of the 42-day experiment (Table 2.3), mean survival was significantly higher ($p=0.03$) for those fed *N. oculata* (16.3 %) than those fed *S. quadricauda* (2.8 %) (Table 2.2). Had the experiment continued, though, it is unlikely that I would have been able to discern differences in survival at later sampling dates, as it appears that survival in both treatments would have continued to decline towards a low level of survival (Figure 2.2). The rates of mortality were most likely due to inadequate diets.

Survivorship of juveniles is likely due to the size range of quality food available, and colonies of *S. quadricauda* was most likely too large for the juveniles to ingest. Visual observations of the gut contents of juveniles fed *S. quadricauda* showed a lack of chlorophyll coloration, which indicates that juveniles fed this species did not ingest it. These observations are supported by an earlier feeding behavior study, where *S. quadricauda* was not ingested by juveniles of 53 days old (See Chapter 1).

In contrast to the lack of chlorophyll color observed in the guts of juveniles fed *S. quadricauda*, guts of those fed *N. oculata* were typically of chlorophyll coloration. The juveniles likely ingested *N. oculata,* but they may not have been able to assimilate it well. No studies have confirmed this, but poor digestibility of *N. oculata* cells has been reported as a potential problem for using this species to raise oyster spat (Numaguchi 2000), and copepods (Payne and Rippingale 2000). It is unknown whether the digestive

system of juvenile freshwater mussels is capable of mechanical or enzymatic digestion of algae with rigid cell walls, such as *N. oculata*. In addition, the presence of fine substrate (<350 μm) has been shown to improve growth and survival of juvenile mussels in laboratory studies (Hudson and Isom 1984, Gatenby *et al.* 1996, O'Beirn *et al.*1998). I added a coarse limestone sediment (450-1000 μm) to the mesh culture containers for ease of sampling. A limestone sand substrate was used successfully to culture *Lampsilis fasciola* in a river-fed hatchery raceway, achieving survival as high as 86 % by 32 days (Hanlon 2000). However, Rogers (1999) found significantly lower growth for juveniles of *L. fasciola* reared in fine sand (500-800 μm) versus fine sediment (<120 μm). Therefore, a sediment layer seems important in the early development of juvenile mussels, other than as a generic substrate layer for burrowing and positioning themselves. Juveniles may ingest fine sediment to aid in the mechanical digestion of algal species with rigid cell walls, and perhaps the lack of fine sediments prevented *N. oculata* from being efficiently assimilated. In addition, sediment was obtained from a dolomitic limestone quarry, and it may have lacked the organic materials in fine river sediment, such as colloidal particles and bacteria that can be used as food (Hudson and Isom 1984, Lopez and Holopainen 1989, Gatenby *et al.* 1997).

From the repeated measure analysis, survivorship did not differ significantly between treatments ($p>0.05$) (Table 2), and neither *N. oculata* nor *S. quadricauda* was shown to be an adequate food source for rearing juvenile mussels. Under the culture conditions I provided, overall survival and growth of juveniles did not compare favorably to those in other reported studies with juveniles of *V. iris* (Table 2.4). While developing an adequate diet for cultivating juvenile mussels is important, there are many other variables that can affect juvenile development in the laboratory. For example, adequate water quality is important for successful culture (Buddensiek 1995, O'Beirn *et al.* 1998). I replaced water weekly, which was twice as frequent as the standard protocols require for these culture systems (Henley *et al.* 2001). The pH ranged from 8.4 to 8.8, which meets standard protocols for water quality within these systems (Henley *et al.* 2001). The water temperature averaged 16.2^0C (SD $\pm$ 1.4) over the course of the experiment. Low water temperatures may have slowed juvenile growth, but probably would not have affected survival since the temperature average was higher than 15^oC (Beaty 1999,

Hanlon 2000). In addition to water quality parameters, the sediment requirements of juveniles need to be explored, and my results may indicate that the complementarily between the algal food and fine sediment, which was lacking in my study, may be extremely important for successful culture.

Table 2.1. Mean shell length (μm) (±SD) and range among replicates for juveniles of *Villosa iris* fed uni-algal diets of either *Nannochloropsis oculata* or *Scenedesmus quadricauda* over a 42-day period.

Species		Days						
		0	7	14	21	28	35	42
Nannochloropsis oculata	Mean (SD)	313 (11.31)	396 (8.83)	411 (9.13)	422 (12.99)	435 (6.60)	453 (18.32)	446 (9.79)
	Range	308-333	387-405	398-419	407-442	427-447	433-487	436-459
Scenedesmus quadricauda	Mean (SD)	313 (11.31)	398 (15.60)	417 (9.99)	429 (14.39)	431 (14.12)	437 (16.00)	444 (0.00)
	Range	308-333	368-410	402-433	416-456	416-450	422-456	444-444

Table 2.2. A summary of the statistical tests and p-values used to compare suitability of two uni-algal diets, *Nannochloropsis oculata* and *Scenedesmus quadricauda*, fed to juveniles of *Villosa iris* for 42 days.

Comparison	Statistical Test	Observations	p-value
Differences in survival between treatments over the course of the experiment	2 factor repeated measures analysis on the diet treatment	84	0.5151
Differences in mean survival between treatments after 42 days	2-sample t-test for unequal variances Nanno > Scen	12	0.0308*
Differences in growth between treatments over the course of the experiment	2 factor repeated measures analysis on the diet treatment	84	0.8289
Differences in mean shell length between treatments after 42 days	1-sample t-test against 444 *u*m	12	0.7360

* Significant difference between treatments

Table 2.3. Mean percent survival (±SD) and range among replicates of *Villosa iris* juveniles fed uni-algal diets of either *Nannochloropsis oculata* or *Scenedesmus quadricauda* over a 42-day period.

Species		Days						
		0	7	14	21	28	35	42
Nannochloropsis oculata	Mean	100	87.8	89.0	78.7	65.8	37.7	16.3
	(SD)	(0.00)	(6.62)	(6.57)	(13.91)	(28.92)	(26.97)	(15.76)
	Range	100-100	77-97	80-95	52-93	16-88	3-74	0-43
Scenedesmus quadricauda	Mean	100	92.7	85.5	85.5	76.3	23.2	2.8
	(SD)	(0.00)	(6.66)	(12.42)	(7.74)	(10.52)	(21.90)	(5.60)
	Range	100-100	85-100	63-98	72-96	66-93	0-44	0-13

Table 2.4. Summary of propagation studies with *Villosa iris*.

Author	Days	Length	Survival	Temperature
Beaty, 1999	30	450 μm	71%	18^{O}C
Beck, this study	42	445 μm	3 – 16%	16.2 ^{O}C
Gatenby et al., 1996	45	386 – 462 μm	3 – 51%	20-25 ^{O}C
Gatenby et al., 1996	45	552 μm	66.5%	20-25 ^{O}C
Beaty, 1999	60	440 μm	33%	18 ^{O}C
Gatenby et al., 1997	60	638 – 744 μm	16 – 51%	Not reported

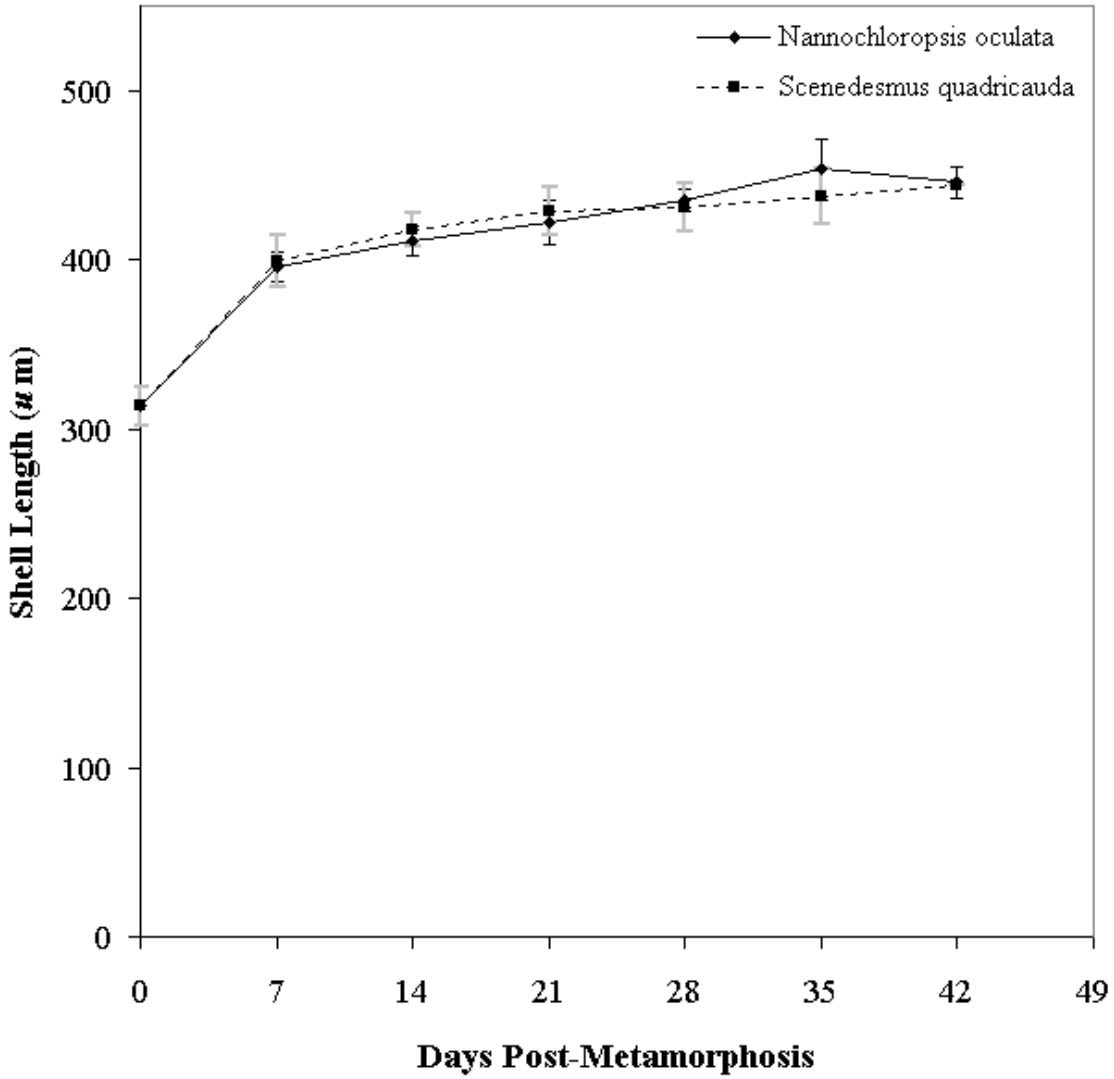


Figure 2.1. Mean shell length (μm) (±SD) for juveniles of *Villosa iris* fed uni-algal diets of either *Nannochloropsis oculata* or *Scenedesmus quadricauda* for 42 days.

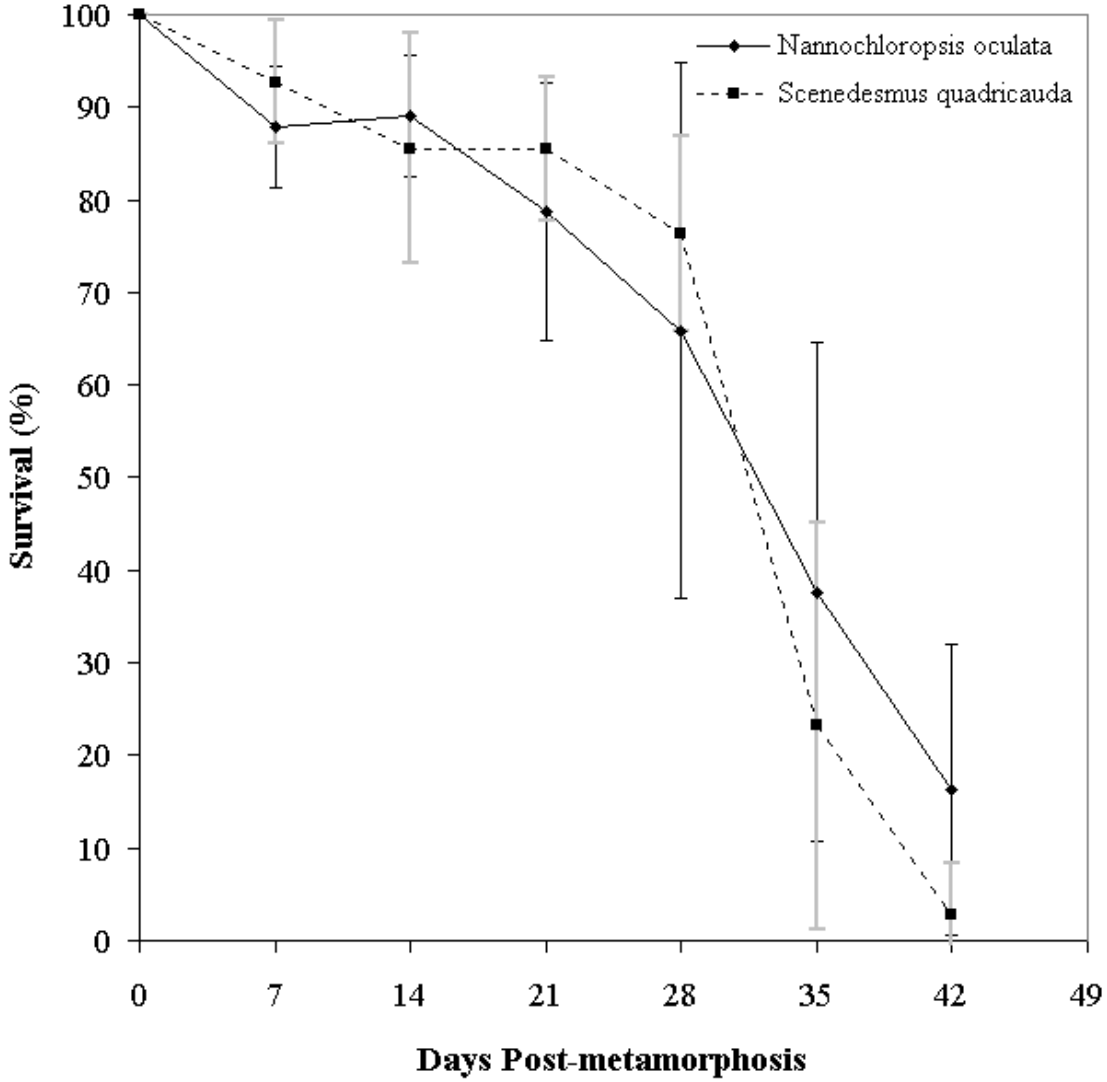


Figure 2.2. Survival (±SD) for juveniles of *Villosa iris* fed uni-algal diets of either *Nannochloropsis oculata* or *Scenedesmus quadricauda* for 42 days.

Chapter 3: An Evaluation of Two Sediment Depths for the Laboratory Culture of Juvenile Mussels

INTRODUCTION

Earlier propagation studies provided evidence that juvenile freshwater mussels require substrate; however, addition of substrate to a culture system increases maintenance time and sampling effort. This addition of substrate promotes enhanced growth and survival of juveniles (Hudson and Isom 1984, Gatenby et al. 1996, O'Beirn et al. 1998). There are numerous hypotheses for why substrate increases the success of culturing juveniles. Additions of substrate may facilitate pedal-feeding activity of juvenile mussels by supplying a medium to burrow (Yeager *et al.* 1994). Substrate is often obtained from a river source, and may contain fine organics, such as colloidal particles, and algae, that can supplement an algal diet (Hudson and Isom 1984, Gatenby *et al.* 1996). It is also possible that juveniles ingest fine substrate to aid in the mechanical digestion of algae (See Chapter 2). In addition, substrate may provide cover or add stability to the culture chambers, buffering the juveniles from outside vibrations and turbulent water flow (O'Beirn *et al.* 1998).

In streams, Neves and Widlak (1987) reported that juveniles typically inhabit depositional areas, which would likely contain moderate levels of sediment. However, the decline of native freshwater mussels is often attributed in part to excessive sedimentation (Neves 1991). Buddensiek (1995) found that moderate levels of sedimentation were beneficial for culture of juveniles *Margaritifera margaritifera*, but after a certain threshold, survival and sedimentation were antagonistic. Alternatively, Lasee (1991) reported that growth and survival for juveniles of *Lampsilis ventricosa* were not positively affected by additions of sediment.

The purpose of this study was to evaluate two different sediment depths for rearing juvenile mussels. I compared survival and growth over 40 days for juveniles of *Villosa iris* reared in a 5 mm and 15 mm layer of sediment (<600 *u*m). A sediment depth of 5 mm is typically added to culture chambers for rearing juvenile mussels (See Chapter 2). I chose a sediment depth of 15 mm for comparison so that the difference between depths would be easy to quantify.

METHODS

Newly metamorphosed juveniles of *V. iris* were obtained from the Virginia Tech Aquaculture Center. The juveniles were less than 2 days old and had a shell length of between 231 and 333 μm. The juveniles were distributed randomly between the two sediment depth treatments, (5 mm or 15 mm), both of which were randomly assigned six replicate 145-L recirculating culture systems (See Chapter 2).

To retain juveniles, each system had a circular container made from 150 mm polyvinyl chloride (PVC) piping. The piping was cut into roughly 55 mm thick slices, and then 150 μm nylon mesh screening was cemented to one of the two open sides of the slice. The containers were filled with the assigned layer of sediment. Sediment was collected from raceways at Buller Fish Hatchery, Marion, VA, watered by the South Fork of the Holston River. Sediment was sieved to < 600 μm, and contained mostly fine silt (< 300 μm) and organic matter, with a minimal amount of fine sand. The containers were placed in their assigned trough, and a total of 150 juveniles were then placed within each container, for a total of 1800 juveniles in the experiment.

Juveniles were fed a bi-algal diet of *Nannochloropsis oculata* and *Neochloris oleoabundans*. Monocultures of the algal species were grown at the Virginia Tech Aquaculture Facility (See Chapter 2), then combined at the time of feeding. Algae were added to the systems twice daily to raise the overall density within the trough of the system to approximately 30,000 cells / ml. Feeding rations were increased every 10 days over the course of the experiment to account for juvenile growth. By the end of the experiment, juveniles were fed a ration of 90,000 cells/ml. Onset Optic Stow Away temperature loggers were used to monitor and record water temperature within the culture systems every 30 min. The pH and NH_3 levels were monitored regularly.

Sampling of juveniles occurred at 10-day intervals over a 40-day period. The contents of the containers were siphoned, and then sieved to collect juveniles. Excess sediment was rinsed through sieves, and then the juveniles were placed in a water-filled Petri dish. Percent survival and mean growth of the juveniles were assessed at each sampling interval. Live juveniles were counted using a dissection microscope. The mean shell length was then obtained from shell measurements of 10 randomly selected individuals using a calibrated ocular micrometer on the dissection microscope. For day

0, the initial mean shell length of 10 juveniles from the overall batch was used for all replicates. Visual observations of the gut color of juveniles under high back-lighting during the sampling were used to verify that juveniles were ingesting algae. A dense green chlorophyll-colored gut signified that algae were being ingested. While the juveniles were being sampled, the containers and trough were scrubbed, and the water was replaced. After sampling, juveniles were placed back into their original container with fresh sediment. At approximately 3-day intervals between sampling events, roughly one-half of the water volume in the systems was drained, the troughs were scrubbed, and then the systems were refilled with fresh water.

To determine whether there was a difference between treatments, I compared survival and mean shell length of juveniles reared in 5 mm of sediment to those reared in 15 mm of sediment, with a repeated measures analysis of variance for the sediment depth factor at $p=0.05$. For percent survival, I analyzed arc-sine transformed data. At the conclusion of the experiment, I used a two-sample t-test for equal variances to determine whether survival or mean shell length after 40 days differed between treatments at $p=0.05$.

RESULTS AND DISCUSSION

From the repeated measure analysis, I found a significant difference ($p=0.04$) in survival of juveniles between treatments over the course of the experiment (Table 3.1), and survival was higher in treatments containing 5 mm of sediment than in treatments containing 15 mm (Table 3.2). Juvenile survival declined rapidly during the first 10 days of the experiment, then leveled off for both treatments (Figure 3.1). At the conclusion of the experiment (40 days), mean survival of juveniles reared in 5 mm sediment was 50.1 % (SD±9.0), while survival of those reared in 15 mm was 40.8 % (SD±11.3), but I found no statistical difference in survival between treatments after 40 days ($p=0.14$) (Table 3.1).

Hudson and Isom (1984) reported that a silt layer of 10 mm was as beneficial as a cloudy silt suspension for raising juveniles of *Utterbackia imbecillis*. Buddensiek (1995) noted that additions of a moderate layer of sediment (<6 mm) resulted in higher survival for cultured juveniles of *Margaritifera margaritifera* than additions of a thicker layer (6-9 mm), although not statistically higher. Buddensiek (1995) did not report the depths as previously stated in 1 mm increments, but rather as fractions by which culture chambers

(depth: 9 mm) were filled with sediment. My results were similar to those of Buddensiek (1995), where survivorship of juveniles was higher when reared in sediment depths of approximately 5 mm rather than in 15 mm sediment depths. In my study, the deeper sediment layers may have been more tightly compacted by their own weight. Rogers (1999) attributed poor survival and growth for juveniles of *Lampsilis fasciola* reared in fine sediment to the substrate being tightly compacted, which may have impeded both feeding and mobility of juveniles.

By 40 days, juveniles obtained a mean shell length of 579 μm (± 51.4) for those raised in 5 mm of sediment, and 579 μm (± 23.6) for those in 15 mm (Table 3.3). When an adequate substrate or food source for rearing juveniles of *V. iris* are not administered in the laboratory, juveniles cease to grow beyond a mean length of approximately 450 *u*m (Gatenby *et al.* 1996, See Chapter 2). For both sediment treatments, mussels continued to grow well past 450 μm (Figure 3.2). I found no statistical differences in growth over the course of the experiment between sediment depths ($p=0.5821$), or mean shell lengths after 40 days ($p=0.9856$) (Table 3.1). This indicates that the juveniles were of the same quality in fitness over the course of the experiment.

From a previous burrowing behavior study (Yeager *et al.* 1994), juveniles of *V. iris* did not burrow deeper than 10 mm in 20 min. Over a 10-day period between sampling in my study, it may have been possible to lose a small number of juveniles towards the bottom layer where sediment was more tightly compacted. Beaty (1999) found that most juveniles of *V. iris* occupied the upper 1-5 mm layer of loose sediment in a study conducted in a river-fed channel. This depth of occurrence may account for the observed difference in survival between treatments, but not in growth.

I was able to maintain adequate water quality in the systems over the course of the experiment. The pH of the systems ranged from 8.4 to 8.8, and NH_3 levels never exceeded 0.16 mg/L. These parameters have been shown to be sufficient for maintaining adults of *Elliptio complanata* in similar systems (Henley *et al.* 2001).

There are several important implications of this study. The shell length of the largest individual measured was 769 μm. As stated previously, the mean shell length of juveniles in my study was 579 μm at 40 days, which is the largest reported mean shell length for juveniles of *V. iris* at that age (Table 3.4). Beaty (1999) reported that juveniles

of *V. iris* reared at 18°C had attained a mean shell length of 440 μm at 60 days. Either the feeding or substrate requirements were probably not met within the culture chambers of that study, as the juveniles failed to exceed 450 μm. In another study, Gatenby *et al.* 1996 reported mean length for juveniles of *V. iris* at 45 days to be 552 μm. Mean shell length for juveniles in my study was almost 550 μm at 30 days.

It is well known that growth in marine bivalves is directly related to temperature until an upper temperature limit is reached (Beiras *et al.* 1994, Sicard *et al.* 1999, Laing 2000). In addition, Hudson and Isom (1984) found significantly higher growth for juveniles reared at 30°C than at 23°C. Beaty (1999) reported better growth for juveniles of *V. iris* at 25°C than at 18°C. The mean water temperature during my experiment was 18.6°C (SD±1.4), and a low mean temperature likely resulted in reduced growth rates when compared to other studies of *V. iris* (Table 3.4). Constant temperatures have also been shown to accelerate growth of juvenile sea scallops when compared to rapidly fluctuating temperatures (Pilditch and Grant 1999), and water temperature fluctuations may have affected growth rates in my study (Appendix B).

It is difficult to further compare my growth data to other reported studies using *V. iris*. Gatenby *et al.* (1997) reported mean shell lengths of *V. iris* at 60 days to range between 638 and 744 *u*m. They did not report any growth data at or around 40 days. O'Beirn *et al*. (1998) used *V. iris* for culture experiments, but they did not sample between days 28 and 56. In addition, it is unlikely that they started with newly metamorphosed juveniles (<3 days old), since the reported mean shell length of the mussels was 400 *u*m at the beginning of the experiment.

Survival and growth of juveniles in my study compare favorably to those of other studies (Gatenby *et al.* 1996, Beaty 1999). It is possible that an algal diet containing both *N. oculata* and *N. oleoabundans* is superior to other algal diets that have been used, even those containing *N. oleoabundans* and different algal species. *Nannochloropsis oculata* is extremely high in polyunsaturated fatty acids (Okauchi 1991, Zou *et al.*2000), which are reportedly important in the diet of juvenile mussels (Gatenby *et al.* 1997). It is also possible that the fine sediment used to supplement the algal diet was much higher in organics that can be used as a food source (Yeager *et al*. 1994). The recirculating culture systems used were much larger than the systems used by Gatenby *et al.* (1996) and

Gatenby *et al.* (1997), which may have also contributed to the overall success of this study.

Survival was higher in treatments containing 5 mm of sediment than in treatments containing 15 mm (Table 3.2), and I found a significant difference ($p=0.04$) in survival of juveniles between treatments over the course of the experiment (Table 3.1). My laboratory findings tend to support hypotheses reporting excessive sedimentation as a contributing factor to the decline of wild freshwater mussel populations. If survivorship of juvenile mussels decreases with increased sediment load in streams, then recruitment will be impaired. For future propagation studies, I would recommend rearing juveniles in a 5-mm layer of sediment as opposed to a thicker layer since survival was higher in less sediment. In addition to higher survival, sampling time and effort was greatly reduced when using less sediment.

Table 3.1. A summary of the statistical tests and p-values used to compare suitability of sediment (< 600 *u*m) at two depths (5 mm and 15 mm) for rearing juveniles of *Villosa iris* for 40 days.

Comparison	Statistical Test	Observations	p-value
Differences in survival between treatments over the course of the experiment	2 factor repeated measures analysis on the sediment depth treatment	60	0.0423*
Differences in mean survival between treatments after 40 days	2-sample t-test for equal variances 5 mm > 15 mm	12	0.1443
Differences in growth between treatments over the course of the experiment	2 factor repeated measures analysis on the sediment depth treatment	60	0.5821
Differences in mean shell length between treatments after 40 days	2-sample t-test for equal variances 5 mm > 15 mm	12	0.9856

* indicates significant difference between treatments

Table 3.2. Mean percent survival (±SD) and range among replicates for juveniles of *Villosa iris* reared in sediment (<600 *u*m) at two depths (5 mm or 15 mm) over a 40-day period.

Sediment depth		Days				
		0	10	20	30	40
5 mm	Mean (SD)	100 (0.00)	66.3 (8.72)	63.4 (7.09)	60 (7.24)	50 (9.02)
	Range	100-100	55-81	59-73	51-69	35-61
15 mm	Mean (SD)	100 (0.00)	58.8 (11.4)	49.7 (9.11)	50.3 (10.6)	40.8 (11.3)
	Range	100-100	41-75	35-59	38-59	24-54

Table 3.3. Mean shell length (*u*m) (±SD) and range among replicates for juveniles of *Villosa iris* reared in sediment (<600 *u*m) at two depths (5 mm or 15 mm) over a 40-day period.

Sediment Depth		Days				
		0	10	20	30	40
5 mm	Mean (SD)	267 (34.6)	413 (10.3)	464 (10.4)	539 (38.4)	579 (51.4)
	Range	231-333	400-425	446-474	502-587	523-664
15 mm	Mean (SD)	267 (34.6)	416 (12.2)	475 (12.7)	547 (19.2)	579 (23.6)
	Range	231-333	400-428	451-490	526-572	553-615

Table 3.4. Summary of propagation studies with *Villosa iris*, including results from Objectives 2 and 3 of this study.

Author	Days	Length	Survival	Temperature
Beaty, 1999	30	450 μm	71%	18 ^{O}C
Beck, this study	30	550 μm	50 – 60%	18.6 ^{O}C
Beck, this study	40	579 μm	41 – 50%	18.6 ^{O}C
Beck, this study	42	445 μm	3 – 16%	16.2 ^{O}C
Gatenby et al., 1996	45	386 – 462 μm	3 – 51%	20-25 ^{O}C
Gatenby et al., 1996	45	552 μm	66.5%	20-25 ^{O}C
Beaty, 1999	60	440 μm	33%	18 ^{O}C
Gatenby et al., 1997	60	638 – 744 μm	16 – 51%	Not reported

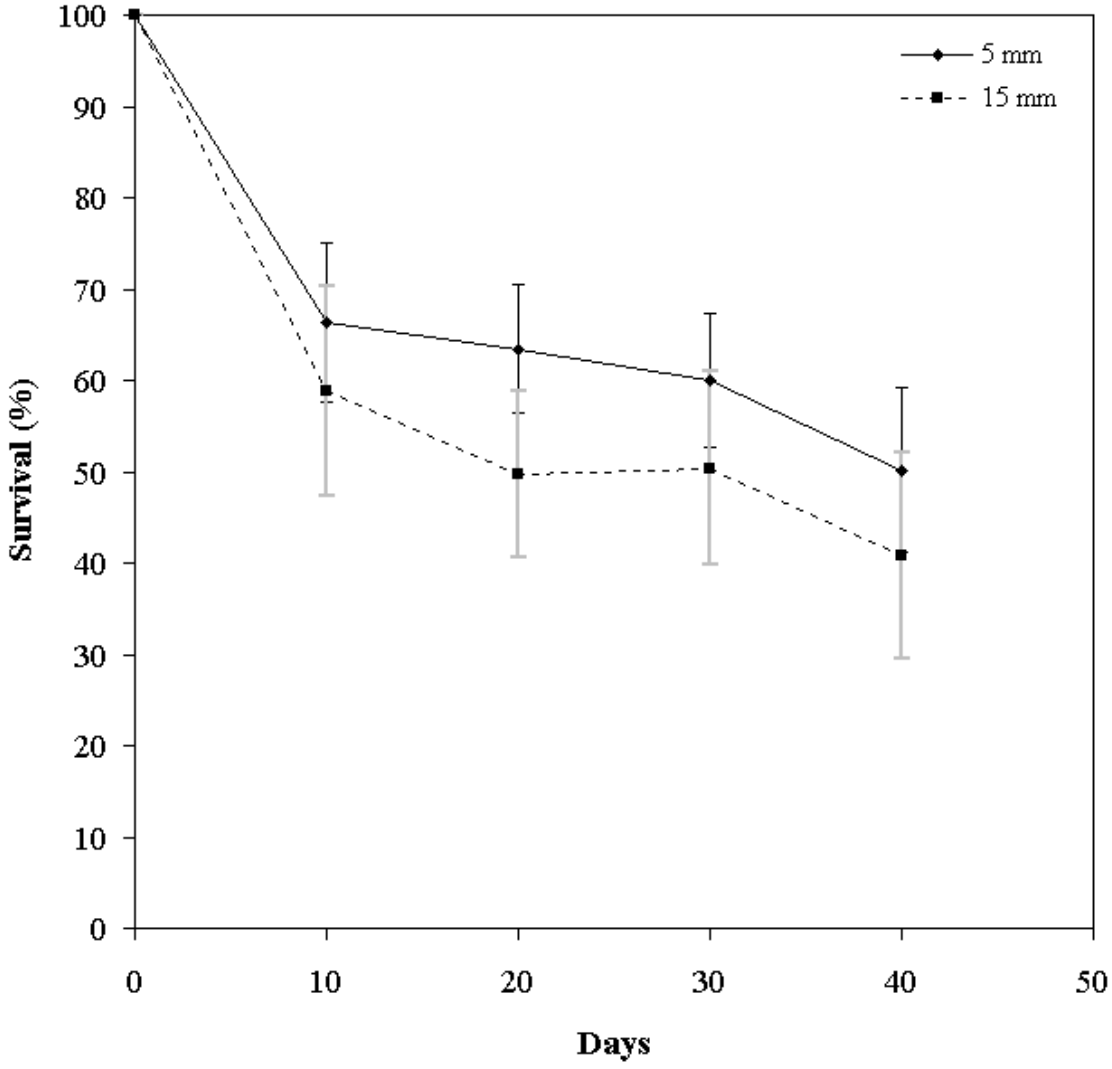


Figure 3.1. Survival (±SD) for juveniles of *Villosa iris* reared in sediment (<600 *u*m) at two depths (5 mm or 15 mm) over a 40-day period. Over the course of the experiment, treatments were significantly different ($p<0.05$).

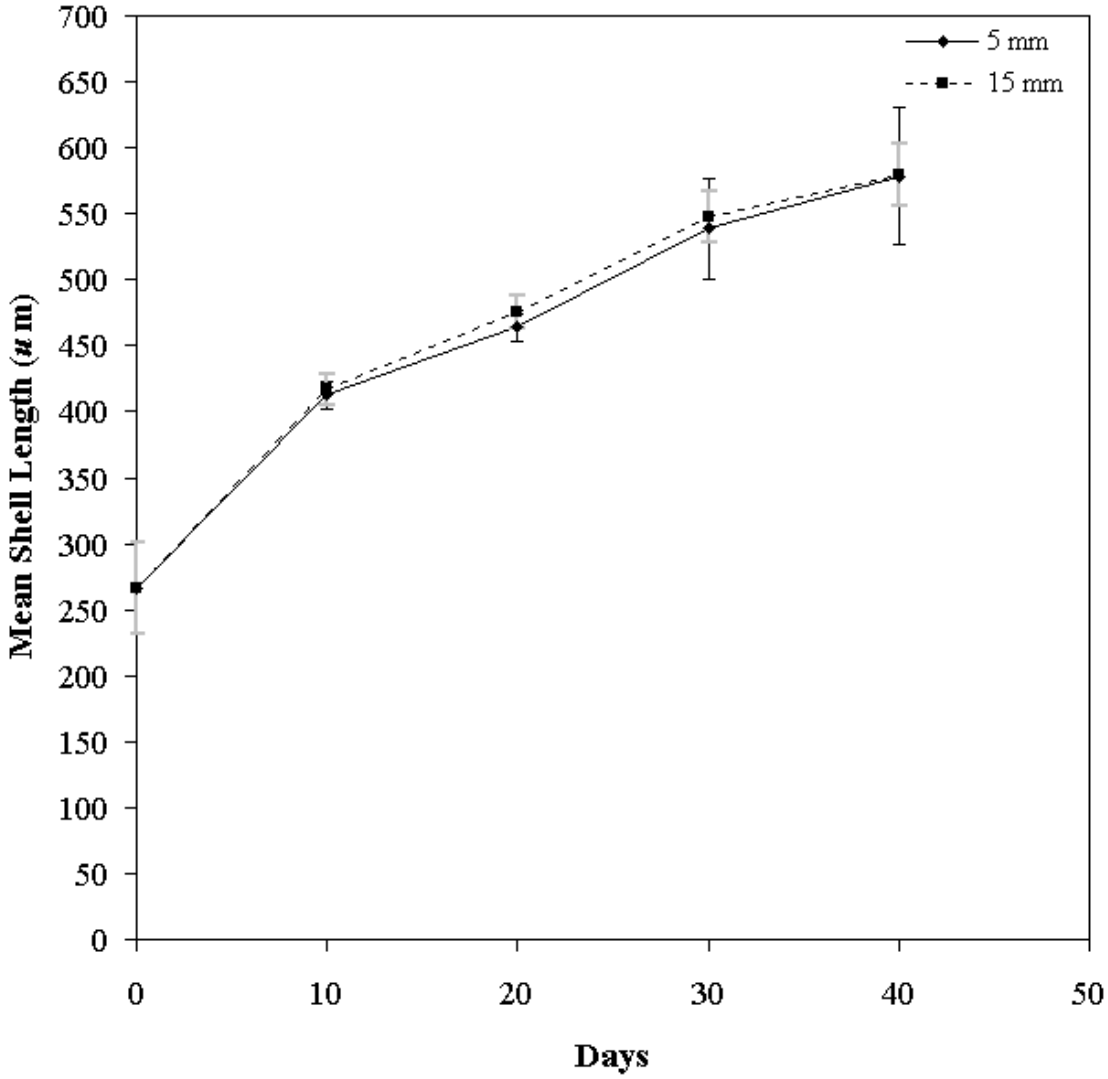


Figure 3.2. Mean shell length (±SD) for juveniles of *Villosa iris* reared in sediment (<600 *u*m) at two depths (5 mm or 15 mm) over a 40-day period.

SUMMARY

1. *Scenedesmus quadricauda* is not an appropriate algal species for feeding juvenile mussels. Juvenile mussels selected for *Nannochloropsis oculata* and *Selenastrum capricornutum* over *S. quadricauda* ($p<0.05$). This may be an indication of particle size preference. In addition, age groups I and II (<53 days old) failed to ingest *S. quadricauda*. Colonies of *S. quadricauda* (22.3 - 44.5 μm) are most likely too large for newly metamorphosed juveniles to ingest.
2. Uni-algal diets of *S. quadricauda* or *N. oculata* were not adequate for rearing juveniles. Juveniles failed to grow beyond 450 *u*m, and survival declined rapidly after 21 days. Visual observations of the gut color of juveniles under high back-lighting indicated that juveniles fed *S. quadricauda* did not ingest the algae. Survivorship was likely dependent on the size range of quality food available. Juveniles fed *N. oculata* ingested the algae, but there was no evidence that the juveniles could assimilate this species.
3. When *N. oculata* was used in conjunction with *Neochloris oleoabundans* and fine substrate (< 600 *u*m), it was shown to be an excellent diet. Until further research is conducted to show otherwise, I would recommend feeding a bi-algal diet of *N. oculata* and *N. oleoabundans*, and providing mussels with a fine substrate, for captive culture. Fine substrate may aid in the mechanical digestion of the algae or contain additional nutrition for juveniles.
4. Sediment depth may affect juvenile survival. Juveniles reared in a 5 mm layer of substrate (<600 *u*m) had slightly higher survival than juveniles reared in a 15 mm layer of substrate ($p<0.05$). Growth of juveniles was not affected by sediment depth ($p>0.05$).
5. By modifying methods for culturing marine algae, I was able to culture large quantities of freshwater algae free of contaminants and "weed" species. The ability to culture freshwater algae in bulk quantities allows testing a suite of algal species for rearing juvenile mussels. Prior to this, cultures of *Scenedesmus* spp. and other "weed" species were used to feed juveniles.

6. Low water temperatures likely reduced growth in my juvenile grow-out studies. My temperatures averaged 16.2^{o}C (SD ± 1.36) (See Chapter 2) and 18.6^{o}C (SD ± 1.37) (See Chapter 3). I was unable to control temperatures, and water temperature dropped to as low as 11.9^{o}C at one point during the first grow-out experiment. During both experiments, temperature fluctuated greatly (Appendix A.). A method for controlling temperature within the recirculating culture systems at the Virginia Tech Aquaculture Facility should be developed.

LITERATURE CITED

Baldwin, B.S. 1995. Selective particle ingestion by oyster larvae (*Crassostrea virginica*) feeding on natural seston and cultured algae. Marine Biology 123(1): 95-107.

Baker, S.M., J.S. Levinton, J.P. Kurdziel, and S.E. Shumway. 1998. Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load. Journal of Shellfish Research 17(4): 1207-1213.

Baker, S.M. and J.S. Levinton. 2000. Feeding selectivity of native freshwater mussels (Unionidae) and competition with zebra mussels (Abstract). Journal of Shellfish Research 19(1): 659.

Beaty, B.B. 1999. Development of juvenile culture techniques and testing of potential biomarkers of environmental stress in freshwater mussels (Bivalvia: Unionidae). Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Beiras, R, A.P. Camacho, and M. Albentosa. 1994. Influence of temperature on the physiology of growth in *Ruditapes decussatus* (L.) larvae. Journal of Shellfish Research 13(1): 77-83.

Buddensiek, V. 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: A contribution to conservation programs and the knowledge of habitat requirements. Biological Conservation 74: 33-40.

Cañavate, J.P. and C. Fernández-Díez. 2001. Pilot evaluation of freeze-dried algae in the mass rearing of gilthead seabream (*Sparus aurata*) larvae. Aquaculture. 193: 257-269.

Defossez, J.-M. and A.J.S. Hawkins. 1997. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum*, and *Tapes decussatus*. Marine Biology 129: 139-147.

Dimock, R.V. and A.H. Wright. 1993. Sensitivity of juvenile freshwater mussels to hypoxic, thermal, and acid stress. The Journal of the Elisha Mitchell Scientific Society 109(4): 183-192.

Duerr, E.O., A. Molnar, and V. Sato. 1998. Cultured algae as aquaculture feeds. Journal of Marine Biotechnology 6(2): 65-70.

Gallager, S.M. 1993. Hydrodynamic disturbances produced by small zooplankton: case study for the veliger larva of a bivalve mollusc. Journal of Plankton Research 15(11): 1277-1296.

Gatenby, C.M., R.J. Neves, and B.C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society 15(4): 597-609.

Gatenby, C.M., B.C. Parker, and R.J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. American Malacological Bulletin 14(1): 57-66.

Gatenby, C.M. 2000. A study of holding conditions, feed rations, and algal foods for the captive care of freshwater mussels. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Hadani, A., S. Beddig, and E. Lubzens. 1992. Factors affecting survival of cryopreserved rotifers (*Brachiomus plicatilis* O.F. Müller). Progress in Aquaculture Research; Proceedings of the 4th German-Israeli Status Seminar held on October 30-31, 1990 GKSS-Forschungszentrum Geesthac.

Hanlon, S.D. 2000. Release of juvenile mussels into a fish hatchery raceway: A comparison of techniques. Masters Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Henley, W. F., L.L. Zimmerman, R.J. Neves, and M.R. Kidd. 2001. Design and evaluation of recirculating water systems for maintenance and propagation of freshwater mussels. North American Journal of Aquaculture 63: 144-155.

Hoff, F.H. and T.W. Snell. 1999. Plankton Culture Manual. Fifth Edition. Edited by: J. Nelson. Florida Aqua Farms, Inc. Dade City, Florida.

Hudson, R.G. and B.G. Isom. 1984. Rearing juveniles of the freshwater mussels (Unionidae) in a laboratory setting. Nautilus 98(4): 129-135.

Isom, B.G. and R.G. Hudson. 1982. In vitro culture of parasitic freshwater mussel glochidia. Nautilus 96(4): 147-151.

Laing, I. 2000. Effect of temperature and ration on growth and conditioning of king scallop (*Pecten maximus*) spat. Aquaculture 183(3-4): 325-334.

Lasee, B.A. 1991. Histological and ultrastructural studies of larval and juvenile *Lampsilis* (Bivalvia) from the Upper Mississippi River. Ph.D. Dissertation, Iowa State University, Ames, Iowa.

Lopez, G.R. and I.J. Holopainen. 1987. Interstitial suspension-feeding by *Pisidium* spp. (Pisidiidae: Bivalvia): a new guild in the lentic benthos? American Malacological Bulletin 5(1): 21-30.

MacDonald, B.A. and J.E. Ward. 1994. Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus* (Mollusca:Bivalvia). Marine Ecology Progress Series 108: 251-264.

Michaelson, D.L. and R.J. Neves. 1995. Life history and habitat of the endangered dwarf wedgemussel *Alasmidonta heterodon* (Bivalvia: Unionidae). Journal of the North American Benthological Society 14(2): 324-340.

Miura, T. and T. Yamashiro. 1990. Size selective feeding of *Anodonta calipygos*, a phytoplanktivorous freshwater bivalve, and viability of egested algae. Japanese Journal of Limnology 51(2): 73-78.

Neves, R.J., L.R. Weaver, and A.V. Zale. 1985. An evaluation of host fish suitability for glochidia of *V. vanuxemi* and *V. nebulosa* (Pelecypoda: Unionidae). American Midland Naturalist 113(1): 13-19.

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.

Neves, R.J. 1991. Mollusks. Pages 251-320 in K. Terwilliger (Coordinator). Virginia's Endangered Species. McDonald and Woodward Publishing Company. Blacksburg, Virginia.

Numaguchi, K. 2000. Evaluation of algal species for the growth of early spat of the Japanese pearl oyster *Pinctada fucata martensii*. Journal of Shellfish Research 19(1): 153-157.

O'Beirn, F.X., R.J. Neves, and M.B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. American Malacological Bulletin 14(2): 165-171.

O'Connell, M.T. and R.J. Neves. 1999. Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). Journal of Freshwater Ecology 14(1): 71-78.

Okauchi, M. 1991. The status of phytoplankton production in Japan. In: Rotifer and Algae Culture Systems. The Oceanic Institute, Hawaii: 247-256.

Parker, B.C., M.A. Patterson, and R.J. Neves. 1998. Feeding interactions between native freshwater mussels (Bivalvia:Unionidae) and zebra mussels (*Dreissena polymorpha*) in the Ohio River. American Malacological Bulletin 14(2): 173-179.

Paterson, C.G. 1984. A technique for determining apparent selective filtration in the fresh-water bivalve *Elliptio complanata* (Lightfoot). The Veliger 27(2): 238-241.

Patterson, M.A., B.C. Parker, and R.J. Neves. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. American Malacological Bulletin 15(1): 47-50.

Payne, M.F. and R.J. Rippingale. 2000. Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. Aquaculture 187: 85-96.

Pilditch, C.A. and J. Grant. 1999. Effect of temperature fluctuations and food supply on the growth and metabolism of juvenile sea scallops (*Placopecten magellanicus*). Marine Biology 134(2): 235-248.

Raby, D., M. Mingelbier, J.J. Dodson, B. Klein, Y. Lagaduec, and L. Legendre. 1997. Food-particle size and selection by bivalve larvae in a temperate embayment. Marine Biology 127: 665-672.

Reid, R.G.B., R.F. McMahon, D. Ó Foighil, and R. Finnigan. 1992. Anterior inhalant currents and pedal feeding in bivalves. The Veliger 35(2): 93-104.

Rogers, S.O. 1999. Population biology of the tan riffleshell (*Epioblasma florentina walkeri*) and the effects of substratum and light on juvenile mussel propagation. Masters Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Sakamoto, K., E. Okimasu, and A. Amemura. 1998. Dietary value of rotifers *Brachionus rotundiformis* cultured with *Synechocystis* sp. SY-4 for larvae of Red Sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus*. Fisheries Science 64(5): 722-726.

Sicard, M.T., A.N. Maeda-Martinez, P. Ormart, T. Reynoso-Granados, and L. Carvalho. 1999. Optimum temperature for growth in the catarina scallop (*Argopecten venticosus circularis*, Sowerby II, 1842). Journal of Shellfish Research 18(2): 385-392.

Silverman, H, S.J. Nichols, J.S. Cherry, E. Achberger, J.W. Lynn, and T.H. Dietz. 1997. Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids. Canadian Journal of Zoology 75: 1857-1866.

Shumway, S.E., T.L. Cucci, R.C. Newell, and C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. Journal of Experimental Marine Biology and Ecology 91: 77-92.

Sokel, R.R., and F.J. Rohlf. 1995. Biometry (3rd Edition). W.H. Freeman and Company, New York. 887 pp.

Solow, A.R. and S.M. Gallager. 1990. Analysis of capture efficiency in suspension feeding: application of nonparametric binary regression. Marine Biology 107: 341-344.

Starkley, R.W., A.G. Eversole, and D.E. Brune. 2000. Growth and survival of juvenile and adult freshwater mussels in the partitioned aquaculture system. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium: 109-114.

Steg, M.L. 1998. Identification of host fishes and experimental culture of juveniles for selected freshwater mussel species in Virginia. Masters Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Tankersley, R.A. and S.W. Butz. 2000. Design and evaluation of a laboratory-scale recirculating aquaculture system for the captive care of freshwater mussels. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium: 127-134.

Vollenweider, R.A. 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. Burgess and Son (Abingdon) Limited. Abingdon, England.

Ward, J.E. and N.M. Targett. 1989. Influence of marine algal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. Marine Biology 101: 313-321.

Ward, J.E., H.K. Cassell, and B.A. MacDonald. 1992. Chemoreception in the sea scallop *Placopecten magellanicus* (Gmelin). I. Stimulatory effects of phytoplankton metabolites on clearance and ingestion rates. Journal of Experimental Marine Biology and Ecology 163: 235-250.

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(9): 6-22.

Yamasaki, S., K. Tanabe, and H. Hirata. 1989. Efficiency of chilled and frozen *N. oculata* sp. (marine *Chlorella*) for culture of rotifer. Memoirs of the Faculty Fisheries Kagoshima University./Kagoshimadai Suisangakubu Kiyo 38(1): 77-82.

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(2): 217-222.

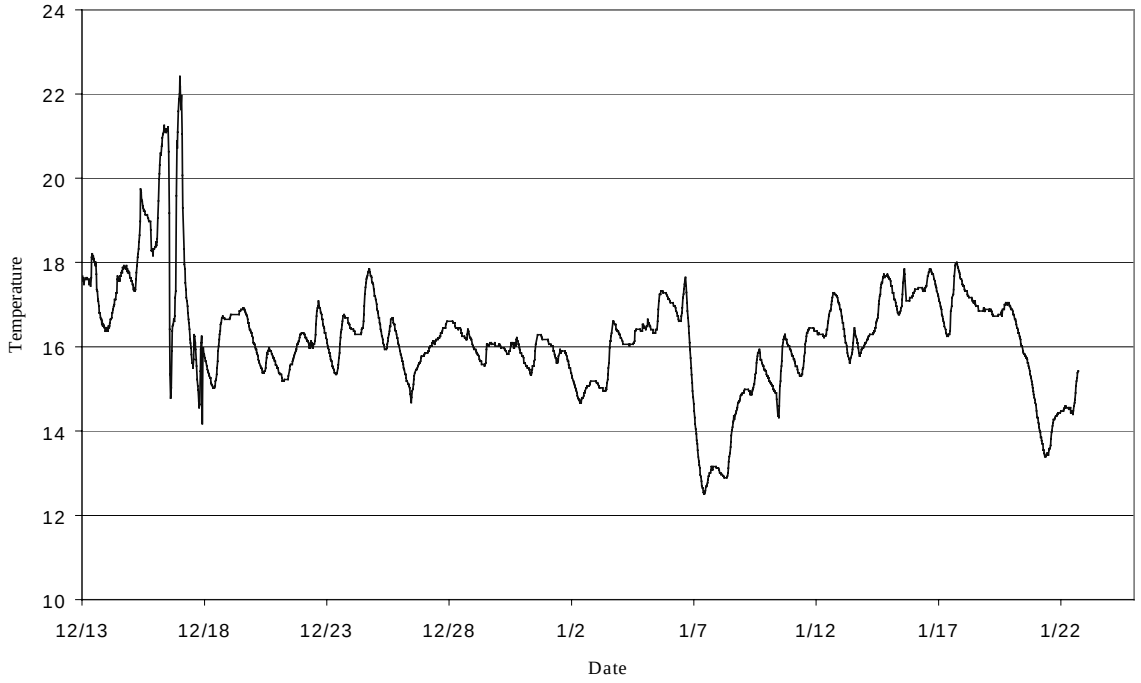
Zale, A.V. and R.J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology 60: 2535-2542.

Zou, N., C. Zhang, Z. Cohen, and A. Richmond. 2000. Production of cell mass and eicosapentaenoic acid (EPA) in ultrahigh cell density cultures of *N. oculata* sp. (Eustigmatophyceae). European Journal of Phycology 35: 127-133.

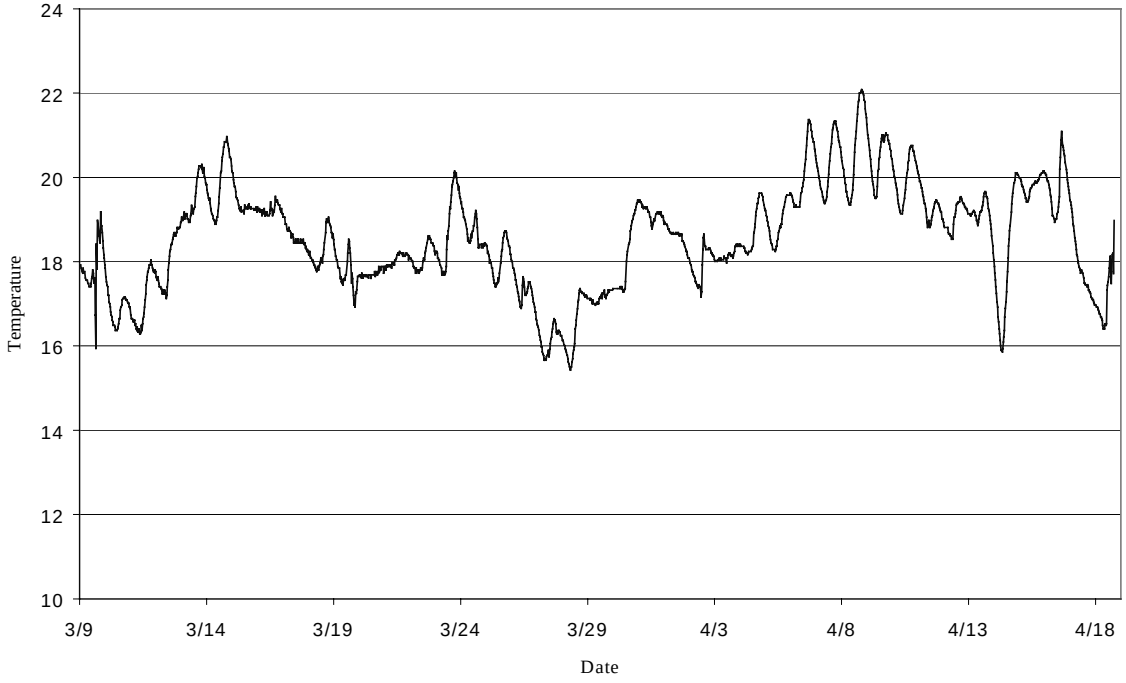
APPENDIX A. Survival data* at 20 days from a diet pilot study.

Treatment	% survival 1	% survival 2	% survival 3	%survival 4	% survival 5
S1	88	71	71	87	54
S2	88	80	81	54	94
R1	0	0	0	0	5
R2	0	0	0	5	13
R+N1	55	39	21	55	55
R+N2	17	25	45	44	46
N1	100	80	85	72	69
N2	28	0	0	46	5

(S) = *Scenedesmus quadricauda*
(R) = Roti-Rich
(R+N) = Roti-Rich and *Nanochloropsis* oculata
(N) = *N. oculata*
* This pilot study used *V. iris*, and ended at the second sampling date, 40 days, with no survival. Each treatment consisted of 2 replicate 145-L recirculating culture systems containing 5 subsamples/system.



B-1



B-2

APPENDIX B. Thermographs of the mean daily water temperature (^{o}C) (Chapter 2: B-1; Chapter 3: B-2) in 2000-2001.

Vita

Kevin Moran Beck was born in upstate New York, in August of 1976. He is the oldest of two boys. He attended Schalmont High School, and graduated in 1994. He obtained his Bachelor of Science in Biology from Southampton College of Long Island University. His love of bivalves and aquaculture brought him to Blacksburg, Virginia in August of 1998 to study freshwater mussel propagation as a candidate for the degree of Master of Science in Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University. He finished his degree in the spring of 2001, and accepted a position as an officer with the United States Coast Guard.