INTRODUCTION

The freshwater mussel assemblage of North America is the most diverse in the world. Many species are endangered, largely due to their highly endemic nature (Kay 1995). Over the past 30 years, native mussel numbers and species diversity have been on a dramatic decline, and now 55% of North American mussel species are extinct or imperiled, partly due to anthropogenic destruction of habitat and degradation of water quality (Williams *et al.* 1993). Most of the mussel species listed as endangered or threatened in Virginia live in the upper Tennessee River drainage of southwestern Virginia (Neves 1991). Therefore, saving mussel species diversity in this region is of utmost importance.

There are many reasons for the decline of freshwater mussels in numerous North American habitats, one of which is the lack of appropriate fish hosts (Neves 1991). To date, the identity of specific fish hosts for most unionacean mussels is largely unknown (Hoggarth 1992). This knowledge gap hinders the success of current and future preservation efforts (White *et al.* 1994). For protection or restoration projects of endangered or threatened mussels to be successful, the host fish species must be identified and known to be present within an area. Conservation and rehabilitation of natural populations of freshwater mussels will be ineffective if they cannot complete their life cycle due to unavailability of the appropriate fish hosts.

Culturing freshwater mussels in artificial conditions for the augmentation of natural populations has proven difficult, primarily for lack of knowledge of physiological and dietary needs. In certain instances, however, artificial culture may be the only option left for saving some mussel species (Neves 1997). Artificial culture may help to increase the size of small mussel populations to where they can reproduce and survive on their own, given the required fish hosts are present and habitat is suitable. Also, it may be possible to re-establish historical populations if

the cause of extirpation has been rectified. The success of past relocation efforts has been inconsistent (Cope and Waller 1995). The more that is known about freshwater mussel reproduction, growth, and survival, the better the chance of success of future conservation efforts (Neves 1997). Artificial culture efforts may be instrumental in achieving these goals.

Mussel Decline in North America

Over the last 100 years, there have been drastic declines in unionacean populations and in species diversity. Two hundred and thirteen of the 297 recognized freshwater bivalve species and subspecies in North America are endangered, threatened, or of special concern (Williams *et al.* 1993). There are many reasons for this decline. At the turn of the century, freshwater mussel populations were damaged by exploitation from the button industry, as well as by seekers of freshwater pearls (Laycock 1983). The impoundment and channelization of streams and rivers adversely affect mussels through changing flow velocity, increasing siltation, changing water temperature regime, depleting oxygen content, changing water depth, and limiting access to host fish (Mathiak 1979; Miller *et al.* 1984; Havlik and Marking 1987). Pollution is also a major threat; major extirpations have been caused by industrial pollution, waste water effluents, acid mine drainage, and siltation. Agricultural pollution is also a problem (Zale and Neves 1982). Mussels, being sedentary animals, are unable to escape these threats. The damage to freshwater mussel populations is especially severe since their dispersal is limited to how far their host fish travel while infested with glochidia; hence, mussel populations are often isolated (Kat 1984). Another major threat to freshwater mussel survival is the invasion of exotics such as the Asian clam, *Corbicula fluminea*, and the zebra mussel, *Dreissena polymorpha*. *C. fluminea* has achieved widespread distribution across coastal and southern North America, becoming the

dominant benthic mollusk in many areas. It is perceived as a threat to unionids because of competitive exclusion, since it is so prolific, quickly dispersing, and tolerant of a wide range of habitats (Baker *et al.* 1994). Even so, there is little direct evidence of negative effects of *C. fluminea* on unionids (Neves 1995). *D. polymorpha,* another highly successful and prolific invader, is a major threat to unionid populations. Since its introduction to the Great Lakes in 1986, it already has caused severe declines and extirpations of unionid populations (Schloesser and Kovolak 1991). *D. polymorpha* is a biofouler, and it kills or debilitates unionids through heavy infestation (Doll 1995). The range of *D. polymorpha* has rapidly extended throughout the Mississippi River basin and possibly could destroy remaining indigenous unionid populations (Neves 1995).

Life History of Unionids

Life history characteristics are quite varied and complex for freshwater unionacean bivalves of North America. Bivalves can be difficult to study because they are generally gonochoristic, long-lived, iteroparous, and have a complex life cycle involving a parasitic glochidial stage. Unionids generally live in stable aquatic habitats where they are buffered from stochastic events In ideal conditions, their populations can reach large numbers and individuals can be long-lived. Some species exhibit sexual dimorphism, and a few are hermaphroditic. They exhibit internal fertilization, where sperm released into the water column by a male is taken in by a female in close proximity. The sperm is carried to the unfertilized eggs held in the gill marsupia (brood chambers), where embryos are held through the early life stages (Richard *et al.* 1991). Water temperature change appears to be an important cue in reproduction. Glochidia generally

are released through the exhalant siphon, specialized gill pores, or a rupture in the ventral portion of the gill (Richard *et al.* 1991).

Most freshwater mussels have a unique parasitic life stage. The larvae, or glochidia, clamp onto, and become encysted in fish host gills, scales, skin, or fins, transform into juveniles, and then drop off after a period of time (Howard and Anson 1923). Freshwater mussels appear specific to their fish host species (Zale and Neves 1982), and some reports suggest that fish host suitability is dependent on the immune system and blood serum components of the host (Kat 1984; Neves *et al.* 1985; O'Connell 1991). Unsuitable fish reject the glochidia, sloughing them off after attachment. There are three types of glochidia: hooked, hookless, and axe-head. Most North American species are of the "hookless" variety, and tend to encyst on the gills of host fish. Unionids, especially those that are tachytictic (short-term brooders), employ a variety of methods to ensure encystment in the proper fish hosts. Glochidial release occurs once a year, and its timing is species-specific, often depending on when the appropriate fish hosts will be available (Fuller 1974; Kat 1984). Depending on the mussel species, glochidia encyst within 2-36 hr of attachment, and fully transform and excyst in about 6-160 days (Kat 1984). It is uncertain what degree of nutrition glochidia may gain from the parasitization of fish; the main purpose for the relationship is thought to be for dispersal to favorable habitats and the reduction of intraspecific competition (Payne and Miller 1989). Survivorship of glochidia is low. For example, in a natural population of *Margaritifera margaritifera,* fewer than one in a million released glochidia successfully establish in the substratum as juveniles (Young and Williams 1984).

It is imperative to prevent mussel populations from becoming too small, or recruitment may not be successful. Unionids have particularly long life spans, occasionally in excess of 100 years, reaching maturity as late as 12 years (Bauer 1983). Relict populations may live on for

many years without substantial successful recruitment, leading to extinction. Information gained from this and other research projects can help ensure that this does not happen. If host fish are identified, their presence, a vital link in the freshwater mussel life cycle, can be protected. If populations have become small or recruitment is otherwise unsuccessful, juveniles can be produced in the laboratory at a higher rate than what is experienced in the wild. Culturing juveniles for a period of time before release also gives them a better chance of survival. Information gained from mussel culture experiments helps us to better understand, and provide for, the unique physical and physiological requirements of mussels. Host fish identification and juvenile mussel culture, the objectives of this study, are important steps in the conservation and rehabilitation of dwindling freshwater mussel populations in Virginia.

Pollution and its Impact on Fishes and Mussels in the Upper Tennessee River Basin

Survival of unionids in the upper Tennessee River basin, where rich unionid and fish faunas co-occur, requires that historically available fish host species continue to be available. The Clinch River, Powell River and Holston River systems of the Virginia portion of the upper Tennessee River basin, where the mussels studied in this project live, have received considerable pollution in the last 30 years. In the early 1970s, the North Fork of the Holston River ranked as one of the most polluted rivers in the U. S. (Jenkins and Burkhead 1993). This was largely due to releases of dissolved solids and mercury from the Olin Mathieson Corporation chemical plant in Saltville, VA. However, the pollution began with commercial salt production there in the 1760s, with fish kills in the lower Virginia reaches of the river in the 1920s and 1940s (Jenkins and Burkhead 1993). The fish assemblage has recovered steadily since the closing of the chemical plant in 1972, although erosion and chemical seepage still occurs at the site (Hill *et al*. 1975;

Freeman 1986). Now extirpated from the North Fork Holston River are *Lepisosteus osseus*, *Moxostoma lacerum* (now extinct), and *Noturus flavipinnis* (federally threatened) (Jenkins and Burkhead 1993). *Cyprinella monacha* is now extirpated above Saltville, and *Ichthyomyzon bdellium* is gone below Saltville. Extirpated from the entire Virginia reach, possibly due to damming, are *Moxostoma carinatum*, *Stizostedion vitreum*, *S. canadense*, and *Aplodinotus grunniens*.

The Virginia portion of the Clinch River suffered a virtually complete fish kill in 1967 due to an alkaline fly ash spill at the Appalachian Power Company plant at Carbo (Jenkins and Burkhead 1993). Recovery was retarded by a sulfuric acid spill by the APCO plant at the site in 1970 (Soukup 1970). Although there has since been substantial recovery of the fish fauna in the main stem, prekill abundances and distributions, particularly of smaller fishes, are virtually unknown (Jenkins and Burkhead 1993). Additionally, tributaries of the Clinch River have been observed to suffer from domestic, coal mine, or industrial pollution (Raleigh *et al*. 1978). Fish species that appear to be extirpated from the Virginia reach of the Clinch River include: *Etheostoma cinereum*, *Moxostoma anisurum*, *Cyprinella monacha*, *Erimystax cahni*, and *Noturus stanauli* (Jenkins and Burkhead 1993).

Sedimentation continues to be a chronic problem in the Clinch and Powell systems. Jenkins and Burkhead (1993) consider it to be a chief cause in the limitation of aquatic life in Virginia. Locally, the Clinch and Powell rivers have accumulated large amounts of silt due to stripmining by the coal industry. Accumulation of coal fines is also a problem, with the largest loads of combined coal particles and sedimentation deposited in the Powell River headwaters (Dennis 1981). Many tributaries to the Clinch and Powell rivers have been degraded by mine wastes as well. Although the Powell River is considered to be in a state of recovery, the

following fish species, which are present in the Clinch River, appear extirpated from the Powell in Virginia: *Cyprinella monacha*, *C. whipplei*, *Moxostoma lacerum*, *Percina burtoni*, *P. macrocephala*, *Etheostoma tippecanoe,* and *E. percnurum* (Jenkins and Burkhead 1984). Also apparently eliminated from the Powell River are *Notropis antheroides* and *Moxostoma anisurum* (Jenkins and Burkhead 1994).

Although the effects of historical episodic and chronic pollution in the upper Tennessee River drainage on the freshwater mussel fauna may not be recorded in the same detail as that of the fish fauna, observations made at the turn of the century show that freshwater mussel density and species richness were once much greater than today (Ortman 1918; Ahlstedt and Tuberville 1997). As early as 1915, Ortman (1918) observed that the mussel fauna of the upper Tennessee drainage was steadily deteriorating due to stream pollution. Pollution and habitat degradation have a greater effect on freshwater mussels, as an indicator species, than fishes, and mussels take a longer time to recover from such events (Ahlstedt and Tuberville 1997). In the Clinch and Powell rivers, mussel distribution and abundance is strongly affected by proximity to mined lands (Ahlstedt and Tuberville 1997). The sediments in these rivers continue to be toxic to mussels, and their toxicity may be magnified during periods of drought (McCann and Neves 1992; Ahlstedt and Tuberville 1997). The tributaries to the Clinch and Powell rivers are in worse condition than the main channels, with the majority of them receiving an Index of Biotic Integrity rating of Poor or Very Poor (Ahlstedt and Tuberville 1997). Surveys by the Tennessee Valley Authority have shown mussel species diversity to be on a steady decline in the Powell River over the last 20 years, with a large die-off in 1983-88. Many of the mussel species observed in the Clinch and Powell rivers are at extremely low densities, with reproduction limited or nonexistent in the Virginia portions of the Clinch River (Ahlstedt and Tuberville 1997).

Surveys of the Tennessee portion of the Holston River (Tennessee Valley Authority 1986a) have shown Cumberlandian mussel populations to be scarce and often comprised of relict individuals. Although damming of the river has had a major impact on mussels in that section of the Holston River, historical pollution and the removal of host fishes may have decimated the mussel fauna to a point where they are unable to recolonize (Tennessee Valley Authority 1986a).

Learning the identity and securing the availability of host fishes for the mussels of the upper Tennessee River basin is vital to their survival. In addition, inputs of mining and industrial pollution into these rivers must be curbed and cleaned up. Reintroduction and population augmentation efforts will be futile if habitat quality is not improved. Only through diligent efforts will the continual decline of mussels in this region be halted and their populations be given a chance to recover. Unfortunately, that chance may already be gone for those fish and mussel species extirpated from the region.

CHAPTER ONE: HOST FISH IDENTIFICATION

Introduction

Specific fish hosts are essential for completion of the unionid life cycle, and hosts, therefore, must be identified before conservation efforts are likely to be successful. Host fish presently are known for approximately only one-quarter of North American mussel species (Hoggarth 1992). One method of identifying fish hosts that has been used in the past consists of finding infested fish, collecting the glochidia, and identifying them. This method requires careful

examination of glochidial morphology. Yet, closely related species are difficult to distinguish from one another, and mis-identification is common (Hoggarth 1994; White *et al.* 1994).

A technique for glochidial identification using genetic characterization recently has been developed. Using restriction fragment length polymorphism analysis and polymerase chain reaction amplification, the DNA of glochidia can be matched with that of its parent species (White *et al.* 1994). Although this method is promising, it requires expensive equipment, time, and expertise, a reference library of mussel DNA samples, and collection of wild fish infested with glochidia.

A traditional method of identifying host fishes is to inoculate them artificially in a laboratory environment. It involves exposing potential fish hosts to glochidia and keeping the infested fish in aquaria for the duration of the transformation period. If mussel juveniles are found in the tanks at the end of this time, transformation on that host species has been successful. If the transformation is not successful, the glochidia will be sloughed off of the host fish and the transformation will not proceed (Zale and Neves 1981). Due to a possible immunological response to repeat infestations, the host fish should be collected from areas where the subject mussel does not occur (Waters and O'Dee 1996). This host identification process takes time, and it does not replicate the ecology and microhabitats of the organisms involved. However, it has proven successful in the past and does provide concrete and useful information that can be helpful in mussel conservation efforts (Neves *et al.* 1985; Yeager and Saylor 1995; Khym and Layzer 1998). The objective of this study was to identify host fish for several mussel species endangered or threatened in Virginia, using artificial infestation methods in a laboratory environment.

Materials and Methods

Mussel Collection

Attempts were made to collect gravid mussels of nine freshwater mussel species. In Virginia, five of the species are endangered, and four are threatened. The species names, status and distribution in Virginia, and periods of gravidity are listed in Table 1, and their preferred habitats are listed in Table 2. Mussels were collected by snorkeling and occasionally by use of a water scope (glass-bottomed bucket). Approximately twenty trips were taken in search of gravid mussels. Search locations and times were limited by access points, weather, water level and clarity, and period of mussel gravidity.

Gravidity was determined by prying open the mussel slightly with reversing pliers, and looking for the characteristic swollen (and possibly differentially colored) gill marsupia, pregnant with glochidia (Mackie 1984). As a precaution against possible glochidial abortion during transport, collected gravid mussels were transported in a cooler equipped with a portable aeration device, river water, and cool packs. Recent work has shown that it is less stressful on mussels held in water during transport, rather than packing them in wet burlap (Chen et al. 1998). The

Table 1. Status, distribution, and periods of gravidity of study mussel species listed in Virginia. *

Species & Status	Distribution in Virginia [& other states]	Period of Gravidity
Lasmigona holstonia (Tennessee heelsplitter) endangered	Holston R. (North & Middle Forks), and upper Clinch R. [rare- Tenn. and Alabama Rivers]	Long-term (Sept.-May)
Ligumia recta (black sandshell) threatened	Upper Tenn. R. drainage: Holston (North and Middle Forks), Clinch, and Powell Rivers [Miss. drainage, St. Lawrence and Alabama River systems]	Long-term (all year)
Elliptio crassidens (elephant ear) endangered	Clinch R. and Powell R. [Miss. R. drainage, Alabama and Tombigbee Rivers]	Short-term (Apr.-July)
Quadrula pustulosa pustulosa (pimpleback) threatened	Clinch and Powell R. [common- Miss. drainage]	Short-term (mid June-late Aug.)
Lexingtonia dolabelloides (slabside pearlymussel) threatened	N. and Middle Forks Holston, Clinch, and Powell Rivers [Clinch, Powell, Elk, Duck, Buffalo R. in Tenn.; Paint Rock R.; Estill Fork and Hurricane Creek in Ala.]	Short-term (mid May-early August)
Pleurobema cordatum (Ohio pigtoe) threatened	Clinch R. (Pendleton Island) [Tenn. side of Clinch R.; common- Tenn., Ohio, Cumberlandian and Miss. Rivers]	Short-term (Apr.-July)
Pleurobema rubrum (Pink pigtoe) endangered	Clinch R. (Pendleton Island) [Mississippi, Ohio, Tennessee, Cumberland Rivers]	Short-term (May-July)
Plethobasus cyphyus (Sheepnose) threatened	Clinch R. (Dungannon), Powell R. [Mississippi R. drainage]	Short-term (May-July)
Cumberlandia monodonta (Spectaclecase) endangered	Clinch R. [Cumberland, Tennessee, Mississippi Rivers, also in Illinois, Indiana, Kentucky, Missouri, Minnesota]	Two broods per season (?)

* All Records from Neves, 1991

Table 2. Preferred habitat types of mussel species listed in Virginia. *

Species	Preferred Habitat Type
Lasmigona holstonia (Tennessee heelsplitter)	Extreme headwaters of rivers, usually in small streams
Elliptio crassidens (elephant ear)	Large rivers, coarse sand and gravel, rivers at least 2 m deep with strong currents
Lexingtonia dolabelloides (slabside pearlymussel)	Shoal and riffle habitats, intermediate sized streams, moderate to fast flowing water, clean heterogeneous substratum
Pleurobema cordatum (Ohio pigtoe)	Shoals in medium to large rivers with mud, sand, gravel, and cobble substrata-sometimes in transition zone between pool and riffle.
Quadrula pustulosa pustulosa (pimpleback)	Silt , to gravel, to cobble substrata, all stream habitats (except those with shifting sand), medium and larger waterways, standing to swiftly flowing water usually greater than 10 cm. deep.
Ligumia recta (black sandshell)	Medium to large rivers, strong current, rare in headwaters, associated with shoals of sand and gravel, occasionally in muddy substrata, depth a few centimeters to 3 m.
Cumberlandia monodonta (spectaclecase)	Clean, free flowing rivers, tucked under the bases of large boulders or along ledges, at the edge of the swift mainstem current.
Pleurobema rubrum (pink pigtoe)	Mud, sand, gravel, and cobble substrata in shoals and riffles of medium to large rivers.
Plethobasus cyphyus (sheepnose)	Sand and gravel substrata in riffles and shoals of large rivers, sand and mud in deeper water in swift current

*Records cited in Neves 1991

collected mussels were held in individual plastic or glass containers in Living Streams (Frigid Units, Inc., Toledo, Ohio), kept between 10 and 15 ^{o}C. The mussels occasionally were fed algae (*Neochloris oleoabundans)* while being held, and they were returned to the site of collection after use. Occasionally, disturbance during collection caused mussels to prematurely abort their glochidia. In such cases the glochidia were collected, the mussels returned to their original site, and viable glochidia were used as soon as possible for artificial infestation. Occasionally, target mussel species were located but were never gravid when checked. When gravid individuals were located, glochidia were often not mature, and either prematurely aborted or never matured when held in the lab at elevated temperatures for a period of time. In such cases, host fish identification tests for those species could not proceed (Table 3).

Host Fish

Some potential fish hosts have been cited for the mussel species used in this study and for their congeners (Table 4). For a fish species to be considered as a natural host, it must co-occur with present populations of these mussels, or have co-occurred historically. Many of the host fish species cited by Watters (1994) are native to the Tennessee River drainage (Table 4), although some, such as *Lepomis gulosis*, *Pomoxis nigromaculatus*, and *Perca flavescens*, *Anguilla rostrata*, and *Scaphirhynchus platorynchus*, are not (Jenkins and Burkhead 1993). Most of the species native to the Clinch River and Powell River are also native to the Holston River system. Of historical note, of the fish species encountered by Cope (1868) on his extensive survey of the upper Holston River, *Cyprinella galactura*, *Notropis telescopus*, *Notropis leuciodus*, *Notropis rubricroceus*, and *Campostoma anomalum* are mentioned as suitable hosts for these mussel species. The Virginia Department of Game and Inland Fisheries Wildlife Information Database

Table 3. Collection locations of target mussel species and gravidity observations.

Mussel Species	Location	Observation (date)
Lampsilis fasciola	Indian Cr., Tazewell Co. Va.	gravid (6/96, 4/97)
Lasmigona holstonia	Mid. Fk. Holston R., Smyth Co. Va.	not gravid (7/96, 7/97,9/97); immature gravid (10/97)
	N. Fk. Clinch R., Scott Co., Va.	not gravid (7/96, 6/97)
Elliptio crassidens	Powell R., Lee Co. Va.	not gravid (5/97)
Lexingtonia dolabelloides	Powell R., Lee Co. Va.	immature gravid (5/97)
	N. Fk. Holston, Smyth Co. Va.	immature gravid (8/97)
Pleurobema cordatum	Ohio R., WV.	immature gravid, aborted (7/96)
Quadrula pustulosa	Ohio R., WV.	partially charged, immature (6/96)
pustulosa	Kentucky Lake, Tenn.	not gravid (7/97)
Ligumia recta	Lower mainstem Tenn. R., Ky.	gravid, aborted (6/96)
	Powell R., Lee Co. Va.	not gravid (5/97)
Pleurobema rubrum	Clinch R., Hancock Co., Tenn.	immature gravid, aborted (7/97)
Plethobasus cyphyus	Clinch R., Hancock Co., Tenn.	immature gravid, aborted (7/97)
Cumberlandia monodonta	Clinch R., Scott. Co. Va.	not gravid (7/96, 6/97), immature gravid,
	Powell R., Lee Co. Va.	aborted (5/97), not gravid (5/97)

Table 4. Cited host fish for target mussel species. Those not native to the Tennessee River drainage are followed by an asterisk. *

Mussel Species	Cited Host Fish Species (those for congeners are in parentheses)
Lasmigona holstonia (Tennessee heelsplitter)	*(Carpiodes carpio*, Pomoxis annularis*, Micropterus salmoides, Lepomis cyanellus)*
Ligumia recta (black sandshell)	*Anguilla rostrata*, Pomoxis annularis*, Micropterus salmoides, Lepomis macrochirus, Stizostedion canadense, (Lepomis gulosis*, Lepomis cyanellus)*
Elliptio crassidens (elephantear)	*Alosa chrysochloris*, (Dorosoma cepedianum, Pylodictis olivaris, Pomoxis annularis*, Pomoxis nigromaculatus*, Stizostedion canadense, Fundulus diaphanus*, Perca flavescens*)*
Quadrula pustulosa pustulosa (pimpleback)	*Scaphirhynchus platorynchus*, Ictalurus punctatus, Ameiurus nebulosus*, Ameiurus melas, Pylodictis olivaris, Pomoxis annularis*, Ictalurus punctatus, (Cyprinella galactura, Cyprinella spiloptera, Lepomis cyanellus, Lepomis macrochirus, Stizostedion canadense, Erimystax dissimilis, Erimystax insignis, Pomoxis nigromaculatus*, Micropterus salmoides)*
Lexingtonia dolabelloides (slabside pearlymussel)	*Notropis ariommus, Notropis rubellus, Notropis rubricroceus, Notropis photogenis, Notropis telescopus, Notropis leuciodus*
Pleurobema cordatum (Ohio pigtoe)	*Lythrurus ardens, Lepomis macrochirus,(Cyprinella galactura, Luxilus cornutus*, Nocomis micropogon, Campostoma anomalum)*
Pleurobema rubrum (pink pigtoe)	*(Lepomis macrochirus, Cyprinella galactura, Luxilus cornutus*, Nocomis micropogon, Campostoma anomalum, Lythrurus ardens)*
Plethobasus cyphyus (sheepnose)	*Stizostedion canadense*
Cumberlandia monodonta (spectaclecase)	none

* Host fish records from Watters (1994); native fish distributions from Jenkins and Burkhead (1993).

was consulted for fish distributions. As a precaution against possible immune responses in the fish due to repeat infestations, host fish used in these experiments were collected from areas where the

mussels presently do not occur (O'Connell 1991, Watters and O'Dee 1996). Also for this reason, collected fish were used once and then returned to their site of collection if possible.

Most potential host fish were collected by electroshocking and seining the tributaries, rivers and lakes of southwestern Virginia. A few of the fish species were obtained from hatcheries. The numbers and species of fish used in the trials depended on their size, availability, and the number of glochidia available for infestation. Fish were transported in coolers containing river water with portable aerators, until they were placed in tanks in the lab. Twenty-four 40 L aerated tanks were used for holding the infested fish, with riffle species held in a Living Stream with a chiller unit until tested.

Municipal water was used in laboratory tanks, but it was allowed to age with aeration for at least 24 hr to remove chlorine residuals. Low concentrations of salt (20-40 g/L) were added to the water to relieve stress while the fish were transported and while acclimating to laboratory conditions. All saline water was changed before infestations began. For minimization of stress on fish, the tanks also were covered with black plastic and provided with halved pieces of PVC pipe as cover for benthic fish. Tank filters were not used to prevent the loss of juvenile mussels. Instead, partial water exchanges were carried out approximately every other day. Fish held for an extended period of time were fed blood worms, mealworms or feeder fish several times per week.

Infestations

Infestations were carried out in the laboratories of Cheatham Hall, Virginia Polytechnic Institute and State University. Laboratory infestation techniques of Zale and Neves (1982) were followed, with the following modifications: gravid female mussels were not sacrificed, tricaine methanosulfate (MS-222) was not used, and larger fish were exposed to glochidia in buckets

rather than through pipetting to reduce fish handling. Glochidia were removed from gravid females by puncturing the gill marsupium with an 18 gauge hypodermic needle, injecting water into the marsupium, and collecting expelled glochidia into a petri dish. Rupturing of the marsupium occurs naturally in many species with the ejection of the glochidia, and this rupture subsequently heals (Richard *et al.* 1991). Glochidia were tested for viability by placing a subsample in a petri dish with a few grains of table salt, and observing the glochidia snap shut through a dissecting microscope. Glochidia that responded quickly were considered fully developed. If the glochidia responded slowly, or a small percentage responded, they were considered immature. Glochidia that did not respond were considered non-viable (Zale and Neves 1982). Immature glochidia were used only if their availability was limited. Due to the observation that the glochidia response time is slowed by low temperatures, they were brought to room temperature before use.

Test fish were placed in a 20 L bucket with just enough water to cover them. Several airstones were placed in the bucket to keep the glochidia in suspension and to oxygenate the water. Approximately one quarter of a gill full of glochidia (estimated at several thousand) was used in each bucket, depending on the size of the mussel, glochidia availability, and the sizes and numbers of fish. Glochidia were washed into the bucket and kept in contact with the fish for approximately 30 min, depending on the numbers of glochidia and fish used. Periodically during infestation, fishes gills were checked for attached glochidia with the use of a hand lens and dissection light. The infestation was terminated if approximately several dozen glochidia were observed attached, with numbers proportionate to the fishes size. The fish were then returned to their 40 L tanks. Water temperatures were monitored to ensure that they did not exceed 25^{o} C.

Every other day until all the juveniles/ glochidia were collected (either transformed or sloughed off), the bottoms of the tanks were siphoned and filtered through a 120 μm nylon mesh (Nitex®). The contents were placed in a petri dish and examined using a dissecting microscope. Recorded were: date and day post-infestation, water temperature, and number of metamorphosed juveniles present. Juveniles were considered living if internal anatomy and/ or movement was observed (Hudson and Strathmore 1990). Fish were considered a suitable host if attachment, encystment, transformation, and excystment of the glochidia occurred on that species (Neves *et al.* 1985). Host suitability was considered probable in cases where fish died at a post-encystment stage, and glochidia were still attached. Fish were considered not suitable if the fish died at a pre-excystment stage, but no glochidia were found attached. Gills and fins of deceased fish were preserved in alcohol and later examined with a dissecting microscope for possible remaining encysted glochidia.

Results

Host fish identifications could not be attempted for a number of the target mussel species due to the inability to locate mature gravid females or due to abortions. Two species of mussels, *Lasmigona holstonia* and *Ligumia recta,* were successfully tested (Table 5). Some fish host species proved more suitable for glochidial transformation than others. Three non-native fish species were tested, *Cichlasoma nigrofasciatum* (convict cichlid), *Xiphophorus maculatus*

Table 5. Host fishes, number of transformed juveniles, and transformation periods for two mussel species.*

Host fish (No.)	Mussel Species				
	L. holstonia			***L. recta***	
	No. of juveniles	Days to transform	No. encysted (no. days post-infestation)	No. of juveniles	Days to transform
Micropterus salmoides (12)				198	(11-30)
Lepomis cyanellus (1)				4	(19-29)
Ambloplites rupestris (3)	1	14		4	23
Lepomis auritus (2)				2	11
Morone americana (1)				1	27
Perca flavescens (1)				18	(10-21)
Chichlasoma nigrofasciatum (1)				58	(15-26)
Xiphophorus maculatus (20)				21	24
Luxilus chrysocephalus (2)			75 (10)		
Cottus carolinae (1)	19	(12-14)	28 (15)		
Campostoma anomalum (5)			24 (10)		
Luxilus coccogenis (1)			49 (10)		

* Numbers encysted are from fish post-mortem examination observations.

(platy), and tilapia *spp.*(hybrid), for possible use in future captive-propagation efforts. Although live juveniles were not collected from some fish, they had encysted glochidia on their gills after the typical rejection (sloughing-off) period of a few days (Zale and Neves 1982). The complete infestation results, including species that were determined to be non-hosts, are shown in Table 6. Successful transformations for *L. holstonia* were achieved on rock bass (*Ambloplites rupestris*) and banded sculpin (*Cottus carolinae*) in 12 to 14 days. The striped shiner(*Luxilus crysocephalus*), central stoneroller (*Campostoma anomalum*), and warpaint shiner (*Luxilus coccogenis*) all died at 10 days post-infestation with numerous glochidia encysted, and are considered possible hosts (Table 5). Host fish identified for *L. recta* are largemouth bass *(Micropterus salmoides),* green sunfish *(Lepomis cyanellus),* rock bass, redbreast sunfish *(Lepomis auritus),* white perch *(Morone americana),* yellow perch *(Perca flavescens),* convict cichlid, and platy. The transformation period required from 10 to 30 days (Table 5), at an average water temperature of 21.5 ^{o}C.

Infestations attempted for the spectaclecase (*Cumberlandia monodonta)* and slabside pearlymussel (*Lexingtonia dolabelloides)* were unsuccessful (Table 6). The glochidia of these two species were not fully developed; therefore, their viability and the test results are unreliable. No encystment or transformation was observed for any fish species tested with *L. dolabelloides.* Two fish species, rock bass and bluegill (*Lepomis macrochirus),* were determined to be possible hosts for *C. monodonta,* with numerous encysted glochidia observed at 11 days post-infestation when the fish died.

Table 6. Results of infestations of various fish species with glochidia of 4 mussel species..

	Mussel Species			
Fish Species	*L. holstonia*	*L. recta*	*C. monodonta**	*L. dolabelloides**
Micropterus dolomieu	NSH	NSH	NSH	
Lepomis macrochirus		NSH	Host(?)	
Cottus carolinae	Host	NSH	NSH(?)	NSH
Tilapia *spp.*		NSH		
Ictalurus punctatus		NSH		
Etheostoma flabellare		NSH		
Etheostoma rufilineatum		NSH		NSH
Campostoma anomalum	Host(?)	NSH	NSH	
Notropis leuciodus	NSH(?)	NSH	NSH(?)	
Luxilus chrysocephalus	Host(?)	NSH(?)	NSH(?)	
Etheostoma blennioides		NSH(?)		NSH
Etheostoma vulneratum		NSH		NSH
Etheostoma simoterum		NSH(?)		
Nocomis micropogon		NSH		
Noturus insignis		NSH	NSH	
Hypentelium nigricans	NSH(?)	NSH	NSH(?)	NSH
Micropterus salmoides		Host		
Lepomis cyanellus	NSH	Host		
Ambloplites rupestris	Host	Host	Host(?)	
Lepomis auritus		Host	NSH(?)	
Morone americana		Host		
Perca flavescens		Host		
Cichlasoma nigrofasciatum		Host		
Xiphophorus maculatus		Host		
Luxilus coccogenis	Host(?)		NSH(?)	
Etheostoma camurum				NSH
Etheostoma zonale				NSH
Lepomis microlophus				NSH

NSH= Not Suitable Host; NSH(?)= Not Suitable, although fish died before mussel transformation;
Host= Successful host; Host(?)= Host, although died before transformation
**C. monodonta* and *L. dolabelloides* glochidia were somewhat immature, and may not have transformed for that reason.

Discussion:

In searching for gravid female mussels of target species, there was some difficulty in finding and in preventing abortion of glochidia. Based on the record of gravidity observations in Table 3, almost all targeted mussel species were observed to be gravid during my study, but many broods were immature and aborted prematurely. *C. monodonta* was particularly sensitive to disturbance, and could hardly be moved without aborting. Other researchers have had similar experiences with *C. monodonta*, and its host fish species remain unknown (Mark Hove, Univ. of Minnesota, pers. comm., 1997). Attempts to allow glochidia to mature by holding gravid adults for a few weeks in the laboratory were unsuccessful; abortions occurred before the glochidia became mature. Attempts were made to use aborted glochidia, but they were typically not viable. Care was taken during transport to minimize temperature or oxygen fluctuations, but it is difficult to precisely simulate the stream environment and not disturb sensitive species. Kitchel (1985) induced abortions in *Fusconaia cor* in the laboratory for host fish work, and found that abortions would occur with or without temperature or flow manipulations. Increased sensitivity to lowered oxygen levels and elevated temperatures has been noted in gravid female mussels (Matteson 1955).

A number of the suggested fish host species in Table 4 were not tested in artificial infestations, or tested in small numbers. There are several reasons for this. One is the difficulty in obtaining large numbers of viable glochidia. A compromise was reached between numbers of fish species and numbers of fish used. Some fish species were not used because they were not available, or not available in suitable sizes or at suitable times. Also, some fish species were not tested because they were not reported to occur in the same location as the mussel species being tested, despite having proven a host for a congeneric mussel. For example, the sauger

(*Stizostedion canadense)* was not available and was not tested as a host fish for *L. recta.* It has since been identified as a host by Khym and Layzer (1998). Nonetheless, congenerics may not always be a good clue as to what host fish species may be suitable. It was recently observed by Riusech and Barnhart (1998) that host specificity may not be consistent between closely related unionid taxa.

During the fish host identification experiments, a water quality problem developed in the laboratory, and many fish died as a consequence. This problem was possibly due to heavy metals leaching out of the pipes, which has been reportedly a problem in laboratories at other universities where old pipes were present. Alternatively, changes in the quality of the municipal water may have caused the problem. When the water was placed in a Living Stream with rocks for a few days, and them placed back into the aquaria, fish exposed to it did not die. In addition, the placement of a water conditioner that removed chlorine and heavy metals in the water seemed to solve the problem. My water quality tests revealed that copper content in the aged city water was over three times the U.S.E.P.A. recommended acute toxicity level for fish and invertebrates, at 0.06 mg/L. Iron was close to the recommended chronic toxicity level, at 0.07 mg/L, and chlorine levels were close to the recommended chronic toxicity level, at 0.008 mg/L (U.S.E.P.A. 1986). The observed levels of these contaminants were below toxic levels reported for naiad mollusks (Havlik and Marking 1993), although the sensitivity of glochidia to contaminants is not known. Goudreau *et al*. (1993) found glochidia to be highly sensitive to monochloramine and unionized ammonia. After the discovery of the water quality problem, water conditioner was always added to the aquarium water before use.

Fish health may have been a factor in infestation success in the laboratory. Following handling and holding in stagnant, relatively warm, unfiltered water, many fish died from infections.

Some fish species also were prone to fighting, and, when kept together, killed each other or prevented each other from feeding. Medicines could not be used to treat fish due to potential adverse reactions of mussel glochidia or juveniles. Salt occasionally was used, but not when there was a possibility of exposure to mussels. Bacterial infections on fish have been noted to possibly encourage sloughing of encysted glochidia (Howard and Anson 1922) and may have affected host fish infestation success. Waters and O'Dee (1996) found that prior exposure to glochidia can illicit long-term serum responses (acquired immunity) in otherwise suitable hosts, but how long this reaction lasts and whether it is species-specific is unknown. It is unlikely, however, that the fishes used in my study had previously developed acquired immunity due to the low levels of infestation in natural settings (and not at all in hatcheries), and the apparent requirement of repeat infestations to acquire immunity (Zale and Neves 1982, Waters and O'Dee 1996).

Fuller (1974) noted that many fish species identified as hosts for certain mussels were related, but this does not appear to be the rule. Of the fish identified as hosts during this project, there did not seem to be a strong preference for any particular taxonomic group. For *L. holstonia,* host fish and possible host fish were in the families Centrarchidae, Cottidae, and Cyprinidae. M. Hove (Univ. of Minnesota, pers. comm., 1997) identified host fish for the congeneric *Lasmigona compressa* in the families Cyprinidae, Cottidae, Centrarchidae, and Percidae, but also had transformation failures on fish species in these families. Host fishes identified for *L.* recta were in the families Centrarchidae, Moronidae, Percidae, Cichlidae, and Poeciliidae. In contrast, host fish identifications carried out by B. Watson (V.P.I. and S.U., pers. comm., 1997) for other mussel species of the Tennessee River basin (*Epioblasma florentina walkeri*, *Villosa perpurpurea*, *Dromus dromas*, and *Lemiox rimosus*) were only in the families Cottidae and Percidae. Hove *et al*. (1998) found four ictalurids to be suitable hosts for

Cyclonaias tuberculata, yet host fish identified by O'Dee and Watters (1998) for *Megalonaias nervosa* included gar, bass, trout, perch, sunfish, and a darter, showing no clear preference for any taxonomic group. Weaver (1981) and Kitchel (1985) showed that short-term brooders living in the Tennessee River drainage tended to use cyprinids as host fishes. M. Hove (Univ. of Minnesota, pers. comm., 1997) identified bluegill and largemouth bass as hosts for *L. recta*, but had difficulty in replicating his own results. This exemplifies how possible differences among individual fish and testing conditions necessitate testing greater numbers of fish, and the need for repeating tests to confirm previous results.

Although the original intent of my study was to test fish species that occurred only in areas where the mussel species lived, sometimes this guideline was not followed. If a fish species and viable glochidia were available, sometimes that fish species was tested. Testing of allopatric fish hosts may offer clues as to what sympatric species might serve as hosts, and they may also be used in captive propagation efforts if the resident hosts are difficult to obtain, are not yet identified, or are extirpated from the mussel's habitat. It might also be the case that the putative host was historically present and important in the ecosystem. Additionally, recent work has shown some success with the transformation of glochidia on amphibians and exotic fish (Watters and O'Dee 1998). The use of these surrogate hosts may prove valuable for the production of juveniles for culture. The use of cortisol, an immunosuppressant, has been used to induce glochidial transformation on non-host fish, with some success (Kirk and Layzer 1997), although this success may depend on the species of fish used (Khym *et al.* 1998). There has also been some success in increasing transformation percentages by decreasing the exposure temperature of the host fish to 10 ^{o}C, presumably by suppressing the immune function of the fish (Barnhart and Roberts 1998).

There was a large range in transformation periods observed during my study, for example from 10 to 30 days for *L. recta*. There are several factors that may have influenced this. Duration of the parasitic period long has been noted to correlate inversely with temperature (Schierholz 1888), although this effect often may be modified by other factors such as maturity of glochidia upon release, strength of immune response, and ability of fish to handle stress and disease (Lefevre and Curtis 1912; Waters and O'Dee 1996). The period of glochidial metamorphosis may possibly be influenced by fish health (Coker *et al*. 1921). Fish species closely related to host species often have a longer period of sloughing than non-related species (Zale and Neves 1982).

Due to the constraints of laboratory space, it was not possible to keep individual fish in its own tank. Therefore, several fish of one species infested with glochidia of a mussel species were kept in aquaria together. When there were successful glochidial transformations, it was not known whether all the juveniles came off of one fish or several. All potential host fish received the same infestation treatment, and therefore all originally had approximately the same number of glochidia attached, although to reduce handling only one or two individuals per fish species was checked for glochidia. Over the following weeks, some fish may have retained more glochidia than others. It was impractical to count the number of glochidia attached to each individual fish, due to the stress of examination, and the time and number of aquaria required.

There has been some success in the development of *in vitro* methods of glochidial culture. Although this information may not help in the protection of mussel populations dependent on the natural availability of host fish, it could help in the artificial production of juveniles to be used in reintroduction and population augmenting efforts, where the identity of the host fish is unknown. Experiments with *in vitro* culture of glochidia has increased knowledge of the requirements of glochidia for transformation. It is now known that animal serum is essential to glochidial

development and that rejection is based solely on immunological response (Ellis and Ellis 1926, Meyers *et al.* 1980, Hudson and Isom 1984). Although nearly 80% *in vitro* glochidial transformation was obtained by Isom and Hudson (1982), later attempts at rearing juveniles transformed *in vitro* proved fairly unsuccessful, with only one out of several species living beyond a couple weeks (Hudson and Isom 1984). Hudson and Shelbourne (1990) were able to obtain up to 100% glochidial transformation using fish or rabbit plasma plus amino acids, vitamins, antibiotics, and glucose. *Lampsilis siliquoidea* glochidia were recently transformed in vitro with 81% success using Eagle's minimal essential medium, rabbit serum, antibiotics, and serum replacements (Myers-Kinzie and Spacie 1998). It has also been discovered that glochidial transformation in vitro is most successful at temperatures above 15 ^{o}C, at low pH (7.6) and high CO_2 levels (5%), which is not wholly consistent with conditions in the host fish (Barnhart and Roberts 1998). Information obtained from these studies will help to gain a greater understanding of the requirements of glochidia, and perhaps improve future host fish and culture work.

Hove *et al.* (1998) have made some suggestions for the improvement of host suitability test success. These include: ensuring that fish used are in good health before and throughout the tests, using flow-through aquaria, keeping tanks free of excess food, holding small host fishes in suspended nets, recording the presence of pre-metamorphosed juveniles, washing the siphonate from the tanks as well as the sieves, and increasing the number of hosts tested. I believe that these are all valid recommendations and may improve host fish and juvenile mussel survival. In particular, when testing stream fishes, survival of the fishes may be significantly greater if cool, flowing water is available, and cleaner aquarium conditions with circulating flow may improve survival of newly transformed juveniles as well. The host fish in my study suffered from aquarium conditions that were inappropriate for them. Hove *et al.* (1997) found that certain fish

(*Cyprinella sp*.) produced more juvenile mussels when they were suspended in nets, presumably due to the reduction of host fish predation on the juveniles. Although predation on juveniles was not noted to occur in my study, it is quite possible, and it would be advisable that precautions are taken in future host fish studies to prevent unnecessary loss of juveniles.

Given that the fish fauna in the Tennessee River drainage is the richest in the United States (Etnier and Stairnes 1993), much more work needs to be done in identifying potential hosts of this diverse mussel fauna. Although host fish were identified for *Lasmigona holstonia* and *Ligumia recta* during my study, laboratory identifications may not demonstrate the most important fish species parasitized under natural conditions. Information gained in laboratory, observations in the field, and genetic analysis can be combined to make definitive host identifications. The fish tested during my study were only a small portion of what may be available to these mussel species; therefore, testing of more species is needed to fully describe the suite of host species available to these mussels.

CHAPTER TWO: MUSSEL CULTURE EXPERIMENTS

Introduction

Attempts at freshwater mussel culture date back to the early 1900s, although the environmental requirements of juveniles were not understood and success was poor. Lefevre and Curtis (1908) were unable to keep freshwater mussels of various species alive in the laboratory for longer than four weeks, and Howard (1922) obtained survivorship of less than eight percent over one month. The glochidial life stage makes artificial propagation difficult. Not until much later (with the successful culture of other bivalves) was it realized that clean tanks and water, appropriate temperatures, and quality foods are essential (Castagna and Kraeuter 1981; Buddensiek 1995; Gatenby *et al.* 1996). It also has been observed that proper nutrition in the first month after metamorphosis is vital, and that this food may not be the same as that consumed by mature individuals or cultured marine mollusks (Sprung 1984, Gatenby *et al.* 1996). Temperature in the holding tanks must be regulated, as it affects growth and survival, and for some species seems best kept at around 20^{o} C, with negative effects at 30 ^{o} C and above (Foe and Knight 1986). Although it has been shown that eutrophication has a negative influence on juvenile mussel growth and survival, the details of the effects of associated water chemistry variables have not been isolated (Buddensiek 1995). The tolerances of adult freshwater mussels to certain pollutants have been recorded, but these standards may be different for juveniles (Havlik and Marking 1987). Research into the response of juveniles to the availability of food and oxygen is, however, ongoing (Dimock 1998, Gatenby *et al*. 1998).

Algal cultures are more successful than artificial diets in maintaining bivalve growth, with live algae producing greater survival than dried algae (McMahon 1991; Gatenby *et al*. 1996;

Dimock *et al.* 1998). Mussels require a constant supply of filterable food to grow and remain healthy. Assimilation efficiencies for *Corbicula fluminea* have been documented to exceed 70% (Lauritsen 1986). Suggested algal species for freshwater mussel culture include *Ankistrodesmus* sp., *Chlamydomonas* sp., and *Scenedesmus* sp.(Foe and Knight 1986, Lauritsen 1986). Algal species with oils rich in polyunsaturated fatty acids have been shown to encourage growth (Gatenby *et al.* 1996). Suggested algal concentrations for adequate nutrition are roughly 11,000-15,000 cells/ml (Paterson 1984). It has been observed that some bivalves ingest bacteria, which may increase assimilations of nutrients from detritus, although this is yet to be proven for freshwater unionids (Crosby *et al.* 1990). The addition of silt has been shown to improve juvenile growth rates, which may be due to their pedal feeding behavior when very young (Reid *et al.* 1992, Yeager *et al*. 1994, Gatenby *et al.* 1996). In addition, the presence of silt may be important in helping mussels maintain proper orientation for feeding efficiency, and stabilization from physical disturbance (Neves *et al.* 1998). Coker *et al.* (1921) examined stomach and fecal contents of mussels and concluded that they were ingesting large quantities of organic and inorganic detrital matter, and that the organic matter was undergoing digestion. Silt, along with bacteria and algae, is ingested by young pedal-feeding juveniles, although the components in the silt which are most beneficial is not known (Hudson and Isom 1984; Yeager *et al*. 1994).

The objectives of this study were to assess the effect of culture system design and maintenance on juvenile mussel growth and survival, and to assess the effects of different water hardness levels and sediment organic content levels on juvenile mussel growth and survival. The mussel species used for these culture experiments was the common freshwater mussel *Lampsilis fasciola,* the wavy-rayed lampmussel. This species has been noted to occur commonly in southwest Virginia (R.J. Neves, pers. comm.), and it has successfully transformed on *Micropterus*

dolomieu (smallmouth bass) in previous host fish experiments (Zale and Neves 1982). Attempts were made to transform *L. fasciola* on rock bass *(Ambloplites rupestris)*, largemouth bass (*Micropterus salmoides)*, smallmouth bass, bluegill (*Lepomis macrochirus)*, redbreast sunfish (*Lepomis auritus)*, and banded sculpin (*Cottus carolinae)*, but it was found that greatest transformation success occurred on largemouth bass and smallmouth bass (Appendix A).

The general setup for the culture of bivalves can be quite simple and inexpensive, although attention to detail, and clean, ideal conditions with the availability of ample food (algae) must be maintained (Castagna and Kraeuter 1981). Although not a substitute for a vigorously self-propagating mussel population, captive production is a useful conservation tool to help re-establish those species with declining recruitment (Neves 1997). The goal of this study was to increase the knowledge base necessary for the successful and efficient propagation of threatened and endangered freshwater mussels.

Materials and Methods

Experiment One: Comparison of Culture Systems

Juvenile Mussel Production

Gravid *L. fasciola* were obtained from Indian Creek, Tazewell County, Virginia, and glochidia were transformed on largemouth bass and smallmouth bass collected from Smith Mountain Lake, Virginia, or purchased from fish hatcheries. Briefly, infestation methods consisted of the following: expulsion of glochidia from gravid mussels with a hypodermic needle, placement of several thousand glochidia in buckets with host fish for approximately 20 min, and checking the gills periodically for attached glochidia. The adult mussel was returned to its collection site. The infested fish were kept in 40 L aquaria, and the bottoms of the aquaria were siphoned and filtered every other day until all transformed juveniles were collected. After collection from the infestation tanks, the juvenile mussels were counted and incubated at 15 ^{o}C. They were kept in plastic dishes and provided with aeration and sediment until enough mussels were collected to begin the experiment. Juveniles were collected over a protracted period of time. At the start of the experiment, on November 1, 1996, their ages ranged from 1 to 19 days old, with an average age of 12 days. When placed in the culture systems, approximately 33% of the juveniles were older than 10 days, 43% were between 10 and 5 days old, and 24% were younger than 5 days. Mussel ages were averaged, juveniles were grouped together, and then randomly placed in the culture systems. The relatively wide range of ages of the juveniles may have had an impact on the results of the culture experiment, but it is not quantifiable. Therefore, it is assumed that differences in individuals were accommodated by grouping them together before placement into the culture systems. Juveniles were gradually adjusted to the ambient water temperature in

the culture systems before placement in them. The chemical content of the municipal water used is shown in Appendix B, and the water was aged to remove chlorine before use.

Culture System Design

The culture systems used for this experiment were a 1200 L dish system and the 790 L bed system. Both were located in the greenhouse of the Virginia Tech Aquaculture Center, and both were recirculating systems. In the bed system, the mussels were spread out in the open beds, the algae culture in the system was meant to be self-sustaining (see below for feeding regime), and the system was only cleaned once per month using a partial water change (Figure 1). In the dish system, the mussels were subdivided into small dishes, fed algae two to three times per week, and the system was cleaned weekly (complete water change)(Figure 2). Two thousand juvenile mussels were placed in each system, 250 in each of eight culture beds and 50 in each of 40 culture dishes. There were six bed sections in each half of the system, but only the middle four in each half were used.

Flow rates were 5 ml/min per cm^2 cross-sectional area of each bed section, a rate cited as appropriate for culturing other bivalves (Castagna and Kraeuter 1981). Flow was adjusted by in-line flow valves, and was dispersed evenly over the system through a dispersal channel and a series of holes and weirs in the bed wall (see Fig. 1). The water reservoirs for each system were made of fiberglass, and the culture beds were made of PVC sheeting. Water pumps were submersed in the water reservoir in the bed culture system, and in the dish system they were external in-line pumps. Flow in the dish culture system was about 5 ml/min per cm^2 cross-sectional area of each trough. Flow was controlled with in-line flow meters just prior to the inlet

on each trough (see Fig. 2). The flow was dispersed with a horizontal piece of PVC pipe containing a series of holes. The polypropylene culturing dishes in the dish-culture system had dimensions of 7.6 by 7.6 cm by 5.1 cm, and glass slides were glued to their bottoms with non-toxic silicone glue to weight them. Before use, the culture dishes were soaked overnight in a 0.5 molar solution of HCl to remove any possible chemical residue. They were then rinsed by soaking for several days in water which was changed several times per day.

Stocking densities for both culture systems were 1-1.5 juvenile mussels per cm^2, which has been appropriate in previous experiments (B. Beaty, Virginia Polytechnic Institute and State University, pers. comm., 1996). Two thousand juveniles were placed in each system, and the experiment ran for four months, from November 1996 through February 1997. Culturing mussels in the controlled conditions of the greenhouse, which was equipped with a heater, however, moderates the effect of season. Once per week, the dish culture system was cleaned and given a complete water change. At that time, ammonia, pH, dissolved oxygen and water hardness levels of both systems were measured. Once per month, the bed culture system was given a partial water change. Temperature fluctuations were recorded using Optic Stowaway Temp Loggers (Onset Computer Corporation, Procasset, MA) for the duration of the experiments. In order to better control water temperature, 50% shade cloth was hung over the culturing systems. Styrofoam sheeting was placed over the culture systems and reservoirs to block direct sunlight, decrease evaporation, and discourage excessive (filamentous) algal growth.

Sediment Preparation

The presence of sediment has been observed to enhance juvenile growth rates and

survival, as they pedal-feed during the first few months of life (Hudson and Isom 1984; Yeager *et al.* 1994; Gatenby *et al.* 1996). Therefore, the culture dishes for this experiment contained a layer of fine silt, 1-2 cm deep. It has been observed that young juvenile *Villosa iris* do not burrow further than 1 cm into the substratum (B. Beaty, Virginia Polytechnic Institute and State University, pers. comm., 1996). Additionally, this depth of sediment was used so that mussels could be removed by rinsing off the top layer of sediment, and then returned to the same culture dish. Natural river silt was obtained from the mouth of Tom's Creek, New River, Montgomery County, Virginia. For ease in mussel retrieval and to kill any predatory invertebrates, the sediment was filtered through a 150 μm sieve and sterilized by autoclaving for 25 min at 120 °C before placement in the culture systems. The chemical composition of the sediment, analyzed at the Soil Testing Laboratory at Virginia Polytechnic Institute and State University, was as follows: pH of 7.4, 3.4% organic matter, 3 mg/L P, 33 mg/L K, 1200 mg/L Ca, 120 mg/L Mg, 6.1 mg/L Zn, and 16.1 mg/L Mn.

Algae Culture and Feeding Regime

The algal species fed to the juveniles was *Neochloris oleoabundans*. This species was chosen due to its availability, suitable cell size (approximately 5 microns), and high content of polyunsaturated fatty acids, which may benefit mussel growth (Gatenby *et al*. 1996). Patterson *et al.* (1998) also recently showed on tests with *Villosa iris* that *Neochloris oleoabundans* has a high nutritional value for mussels, with 57% assimilation of ingested carbon. My algae was cultured in 230 L Kalwall tubes at the Virginia Tech Aquaculture Center. The water used for the algal culture was conditioned municipal water, sterilized with a UV filter, and aerated. A chemical analysis of the municipal water is presented in Appendix B. On cloudy days, plant lights were

used on the cultures. The fertilizer used was Fritz F/2 media (Appendix C). Attempts were made to use algae that were at the peak of optically judged maximum density and with the fewest dead cells, although that was not always possible.

The bed culture system was intended to be a self-sustaining food source (algae and bacteria culture). The bed culture system was inoculated with algae (2×10^5 cells per cm^2 substrate), a commercial bacterial inoculate (1 ml Aqua-Bacter Aid, Argent Chemical Laboratories, Redmond, WA- for contents see Appendix D), and Fritz F/2 fertilizer (0.25 ml per liter water) approximately 1 wk before the introduction of the juvenile mussels. This was to allow colonization of the substrate before the mussels were added (C. L. Yang, Virginia Polytechnic Institute and State University, pers. comm., 1996). The dish culture system was not designed to provide a self-sustaining food source. The mussels in the dish system were fed approximately 2×10^4 algal cells per ml of water in the culture system 2-3 times per week, depending on the availability, density, and health of the algae culture. The algal density was determined using a hemacytometer. Given the flow rates in each culture system, the mussels thus received 1×10^5 algal cells per cm^2 cross-sectional area per minute immediately after feeding. Approximately one month after initiation of the culture experiment, the algal contents of the sediment in the bed culture system were examined. Due to the failure of *Neochloris sp*. to colonize the substrate after the first month of culture, it was determined that the bed system should receive the same frequency and concentrations of algae feedings as the dish system from that point onward.

Growth and Survival Measurement and Analysis

Periodically, random subsamples of mussels from each system were collected and measured, using a microscope ocular micrometer, and survival was assessed. Individual culture bed units were sampled on a monthly basis, as it was found that subsampling was too difficult. Three different culture dishes were sampled every 2 wk. From each sample, surviving individuals were counted, and an arbitrary sampling of 10 mussels per dish, 30 per bed (or however many were still alive in the sample) were measured in height and length using an ocular micrometer (Figure 3). At the end of 4 mo, all eight bed sections were sampled and twelve dishes were sampled.

A General Linear Models Procedure Multivariate Analysis of Variance test compared growth data using the computer statistical program SAS. A Logistic Regression Procedure compared the survival data, also using the SAS program; this test was used because survival, as a function of age, compares a proportion with a continuous variable, and the maximum likelihood method fits with the binomial distribution of the dependent variable (Sokol and Rohlf 1995).

Initial conditions in the bed culture system were as follows: temperature 26 ^{o}C, dissolved oxygen 6.7 mg/L, pH 8.15, un-ionized ammonia 0.04 mg/L, and hardness 99 mg/L $CaCO_3$. Initial conditions in the dish culture system were: temperature 24 ^{o}C, dissolved oxygen 7.4 mg/L, pH 8.42, un-ionized ammonia 0.008 mg/L, and hardness 138 mg/L $CaCO_3$.

Materials and Methods

Experiment Two: Sediment Type and Water Hardness Effect

Juvenile Mussel Production

As in the first culture experiment, *Lampsilis fasciola* juveniles were produced by laboratory infestations of glochidia on largemouth bass and smallmouth bass. Again, glochidia were excised from adult mussels with a syringe, fish were infested in buckets, and infested fish were kept in 40 L aquaria until all juveniles were collected by siphoning the bottom of the tanks. Juveniles were kept at 20 ^{o}C until enough were collected to begin the experiment, and they were provided with silt, algae, and aeration. The juveniles were placed in the culture systems on August 1, 1997, at which point their average age was 2 days. A total of 4,800 juvenile mussels were used in this experiment.

Sediment Substrate Preparation

Sediment was prepared in the same manner as in the first culture experiment; however, sediment with two different percentages of organic matter was used. The sediment was obtained from the banks of the New River at the Virginia Tech Kentland Experimental Farm, Montgomery County, VA. Sediment with a high organic content was obtained from newly deposited sediment on the banks of the river (A-horizon soil), and low organic content soil was obtained from digging 0.6 m down to the B-horizon at the same site. Before placement in the culture systems, the chemical content of the sediment was analyzed by the Soil Testing Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA. The A-horizon sediment had 4.1 % organic matter, pH of 7.6, 19 mg/L P, 45 mg/L K, 1200 mg/L Ca, 120 mg/L Mg, 6.1 mg/L Zn, and 16.1 mg/L Mn. The B-horizon soil had 2.5 % organic matter, pH of 8.0, 5 mg/L P, 26 mg/L

K, 1200 mg/L Ca, 120 mg/L Mg, 6.1 mg/L Zn, and 16.1 mg/L Mn. Originally the A-horizon soil had an organic content of 6.2%, but it deteriorated before the second attempt at this experiment could begin.

Algae Culture and Feeding

Algae were cultured in the same manner as in the first culture experiment. The average amount of algae added to each system at each, nearly daily, feeding was 16,600 cells algae added per 1 ml culture system water. Due to differences in the health of the algal culture available, however, the amount of algae added ranged from 3,800 cells to 29,400 cells per ml culture system water (Appendix Q). The predominant algal species fed to the mussels was *Neochloris oleoabundans*, although the culture was occasionally contaminated with *Scenedesmus sp.* and *Microspora sp.* Whenever this occurred, attempts were made to sterilize the culture systems containing the invading species. On occasion it was necessary to restart the *Neochloris* culture from a fresh inoculate. The culture systems were cleaned and given a complete water change every week. At that time pH, dissolved oxygen, and water hardness were measured. The temperature in the systems was monitored using Optic Stowaway Temp loggers (Onset Computer Corp., Procasset, MA).

Culture System Design

The culture system used was similar to the dish system used in the first culture experiment (Figure 2). The only modifications were that four independent replicates of the system were used, the dishes were shallower (3 cm deep), and the glass plates used to weight the dishes in the first experiment were removed. There were 24 dishes in each of the four systems, 12 with high

organic content sediment and 12 with low organic content sediment. Two of the culture systems had high water hardness (250 mg/L $CaCO_3$) and two had low water hardness (40 mg/L $CaCO_3$). Fifty juveniles were placed in each dish, for a total of 4,800 juveniles. The juveniles were mixed together into one group and then randomly assigned to dishes.

Growth and Survival Measurement and Analysis

Each of the four culture systems was subsampled every 3 wk for 15 wk. Two dishes were sampled from each substrate type per culture system at that time, for a total of 16 dishes. On the last sampling date (wk 15), 32 dishes were sampled. To record growth (height and length), 60 mussels were initially measured, and thereafter usually 15 mussels from each dish were measured. Height and length measurements were taken (Figure 3). Percent survival was calculated for each dish by counting the number of live mussels in each subsample at each sampling period. No dish was sampled twice, although juveniles were placed back in their dishes after counting and measuring.

To test for significance, Tukey-Kramer multiple comparisons tests were run on the height, length and survival data at the end of the experiment, using the SAS computer statistical analysis package. Because survival data were not normally distributed, it was again transformed using: arcsin (survival + 0.5) (Hinkelmann and Kempthorne 1994). The adjusted-P Tukey- Kramer multiple comparisons test also adjusts for unequal sample sizes (Sokol and Rohlf 1995).

Results

Experiment One: Comparison of Culture Systems

At the end of the culture period, survival in the bed system was 3.1%, and in the dish system it was 31.3 % (Figure 4). Survival in dish culture system was significantly greater for all weeks ($P < 0.01$). Means, standard deviations, ranges, and sample sizes are shown in Table 7. For each sample period, sample sizes were generally 1 to 2 bed sections and three culture dishes, although on the last sample date 8 bed sections and 12 dishes were sampled. Given the low survivorship and small sample sizes for the beds, standard deviations are larger for them than for the dishes. Note that there are no samples for weeks 6, 10 and 14 for the bed culture system, due to the difficulty of subsampling the culture beds.

At the end of the 16-wk culture period, mean mussel height in the bed system was 1347 (+/- 505) microns, and 861 (+/- 188) microns in the dish system (Figure 5). Mean mussel length in the bed system was 1765 (+/- 762) microns, and 1110 (+/- 271) microns in the dish system (Figure 6). Mussels in the bed system were significantly ($P< 0.01$) larger in both height and length, as analyzed over the entire culture period. Sample sizes consist of the number of mussels measured on each sample date, and generally 10 mussels per dish and 30 mussels per bed section were measured, or how many mussels remained alive (Table 8). Standard deviations were generally larger for the bed system than in the dish system, most likely due to smaller sample sizes as a result of high mortality. Again, there are no samples for weeks 6, 10 and 14 for the bed culture system.

When the contents of the sediment in the bed system were analyzed after 1 mo, few if any *Neochloris sp*. cells were present, and the sediment was dominated by a benthic diatom (*Navicula*

Table 7. Decline of mean percent survival (+/- 1 SD) for juvenile mussels in the dish and bed culture systems.

Week	Mean (+/- 1 SD)	Range	N*
	Bed System		
1	8.0 (-)	-	1
4	9.6 (-)	-	1
8	5.0 (4.2)	(2.0-8.0)	2
12	4.0 (0.57)	(3.6-4.4)	2
16	3.1 (2.8)	(0.4-9.2)	8
	Dish System		
1	90.0 (-)	-	1
4	49.3 (11.0)	(38-60)	3
6	46.0 (18.3)	(26-62)	3
8	42.7 (7.0)	(36-50)	3
10	47.3 (11.0)	(36-58)	3
12	28.0 (19.3)	(6.0-42)	3
14	36.0 (20.9)	(12-50)	3
16	31.3 (15.4)	(2.0-56)	12

* N= number of units (bed section or dish) sampled.

Table 8. Decline in mean percent growth (+/- 1 SD) for juvenile mussels in the dish and bed culture systems. At week 0, mean height was 300.85 (17.73) microns with a range of 256.4 to 333.3 (N=30); mean length was 361.11 (32.85) with a range of 333.3 to 433.3.

Week	Height (μm) (+/- 1 SD)	Length (μm) (+/- 1 SD)	Range	N*
		Bed System		
1	351.28 (41.96)		(307.7-410.3)	10
4	420.41 (44.25)		(333.3-500.0)	24
8	631.79 (130.93)		(384.6-897.4)	25
12	703.84 (173.60)		(538.5-1153.8)	20
16	1346.98 (504.64)		(589.7-2615.4)	62
1		369.23 (72.72)	(256.4-461.5)	10
4		478.63 (85.35)	(359.0-641.0)	24
8		762.05 (192.52)	(384.6-1205.1)	25
12		873.08 (263.66)	(564.1-1589.7)	20
16		1764.68 (762.36)	(769.2-3897.4)	62
		Dish System		
1	362.82 (31.43)		(307.7-410.3)	20
4	440.96 (55.57)		(333.3-589.7)	38
6	488.37 (70.76)		(333.3-717.9)	43
8	626.84 (152.89)		(384.6-1025.6)	47
10	625.13 (128.87)		(384.6-897.4)	50
12	634.45 (126.13)		(384.6-897.4)	39
14	777.17 (143.32)		(461.5-1025.6)	42
16	861.26 (188.30)		(384.6-1333.3)	129
1		405.13(48.22)	(333.3-487.2)	20
4		540.15 (80.32)	(384.6-717.9)	38
6		599.28 (111.35)	(333.3-846.2)	43
8		626.84 (143.44)	(359.0-1025.6)	47
10		803.59 (193.66)	(435.9-1179.5)	50
12		807.36 (193.57)	(435.9-1179.5)	39
16		996.95 (214.02)	(487.2-1384.6)	42
16		1110.12 (270.87)	(512.8-1692.3)	129

* N= number of mussels measured.

sp.). Also present were *Scenedesmus sp.* (planktonic alga), *Cyclotella sp.* (planktonic diatom), *Melosera sp.* (diatom) and *Microspora sp.* (filamentous green alga). (Bruce Parker, Virginia Polytechnic Institute and State University, pers. comm., 1996). *Navicula sp.* appeared to be dominating the dish culture system as well. After the first month, the feeding regime of the bed system was increased to match that of the dish system. The average amount of algae added to each system at each interval was approximately 21,000 cells per ml culture system water (Appendix K). There was no significant difference in algae concentrations added to the bed and dish culture systems over the culture period ($P > 0.05$) Also observed present in both systems after 1 mo of culture were chironomid larvae, which were thought to have been derived from egg-laying adults present in the greenhouse. After consultation with an aquatic entomologist, I concluded that they would not eat the mussels, and that their disturbance of the substrate was minimal (S. Hiner, Virginia Polytechnic Institute and State University, pers. comm., 1996).

Dissolved oxygen, pH, water hardness, unionized ammonia, temperature, and algae feeding records for the duration of the culture trial were summarized. Averaging 8.6 mg/L in the dish system, dissolved oxygen was significantly greater than in the bed system, which was 7.8 mg/L ($P < 0.05$)(Appendix F). There was no significant difference in water pH for the bed system, at 8.5, and the dish system, at 8.4 ($P > 0.05$)(Appendix G). Water hardness levels also were not significantly different, averaging 244 mg/L $CaCO_3$ for the bed system and 219 mg/L $CaCO_3$ for the dish system ($P > 0.05$) (Appendix H). Unionized ammonia levels for the bed system averaged 0.029 mg/L NH_3, and 0.016 mg/L NH_3 for the dish system, and differences between the two systems were not statistically significant ($P > 0.05$) (Appendix I). Averaging 23^{o} C, temperatures in the bed system were significantly greater than in the dish system, which averaged $20\ ^{o}$ C ($P < 0.01$)(Appendix J).

Experiment Two: Sediment Type and Water Hardness Effect

After 15 wk, juveniles in the high water hardness treatment exhibited significantly greater growth (height and length) and survival than those in the low water hardness treatment ($P<0.01$, Tukey-Kramer). The difference in mussel survival between high and low water hardness treatments became larger over time, resulting in an average 9% survival in low water hardness and 44% survival in high water hardness by the end of the study (Figure 7). By the end of 15 wk, juveniles grown in high hardness water were over 350 microns greater in length (at 2128 microns) and 250 microns greater in height (at 1526 microns) than those grown in the low hardness water (Figure 8, Figure 9).

The effects of sediment organic content on mussel growth and survival were less obvious than those of water hardness. Juveniles grown in high hardness water and high organic sediment exhibited greater survival than those in high hardness water and low organic sediment, although the difference was not statistically significant. After 15 wk, mussels in high water hardness and high organic content sediment exhibited a survival rate of 47.25%. This was 6.25% greater than mussels in the high water hardness and low organic content substrate. Survival for both low water hardness treatments was around 9%, approaching that level after 6 wk (Figure 7). Table 9 details the decreasing survival percentages over time, showing standard deviations, ranges, and sample sizes. In the latter part of the study in the low water hardness treatments, when survival was low, standard deviations were comparatively large, reaching levels greater than the means.

Table 9. Decline of mean percent survival (+/- 1 SD) of juvenile mussels in the water hardness and substrate organic content culture treatments.

Week	Water Hardness	Organic Content	Mean (% survival)	Standard Deviation (+/-)	Range	N*
3	high	low	54.5	23.5	(24-74)	4
3	high	high	64.0	26.3	(30-94)	4
3	low	low	47.5	25.2	(10-62)	4
3	low	high	50.5	13.4	(32-64)	4
6	high	low	46.5	16.9	(28-68)	4
6	high	high	32.0	4.6	(28-36)	4
6	low	low	17.5	20.9	(2-48)	4
6	low	high	15.0	9.6	(4-24)	4
9	high	low	37.5	12.8	(24-52)	4
9	high	high	51.5	12.8	(38-66)	4
9	low	low	12.5	10.2	(2-24)	4
9	low	high	16.0	4.9	(12-22)	4
12	high	low	36.0	13.5	(28-56)	4
12	high	high	53.0	4.8	(46-56)	4
12	low	low	12.5	13.0	(0-30)	4
12	low	high	14.0	9.9	(4-26)	4
15	high	low	41.0	9.1	(24-52)	8
15	high	high	47.3	9.5	(32-64)	8
15	low	low	10.5	7.0	(0-22)	8
15	low	high	7.5	8.8	(0-24)	8

*N= number of replicates (culture dishes) sampled from each treatment on each sample date.

Standard deviations for the high water hardness treatments constituted a smaller percentage of the means, likely due to their higher survivorship. Sample sizes for each sample date consisted of 4 dishes per treatment, with 8 dishes sampled on the last date.

Juveniles in high organic sediment generally exhibited greater growth (height and length) than those in the low organic sediment, although the differences were not statistically significant. At 15 wk, the average mussel height and length for both substrate treatments in high water hardness were nearly identical at 1524-1528 microns and 2125-2130 microns, respectively. At 1290 microns in height and 1790 microns in length, mussels in the low water hardness and high organic content sediment were slightly larger than those in low water hardness and low organic content sediment. The differences, however, were not statistically significant (Figure 8). Tables 10 and 11 detail the means, standard deviations, ranges, and sample sizes for mussel height and length measurements. Sample sizes are numbers of mussels measured and were determined by survivorship. There is no obvious correlation of treatment with size of standard deviation or range in either case. However, in the low water hardness and low organic content sediment treatments, there were mussels who did not grow nearly at the rate of those in the high water hardness treatments. For example, the smallest measured mussel in the low hardness water and low organic content sediment grew 2.8 times less in height and 2.5 times less in length than the smallest mussel in any of the other treatments.

Dissolved oxygen, pH, water hardness, unionized ammonia, temperature, and algae feeding records for the duration of the culture trial were summarized (Appendices L-P). During the culture period, dissolved oxygen levels averaged 8.0 mg/L O_2, and were not significantly different between the culture systems. For the high water hardness systems, pH averaged 8.5, and

Table 10. Growth in shell height (+/- 1 SD) for the water hardness and substrate organic content culture treatments.

Week	Water Hardness	Organic Content	Height (+/- 1 SD)	Range	N*
0	-	-	287.61 (18.5)	(256.41-384.62)	60
3	high	low	492.54 (78.5)	(333.3-666.7)	67
3	high	high	469.48 (82.0)	(307.7-666.7)	71
3	low	low	401.97 (55.3)	(307.7-538.5)	65
3	low	high	395.23 (42.5)	(307.7-564.1)	64
6	high	low	598.59 (123.9)	(333.3-897.8)	58
6	high	high	614.94 (131.4)	(333.3-948.7)	58
6	low	low	556.56 (114.4)	(359.0-794.9)	34
6	low	high	526.50 (91.5)	(359.0-717.9)	30
9	high	low	947.92 (181.4)	(615.4-1512.8)	64
9	high	high	973.59 (167.7)	(487.2-1359.0)	67
9	low	low	870.77 (188.2)	(461.5-1205.1)	25
9	low	high	716.39 (183.4)	(461.5-1282.1)	33
12	high	low	1291.78 (331.9)	(641.0-2820.5)	58
12	high	high	1538.90 (266.9)	(1153.8-2948.7)	60
12	low	low	1235.90 (310.4)	(641.0-1897.4)	25
12	low	high	1135.53 (279.3)	(666.7-1897.4)	28
15	high	low	1523.56 (290.0)	(820.5-2282.1)	117
15	high	high	1528.08 (262.2)	(923.1-2282.1)	121
15	low	low	1260.07 (269.4)	(512.8-1769.2)	42
15	low	high	1293.27 (225.0)	(923.1-1820.5)	32

*N= number of mussels measured.

Table 11. Growth in shell length (+/- 1 SD) for the water hardness and substrate organic content culture treatments.

Week	Water Hardness	Organic Content	Length (+/- 1 SD)	Range	N*
0	-	-	259.83 (19.9)	(230.77-307.69)	60
3	high	low	617.30 (121.9)	(359.0-820.5)	67
3	high	high	589.38 (131.5)	(307.7-846.2)	71
3	low	low	493.89 (99.4)	(307.7-717.9)	65
3	low	high	474.76 (82.1)	(359.0-743.6)	64
6	high	low	784.70 (182.3)	(384.6-1179.5)	58
6	high	high	783.82 (214.4)	(333.3-1410.3)	58
6	low	low	745.10 (168.1)	(410.3-1076.9)	34
6	low	high	699.15 (122.3)	(435.9-948.7)	30
9	high	low	1261.62 (273.8)	(769.2-2000.0)	64
9	high	high	1301.57 (242.8)	(589.7-1769.2)	67
9	low	low	1240.00 (276.4)	(615.4-1666.7)	25
9	low	high	934.73 (281.3)	(538.5-1794.9)	33
12	high	low	1778.07 (498.6)	(794.9-4230.8)	58
12	high	high	2156.41 (431.2)	(1435.9-4512.9)	60
12	low	low	1752.82 (479.8)	(871.8-2820.5)	25
12	low	high	1591.58 (416.8)	(846.2-2666.7)	28
15	high	low	2130.07 (438.1)	(1025.6-3256.4)	117
15	high	high	2125.03 (389.0)	(1205.1-3153.8)	121
15	low	low	1758.20 (394.3)	(564.1-2435.9)	42
15	low	high	1790.87 (347.0)	(1179.5-2692.3)	32

*N= number of mussels measured.

for the low water hardness systems, it averaged 8.1; these were significantly different ($P < 0.01$) (Appendix M). The high water hardness systems had a hardness averaging 254 mg/L $CaCO_3$, and

the low water hardness systems averaged 49 mg/L $CaCO_3$, also a significant difference ($P < 0.01$) (Appendix N). Averaging 0.017 mg/L NH_3, the un-ionized ammonia levels in the high water hardness systems were significantly greater than the 0.0036 mg/L NH_3 average in the low water hardness systems ($P < 0.01$)(Appendix O). Temperatures ranged from 17.2 to 26.5 ^{o}C, with an average of 23.0 ^{o}C, and were not significantly different between the culture systems (Appendix P).

Discussion

Experiment One: Comparison of Culture Systems

Given the ten-fold greater survival of juveniles in the dish versus bed culture system, the dish system provides better conditions for the purposes of juvenile propagation. Although there was greater growth observed in the bed system, numbers, and not sizes, of mussels are likely more important to conservation efforts. It is difficult to discern whether there was one dominant feature of the dish culture system that contributed to the higher survival. It was probably a combination of factors resulting from greater maintenance and better overall culture system design, in terms of eveness of flow, cleanliness, substrate stability, water quality, and food availability.

Density and Growth One possible reason for greater growth rates of mussels in the bed system is lower density resulting from low survival. Both culture systems started out with a mussel density of 1-1.5 mussels per cm^2, but by the end of the study densities, they were approximately 0.3 mussels per cm^2 in the dish system and 0.04 mussels per cm^2 in the bed system. The difference in growth between the two systems became greatest toward the end of the 16 wk, however, and the decrease in mussel density in the bed system occurred toward the beginning, so I feel that density is not the causative factor.

Temperature and Growth Temperatures in the bed culture system averaged nearly 3^o C higher than those in the dish system, which is a significant difference ($P < 0.01$). This is probably due to culture system design, with the submersible pumps in the bed system generating heat. With elevated water temperatures, it may be possible to increase growth rates within the dish culture system. Increased growth has been associated with higher water temperatures, oxygen, and calcium levels (Buddensiek 1995). Hudson and Isom (1994) reported a slightly significant

increase in growth in *Anodonta imbecilis* from 23 ^{o} C to 30 ^{o} C. Being ectothermic, an increase in water temperature causes a corresponding increase in metabolic rate in mussels, thus increasing growth. Most species exhibit partial seasonal acclimatization to temperature changes, although degree to which this occurs varies. Individual mussel species have certain minimum and maximum temperature tolerances depending on their native habitat, and this must be taken into account when determining the temperature to culture a species (Hornbach 1985; Foe and Knight 1986). Cold-adapted species, for instance, have increased growth and increased mortality at temperatures higher than what is normal to them (Buddensiek 1995).

Water Chemistry Although there was some fluctuation in water chemistry variables during the course of the experiment, all were within acceptable ranges. At the mean temperatures and pH conditions of my experiment, 4 mm *Mercenaria mercenaria* have a reported lethal level of unionized ammonia of 2 mg/L (Stickney 1994). My ammonia levels never approached this value. *Elliptio complanata*, however, has a reported 50% reduction in ciliary response at an unionized ammonia level of 0.06 mg/L (Anderson *et al.* 1978). This level was exceeded on two occasions in the bed culture system, although the ammonia levels were otherwise safely below 0.06 mg/L. Although such ammonia levels could be a possible stressor to mussels, survivorship in the bed culture system dropped long before the peaks in ammonia levels were recorded, and there was no observed decline in survivorship associated with the elevated ammonia levels. My dissolved oxygen levels were well above the recommended level of 5 mg/L for naiades (Havlik and Marking 1987).

Flow and System Design Mussels require water flow to deliver food and flush wastes. It has been demonstrated with adult marine bivalves and with juvenile freshwater mussels, that a certain degree of constant flow is beneficial to growth and survival (Hudson and Isom 1984; Van

Erkon Schurink and Griffiths 1993). Fluorescent dye tests were conducted on both culture systems used in this study, revealing uneven flow patterns in the bed system. The uneven gravel floor of the greenhouse, in which the culture systems were housed, made it difficult to maintain a perfectly level surface in the bed system, which resulted in some bed sections receiving more flow than others. In addition, within each bed there were distinct areas which received most of the flow, and other areas which received little to none. The location of the mussels within each bed was not controlled, and therefore, some of them may have been located in less than ideal locations with respect to flow. It was also difficult to subsample the culture beds, with the potentially non-random location of the mussels within the beds and the disturbance that the entire bed received during subsampling. The dish culture system design is more versatile in that the dishes can be removed, their orientation in the flow is controllable, and reliable subsamples are taken without disturbing all mussels in the system. The flow meters on the dish culture system also made it easy to regulate the amount of flow moving across the dishes. There were no patterns observed in mussel survival or growth based on dish or bed location within each of the culture systems. Placement of individual mussels may have been a factor, although it would have been impossible to monitor during this study.

The flow patterns within the culture systems as well as the locations of individual mussels may have contributed to the range in survival and growth exhibited by mussels exposed to otherwise similar conditions. In addition, the range in age of the mussels upon placement in the culture systems may have been a contributing factor. Mussels that were 1 day old upon placement in the systems may have had an advantage over those that were 19 days old. Although this may have increased the range of growth and survival exhibited by the juveniles, the effects

should have been equal in both culture systems, due to the mixing of juveniles of all ages and arbitrary placement in the culture systems at the start of the experiment.

The submersed water pumps located in the water reservoir in the bed culture system were a design disadvantage. In addition to raising the water temperature by approximately 3^{o} C, the submersed pumps held the danger of potentially leaking oil into the culture system. One of the two submersed pumps leaked a slight amount of oil into the bed culture system during week 13 of the experiment. The leak was discovered the same day that it occurred; the leaky pump was replaced and any visible oil was removed with oil absorbent cloths placed on the water surface. There was no corresponding drop in growth or survival on or after week 13; therefore, I conclude that this oil leak had no significant adverse effect.

Diurnal Cycle When in artificial culture, mussels seem to benefit from an environment that closely simulates natural conditions. Growing them in a greenhouse with controlled temperatures yet natural lighting is advantageous. Some mussel species have been observed to display circadian rhythms in their metabolic processes, possibly requiring both dark and light periods to function normally (Graves and Dietz 1980; McCorkle-Shirley 1982; Gatenby *et al*. 1998). Diurnal cycles also have been associated with vertical migrations, rhythmic gaping, and respiratory patterns (McCorkle *et al*. 1979). These behaviors may perhaps be associated with avoidance of predation or desiccation. Conditions of constant light have been shown to be deleterious to mussel culture, causing filamentous algae overgrowth and possibly interfering with a mussel's natural circadian rhythm (C.L. Yang, Virginia Polytechnic Institute and State University, pers. comm., 1996)

Biofouling Cleanliness in freshwater mussel culture seems to have a positive effect on juvenile growth and survival. The dish culture system was much easier to clean than the bed

system, leading to less disturbance of the juveniles. When a problem develops, such as an colonization by other small (and potentially predatory) organisms or undesirable algae, being able to clean the culture system well is important. If many insect larvae or other small invertebrates reach large numbers in the culture system, they could become disruptive to the mussels, competing for food and space if not directly harming them. Overgrowth of undesirable algae, insects and other decaying organic matter could create an oxygen deficiency, increase ammonia levels, interfere with respiration, orientation, and feeding, or restrict flow through the clogging of pipes and valves. Undesirable algae (e.g., filamentous) also compete with more desirable, planktonic species that are more easily ingested by juvenile mussels. Although the benthic diatom (*Navicula sp.*), that colonized the substrate during this experiment, has been observed to be ingested by adult mussels (Bruce Parker, Virginia Polytechnic Institute and State University, pers. comm., 1996), it is not known if it is a suitable food for juveniles. Because it is a benthic species, it may be less accessible to filter-feeding mussels than planktonic species. *Navicula sp*. is approximately twice the size of *Neochloris sp*., and may therefore be more difficult for juvenile mussels to ingest. Since juvenile mussels pedal-feed for the first part of their lives, biofoulers clogging the substrate may impede their ability to locomote and collect detritus and algae for consumption. From the initiation of the experiment, tracks in the substrate were observed where juveniles were transversing the substratum.

Food Availability Attempts to establish a self-sustaining algal culture in the bed culture system were unsuccessful, and thus abandoned during this experiment. It would be impractical to raise juveniles in a perfectly sealed environment, and thus algal species other that the one fed to the mussels are able to establish themselves. If conditions in the culture system are not exactly what the inoculated algae thrive in, other species of algae as well as diatoms may have an

advantage in dominating the system. In a greenhouse environment, lush with various flora and fauna, this is particularly the case. Ensuring that there is always sufficient desirous algal species present may increase mussel growth rate, and this can be accomplished with frequent feedings. Leaving food availability up to the chance of whatever species happens to take over is too risky, especially when mussels are young and require a daily supply of algae of the appropriate size and of excellent nutritional value for maximal growth. Gatenby *et al.* (1996) showed that it is beneficial to have a high percentage of polyunsaturated fatty acids (PUFAs) in a mussel diet; therefore, small algal species with high PUFA content, such as *Neochloris oleoabundans*, are desirable for newly transformed juveniles.

Bacteria Although researchers have observed that the presence of bacteria can be beneficial to pedal-feeding young juvenile mussels (Yeager *et al.* 1994; Gatenby *et al.* 1996), and may be a significant source of vitamin B-12 in adult mussels (Nichols and Garling 1998), the addition of bacterial inoculate during this experiment had no measurable effect. Culture system design and maintenance may have been more important in this situation. Both culture systems were operated for over a week before placement of the juveniles, and bacteria likely colonized both systems at that time. It is possible that the higher growth rates observed for mussels in the bed culture system may have been partially attributable to a higher availability of bacteria, at least in the first few weeks when a greater amount of bacteria is ingested by juvenile mussels (Yeager *et al.* 1994). Alternatively, greater growth rate may be due solely to the temperature and culture system design difference.

Experiment Two: Sediment Type and Water Hardness Effect

Juvenile Production Due to high host fish mortality, it took a long time to collect enough juveniles from the remaining fish to begin the culture trial. The first time this experiment was attempted, the average age of the juveniles stocked in the culture systems was 15 days. The mussels were kept in a 12 ^{o}C incubation chamber with aeration, algae, and sediment while awaiting placement in the culture systems. The first attempt at starting this experiment was aborted due to poor juvenile survival, averaging 11 %, after 3 wk of culture. The second time this experiment was initiated, there was much better juvenile production, and they were placed in the culture systems at an average age of 2 days post-metamorphosis. They also were kept in an incubation chamber with aeration, algae, and sediment until the experiment was ready to begin, but this time with the chamber at 20 ^{o}C to better simulate culture conditions.

Water Hardness The results of this culture experiment suggest that for rapid growth and highest survival, mussels should be cultured in high water hardness. The significance of high water hardness to young mussels is likely directly related to the importance of calcium availability. Indian Creek, where the mussels used for this experiment were obtained, is a tributary of the Clinch River - where mean yearly hardness levels measure 248 mg/L $CaCO_3$ (Appendix E)(Tennessee Valley Authority 1986c). Buddensiek (1995) associated calcium availability with growth in *Anodonta imbecilis*. Up to one-third of a freshwater mussel's energy devoted to growth can be spent solely on shell production (Wilbur and Saleuddin 1983). Larger, thicker shells provide greater protection from predation and desiccation, and are, thus, important to survival. Mussel's shells are composed of calcium carbonate, which may not be contained in their food source. With all the other measured water chemistry variables in the troughs being similar, calcium uptake by mussels from the water may be a positive influence for high survival and

growth. Although the relative roles of foods as sources for the important shell calcium are unknown, Ca^{2+} uptake has been observed across epithelial surfaces in freshwater mussels (Burton 1983). Additional uses that mussels have for calcium are as a buffer for respiratory acidosis during hypoxia, and as the main source of maternally derived shell material for brooded glochidia (Silverman *et al.* 1985, 1987; Heming *et al.* 1988)

Substrate Organic Content Although there was no significant difference in growth and survival in mussels grown in high and low organic sediment, there may be a benefit to using high organic content substrate. Substrate composition may particularly be important in the first few months of culture when mussels are reliant on pedal feeding. Gut content analyses of juveniles 2 wk old have shown a significant ingestion of detrital particles (Yeager *et al*. 1994). It is possible that the levels of organic content in the substrates used during this experiment were too similar to differentiate its effect. Alternatively, the difference in substrate composition may have been more than adequate, but the experiment was too short in duration to reveal a different response. The dip in survival observed at week 6 for the high water hardness and high soil organic content treatment may have been an anomaly, uncharacteristic of the overall better survival of those mussels held in high organic content soil and high water hardness. At week 12, juveniles in high hardness water were 247 microns greater in height and 378 microns greater in length if they were in the high organic content soil. They also had 6.5% to 14% greater survival on four of the five sampling periods. The mussels were observed filter-feeding after approximately 8 wk; thereafter the importance of substrate composition may be diminished. By week 15, the gap in survival between low and high organic content narrowed, therefore reflecting but limited advantage to high organic content soil in this experiment.

Juvenile Care Placing newly metamorphosed juveniles in the proper culture environment as soon as possible after excystment ensures the best survival. It is possible that the lengthy waiting period of the juveniles during the first experiment attempt caused survivorship to decline. The mussels were kept at a colder temperature to slow their metabolism awaiting placement in the culture systems, and it is possible that this was detrimental to their survival. The second time the experiment was initiated, juveniles had a very brief waiting period (averaging 2 days). They were kept in conditions similar to that of the culture environment; with moderated temperatures, and with suitable substrate and algae, leading to better survival. Whenever juveniles are moved from one system to another, I recommend that an acclimation time is allowed. I found that to reduce stress on the mussels, at least 1 hr was suitable to slowly adjust them to a new environment. To reduce thermal shock, it is common practice to temper fish at a rate of 5 ^{o}C per hour, although for freshwater mussels further study is warranted (Stickney 1994). When subsampling juveniles, care must also be taken to rinse the substrate through the sieve using water of suitable temperature and chemistry, and to return the mussels as quickly as possible to the culture system. Yeager *et al.*(1994) observed 3-5 d old *Villosa iris* actively feeding, suggesting that it is desirable for them to be placed in a proper environment with available food by that age.

Water Chemistry Although there was some fluctuation in water chemistry variables during the course of the experiment, all were within acceptable ranges. The unionized ammonia level of 0.06 mg/L, which has been reported to reduce cilliary response (Anderson *et al.* 1978), was exceeded on two occasions in trough number three (at 0.074 and 0.066 mg/L), although the ammonia levels were otherwise safely below 0.06 mg/L. There was no observed drop in survivorship associated with the elevated ammonia levels. The dissolved oxygen levels were well above the recommended level of 5 mg/L for naiades (Havlik and Marking 1987).

Growth and Survival Comparison with Other Culture Experiments

It is difficult to compare juvenile growth and survival rates with other studies of differing duration, where other species with their inherent growth rates were used (McMahon 1991). In a 74 d study with *Utterbackia imbecillis*, shell lengths increased 18-fold, but survival was only 1.0%, and attempts to rear *Lampsilis ovata, Fusconaia ebena, Ligumia recta, Pleurobema cordatum,* and *Carunculina moesta* completely failed (Hudson and Isom 1984). Although Buddensiek (1995) was able to culture *Margaritifera margaritifera* to 6.4 cm in cages for 52 mo, there was a high initial mortality. Young and Williams (1984) also reported that most juvenile mussel mortality occurred within the first few months of culture. *Villosa iris* were cultured by Gatenby *et al.* (1996) for 140 d with 30% survival, and for 272 d with 5% survival.

In my first culture experiment, mussels grown in the bed culture system had a 4.4 fold increase in height and 4.9 fold increase in length over 16 wk (112 d), with 3.1% survival. In the dish system they increased 2.9 fold in height and 3.1 fold in length, with 31.3% survival. In the second culture experiment which ran 15 wk (105 d), mussels in the high water hardness had a 5.3 fold increase in height, an 8.2 fold increase in length, and 45% survival. In low water hardness, mussels grew 4.5 fold in height, 6.9 fold in length, and had 9% survival. The increased growth and survival rates observed in the second culture experiment with high water hardness may be attributable to increased feeding rates, rapid placement in the culture system after excystment, cleanliness of the culture system, improved culture system design with relatively even flow patterns, and appropriate water chemistry. It remains to be verified whether substrate composition may affect survival. The resultant growth and survival rates were acceptable, and therefore, my culture techniques would be appropriate for use with a suite of species.

Recommendations for Juvenile Mussel Culture and Suggestions for Future Research

Results from my culture experiments yielded several recommendations for the culture of juvenile freshwater mussels in recirculating systems. These recommendations are based on my experiences with *Lampsilis fasciola* juveniles, and may need to be modified to suit the requirements of other species. Researchers working with other species, however, have come to the same conclusions that daily algae feeding, silt substrate, and flowing well water are beneficial to juvenile mussels (Ruessler and Keller 1998). Presented here are some recommendations for mussel culture techniques and research needs that may help improve future propagation efforts.

- Juveniles can be cultured in small, shallow dishes (containing approximately 1 cm deep sediment substrate), that can be removed for culture system cleaning and can be easily sampled to monitor mussel health, without disturbing other mussels.
- Clean the culture system and complete water changes weekly, keeping the system clear of biofouling.
- Care should be taken that flow in the culture system is even so that all mussels receive the same benefits of fresh water replacement for ammonia removal and the provision of oxygen and algae.
- Juvenile mussels should be fed at least ten to fifteen thousand cells *Neochloris oleoabundans* per 1 ml culture system water daily. Recent work with *Elliptio complanata* has shown that feeding rates can reach 50,000 algal cells/ml before waste (pseudofeces) increases (Bill Henley, Virginia Polytechnic Institute and State University, pers. comm. 1998). Patterson *et al*. (1998)

also showed that feeding at rates of up to 100,000 algal cells/ml may be beneficial. Feeding at higher rates than mine may therefore be warranted.

- Large host fish seem to be better able to withstand the stress of large glochidial infestations than small fish. In my experience, fifty 3-7 cm. largemouth bass produced a total of several hundred *L. fasciola* juveniles, while three 30 cm. largemouth bass produced several thousand juveniles.

- For best juvenile survival, juveniles should be placed into the proper culture environment within days of post-metamorphosis. Special care should be taken during the first 6 wk of juvenile life, when most of the mortality occurs. Further research is needed to determine how disturbance affects the mussels, and how best to acclimate them to new culture conditions.

- Research is needed to determine what levels of feeding, temperature, and flow promote the greatest growth and survival.

- High water hardness (250 mg/L $CaCO_3$) is recommended for good growth and survival. Additional testing may be required to refine the level of hardness that is best suited for freshwater mussel culture, and of species other than *L. fasciola*.

- Average water chemistry variables that were found acceptable during the culture experiment were: dissolved oxygen: 8.0 mg/L; pH: 8.25; un-ionized ammonia: < 0.09 mg/L; temperature: 23 ^{o}C. Further research is required to determine what levels of toxicants, nitrogenous wastes, and low dissolved oxygen are detrimental to juvenile growth and survival.

- Further research is required to determine the most important nutritional and structural components of juvenile mussel's diets and substrates, particularly in the sensitive pedal-feeding

stage. The value of food sources other than *Neochloris oleoabundans* should be explored, as it may not always be available.

- For high intensity production, it needs to be determined when density of juveniles is excessive.

- Research is needed to determine the modified culture requirements of different species of juvenile mussels.

- For the continued conservation of freshwater mussels, it will be most beneficial to restore their native habitats, if possible, and help build their populations to stable levels through captive propagation. In addition, in order to ensure long-term viability, host fish species must be identified and determined to be in sufficient abundance to the mussel population.

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Appendix A: Juveniles of *Lampsilis fasciola* produced from host fish species.

Fish Species (Number)	Number of Juveniles Collected (approximate)
Ambloplites rupestris (7)	77
Lepomis macrochirus (13)	109
Lepomis auritus (2)	10
Cottus carolinae (12)	0
Micropterus dolomieu (6) and *Micropterus salmoides* (199)	15,000

Appendix B: Quality analysis for water at Virginia Tech Aquaculture Center*
(nd= not detected at less than or equal to detection levels)

Measurement (mg/L)	Municipal Water	Well Water
total coliforms	nd	nd
arsenic	nd	nd
barium	nd	nd
cadmium	nd	0.005
chromium	nd	0.004
copper	0.014	0.083
iron	0.031	0.096
lead	nd	0.020
magnesium	0.008	0.014
mercury	nd	nd
nickel	nd	nd
selenium	nd	nd
silver	nd	nd
sodium	9.5	nd
zinc	0.004	0.018
alkalinity	70	270
chloride	nd	nd
fluoride	0.6	nd
nitrite	nd	nd
sulfate	nd	20
hardness	120	370
pH	8.40	7.90
dissolved solids	165	520
turbidity (turbidity units)	0.3	0.2
total trihalomethanes	0.076	nd
organic chemicals	nd	nd

*****Test performed at: The Water Check Division, National Testing Laboratories, Inc., Cleveland, Ohio

Appendix C: Ingredients in the Fritz F/2 algae fertilizer *

Part "A"		Part "B"	
Fe	1.3%	Available Nitrogen	15.0%
Mn	0.034%	Available Phosphate	2.0%
Co	0.002%	Vitamin B1	0.07%
Zn	0.0037%	Vitamin B12	0.0002%
Cu	0.0017%	Biotin	0.0002%
Mo	0.0009%		
Ingredients: Ferric Chloride, EDTA, Cobalt Chloride, Zinc Sulfate, Copper Sulfate, Sodium Sulfate, Sodium Molybdate		Ingredients: Sodium Nitrate, Monosodium Phosphate, Thiamin Hydrochloride (Vitamin B1), Vitamin B12, Biotin	

* Fritz Industries, Inc. Dallas, TX

Appendix D: Composition of Aqua Bacta-Aid (ABA)*

Ingredient	Concentration
Aerobacter, Bacillus (2 different species), *Cellumonas, Nitrobacter, Notrosomonas, Pseudomonas, Rodopseudomonas*	180 billion bacteria per gallon
NH_4Cl	0.10%
K_2HPO_4	0.10%
$MgSO_4\ 7H_2O$	0.05%
Na Acetate	0.10%
Yeast extract	0.10%
Na_2S	0.50%

***** Water Quality Science, Inc. Bolivar, MO. Concentrations of solids given on a weight per volume basis.

Appendix E: Analysis of water chemistry in the Clinch River, Virginia, (River Mile 227.4)*

Parameter	Mean Value
Temperature, ^{o}C	15.9
Color, Units	11.9
Dissolved oxygen, mg/L	8.8
pH, SU	7.7
Alkalinity, mg/L	112
Hardness, mg/L $CaCO_3$	248
NH_3 +NH_4, mg/L	0.07
Suspended solids, mg/L	20.2
Chlorides + Sulphates, mg/L	38.7
Chlorides, mg/L	8.4
Sulphates. mg/L	30.3
Iron, dissolved, µg/L	47.4
Arsenic, µg/L	2.2
Cadmium, µg/L	0.12
Chromium, µg/L	1.1
Copper, µg/L	10.3
Lead, µg/L	2.8
Mercury, µg/L	<0.2
Selenium, µg/L	<1
Zinc, µg/L	15.1
Fecal coliform, No./ 100 ml	44

*Source: Tennessee Valley Authority, 1986c. Values are the means of monthly samples taken from July 1980 to May 1981.

VITA

Michelle Birgit Steg was born in Omaha, Nebraska on January 28, 1973. She grew up in McLean, Virginia and graduated from McLean High School in 1991. She attended the University of Virginia in Charlottesville, Virginia form 1991 to 1995, graduating with a bachelor's degree in Environmental Science. While at UVA, she was involved in research with the F.I.S.H. (Fish In Sensitive Habitats) project, measuring the effects of atmospheric acidic deposition on brook trout and blacknose dace in streams in the Shenandoah National Park. Feeling a calling to the study of aquatic organisms, in August 1995 she began her work as a graduate student at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. There she worked on life history studies and culturing techniques for freshwater mussels threatened and endangered in Virginia. In November 1998, her requirements for the Master of Science degree in Fisheries Science were completed.

Though her first love remains freshwater fishes, her professional aspirations are to eventually work in the conservation of freshwater ecosystems on a broader scale. Seeing the increasingly perilous state of endangerment of many freshwater organisms, she hopes to work in their protection, and in the conservation and rehabilitation of their habitats and populations.