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# Preliminary Studies on the Life History and Propagation of Several North Carolina Freshwater Mussels

(Final Report)

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Prepared for:
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Raleigh Ecological Services
P.O. Box 33726
Raleigh, North Carolina
27636-3726

December 2001

45 pp.

# ABSTRACT

We modified a pre-existing research laboratory located at the North Carolina State University, College of Veterinary Medicine for the propagation and captive culture of native freshwater mussels. Modifications to the facility included the construction of a greenhouse to culture algae as a food source, design and construction of closed recirculating systems for holding fish hosts and for captive culture of freshwater mussels. In conjunction with renovating laboratory space, research was conducted to further develop propagation protocols including life history and captive culture studies.

We tested two common hatchery reared fish species as suitable hosts for 4 species of mussel. Hatchery reared largemouth bass (*Micropterus salmoides*) and Hybrid blue gill (*Lepomis macrochiru cyanelluss*) were infested with glochidia of the yellow lance (*Elliptio lanceolata,* I. Lea), carolina creekshell (*Villosa vaughaniana,* I. Lea), notched rainbow (*Villosa constricta,* Conrad), and eastern floater (*Pyganodon cataracta,* Say). Hybrid bluegill tested positive as a suitable host for *V. vaughaniana*. Glochidia of *Elliptio lanceolata* extracted from mussels of the Tar R. drainage successfully metamorphosed on hybrid bluegill and largemouth bass. However, glochidia of *E. lanceolata* extracted from mussels of the Nuese R. drainage did not successfully metamorphose on largemouth bass or hybrid bluegill. Successful metamorphosis of glochidia of *V. constricta* and *P. cataracta* was negative on hybrid bluegill and largemouth bass.

Juveniles of the eastern lampmussel (*Lampsilis radiata radiata*, Gmelin 1791) were artificially propagated and initially cultured in a closed recirculating system using 3 different flow regimes to identify affects of different flow rates on growth and survival of juvenile mussels. In addition, juveniles of the carolina creekshell (*Villosa vaughaniana*, Conrad), eastern

elliptio (*Elliptio complanata*), and green floater (*Lasmigona subviridis*) were propagated in the culture system. The recirculating culture system was designed and constructed with 6 separate flow channels. Water velocity for each channel was maintained at either high (~ 4.2 cm/sec), medium (~ 2.3 cm/sec) or low (~ 0.7 cm/sec). Juveniles were fed once a day, 5 times a wk, with a combination of two algal species (*Scenedesmus sp.* and *Chlorella vulgaris*). Growth and survival were compared among juveniles reared under different flow regimes after 62 days. Mean growth (±1 SD) was 0.76 ± 0.15 mm, 0.74 ± 0.16 mm, and 0.79 ± 0.17 mm for juveniles exposed to high, medium, and low flow respectively, and was not statistically different among groups ($p = 0.292$, $\alpha = 0.05$). Survival (±1 SD) was variable among groups with 13.0 ± 2.6, 4.3 ± 3.5, and 12.0 ± 6.4%, for high, medium, and low flow respectively with no statistically significant differences among groups ($p = 0.252$, $\alpha = 0.05$). Preliminary results suggest that, within the range tested, different flow rates in our rearing system do not substantially affect juvenile mussel growth or survival. Long-term mean shell length and survival for *L. r. radiata* was 6.64 ± 1.83 mm and 5.68% at 370 d. Long-term mean shell length and survival for *V. vaughaniana*, was 4.28±1.28 mm and 19.71% at 294 d. Growth and survival of juveniles of *E. complanata* at 31 d was 0.46±0.05 mm and 3.57%. Growth and survival of *L. subviridis* was poor, resulting in only two survivors at 72 d with a mean shell length of 0.61±0.01 mm.

We conducted a study to establish the reproductive strategy and identify suitable fish hosts for *Toxolasma pullus* from University Lake, North Carolina. In addition we examined internal growth lines of shells to establish age-class structure of *T. pullus*. Females of *T. pullus* appear to be bradytictic and seem to maintain their brood into August. Hybrid bluegill and redbreast sunfish tested positive as suitable hosts for *T. pullus*, however, other *Lepomis* species may serve as suitable hosts. Most specimens of *T. pullus* we aged were between 4 and 6 years old. We

speculate that the longevity of *T. pullus* is short-lived and may rarely exceed 15 years of age. Predation may be the predominant limiting factor of age.

## ACKNOWLEGEMENTS

We appreciate and thank Robert Glosson, Rachel Monschein, and the Orange County Water and Sewer Authority for providing information and access to University Lake. Special thanks to John Holland and Morgan Raley for their field and technical assistance. This project was funded by the U.S Fish and Wildlife Service and North Carolina State University, College of Veterinary Medicine.

## Table of Contents

## LIST OF TABLES AND FIGURES

## SECTION 1: General Introduction

### Status of freshwater mussels in North America

Although the greatest diversity of freshwater mussel fauna exists in North America, modern anthropogenic activity has imperiled many species and has decimated this fauna dramatically over the last 50 years. Of the 297 freshwater mussel species recognized in the continental United States, 67% are vulnerable to extinction or are already extinct (Williams *et al.*, 1993). Impoundments, water pollution, channelization, dredging, and the recent proliferation of the exotic zebra mussel (*Dreissena polymorpha*) are thought to be the major reasons for declining native mussel populations.

Historically, freshwater mussels were an important food resource for Native Americans (Parmalee and Klippel, 1974). In the early 1900's, many mussel species were extensively exploited for button manufacture (Coker, 1921). Today, freshwater mussels are an important economic resource in the cultured pearl industry (Williams, 1993). Despite their economic importance, it is the ecology of the freshwater mussel that society benefits from the most. Unionids are a significant component of aquatic ecosystems, comprising as much as 90% of the benthic community biomass (Ökland, 1963). They are an important food source for aquatic and terrestrial wildlife, and they improve water quality by removing sediment, nutrients and contaminants from the water column. Because unionids are long-lived benthic filter-feeders, they serve as excellent bioindicators of environmental degradation of rivers and streams. This is especially pertinent to many water quality and public health issues.

Realizing the ecologic, economic, and scientific value of freshwater mussels, a national strategy was developed in 1998 by the National Native Mussel Conservation Committee to

curtail the continued demise of freshwater mussel fauna. Despite state of the knowledge, our understanding of ecology and biology of freshwater mussels is still in its infancy.

## Basic Life History

The complex reproductive cycle begins with the production and release of sperm by mature males. The sperm are released through the excurrent aperture into the ambient water column where it is received through the incurrent aperture of a mature female. Although fertilization presumably takes place within the superbranchial chamber, embryonic development occurs within the marsupial gills until embryos fully mature into parasitic larvae called glochidia. Glochidia are retained within the gills of the female for various lengths of time. Brooding of larvae is categorized by two different reproductive strategies (Ortmann, 1911). Long-term brooders (bradytictic) typically spawn in late summer, brood young over the winter, and release glochidia the following spring and early summer. Short-term brooders (tachytictic) spawn in the spring and release glochidia that summer. Some mussels are sexually dimorphic and can be easily identified as male or female, while others have no obvious sexually dimorphic characteristics and can be extremely difficult to sex. Although the accepted paradigm suggests a dioecious fertilization process, controversial evidence suggests hermaphroditism as a possible adaptive fertility mechanism in situations of adverse reproductive conditions (van der Schalie, 1966; Downing *et al.*, 1993; Neves, 1997). Neves (1997) suggests that extrinsic conditions such as low densities, or highly skewed sex ratios may induce cross- or perhaps self-fertilization among hermaphroditic females.

With the exception of a few species, glochidia of most mussel species are obligate parasites of fish. A few species are facultative parasites and can undergo direct development. Nevertheless, the parasitic process is species specific; each species of mussel requires specific

species of fish to complete their lifecycle. Gravid mussels use a variety of strategies to increase the success of infecting fish hosts. Some mussels exhibit behavior and have special anatomical features, which attract and lure certain species of fish. Through possible chemosensory ability, some species of mussel may be stimulated to discharge glochidia when a suitable fish host is nearby (Henley and Neves 1997). Other species may simply disperse large numbers of glochidia into the water column at random.

Depending on the mussel species, glochidia are released from the marsupial gill either individually or as conglutinate clusters. In some taxa, glochidia exit the gills through small pores located on the ventral margin of the gill. In other taxa, glochidia exit through the excurrent aperture via the superbranchial chamber. The number of glochidia held within the marsupial gills varies among the species and the size of an individual female, and has been estimated to range from 100,000 to 3.5 million (Yeager and Neves, 1986; Neves and Wildak, 1988). Despite the high fecundity of females, the probability of glochidia coming into contact with a suitable host is likely to be extremely low, resulting in the highest level of mortality occurring at this life stage. In some subfamilies glochidia that succeed in contacting a suitable fish host will attach to the gills of the fish. In other subfamilies glochidia will attach to skin and/or fins. Once the glochidia attach to the host fish they become encysted within the tissue and remain encysted over a period of one to three weeks or even a few months until metamorphosis is complete (Zale and Neves, 1982b). When metamorphosis is complete, the glochidia drop from the host fish and become established within the substrate where they will grow and develop into sexually mature individuals, if conditions are conducive to their survival. Individuals of some species have been reported to live for more than 50 years (Neves and Moyer, 1988), and margaritiferid species have been reported to extend there life span beyond 210 years (Ziuganov *et al.* 2000).

## North Carolina's Freshwater Mussels

Twenty-nine of the 49 recognized freshwater mussel species of North Carolina are listed as state endangered, threatened, or of special concern (John Alderman, perc. com. The North Carolina Wildlife Resources Commission). Thirteen of these species are federally listed. Habitat destruction and pollution from development, impoundments, and poor land management practices have contributed to declines in these mussel populations. In 1998, the National Native Mussel Conservation Committee proposed a national strategy to respond to the decline of mussel populations (National Native Mussel Conservation Committee 1998). Captive propagation and subsequent release of imperiled mussel species was identified as a potentially viable conservation tool for enhancing mussel populations.

Initial efforts have focused predominately on the propagation of mussel species west of the Appalachians (Zale and Neves 1982a, 1982b, Neves et al. 1985, Yeager and Saylor 1995, Haag and Warren 1997, Keller and Ruessler 1997, Gatenby 1996, 1996, O'Beirn 1998). Little attention has been given to North Carolina's mussel fauna, many of which are found exclusively within the Atlantic slope region. Furthermore, comprehensive host fish identification studies have not been conducted for many North Carolina mussel species. Consequently, protocols for artificial propagation and culture of many of these species have not been developed. The life-history information needed for captive propagation must be ascertained before these species become so rare that the collection of gravid individuals becomes difficult and potentially deleterious to the existence of remaining populations.

## North Carolina Freshwater Mussel Conservation Partnership

The North Carolina Freshwater Mussel Conservation Partnership was formed in 2000 in response to the declines in freshwater mussel populations in North Carolina. The mission of the

partnership is to conserve North Carolinas' freshwater mussel fauna through educational outreach, research and collaboration with private and state and federal agencies. The partnership strives to bring government and public attention to the value of freshwater mussel conservation and it's impact on public health issues. Research is currently underway at North Carolina State University Freshwater Mussel Research Facility (NCSU-FMRF), located at the College of Veterinary Medicine.

Since March 2000 research at this facility has included fish host investigations and artificial propagation of freshwater mussels. What follows is the first annual progress report on the results of this research.

The goal of the freshwater mussel propagation program is to produce juvenile mussels of North Carolina's mussel species for purposes of augmenting declining populations as well as reestablishing extirpated populations. In addition, we hope to produce and maintain juveniles mussels for experimental purposes and provide a facility to conduct research which aids in the conservation of freshwater mussels.

## The NCSU Freshwater Mussel Propagation Facility

In 1990 a facility was constructed at North Carolina State University, College of Veterinary Medicine for the purposes of conducting marine bivalve research. In 1998, Dr. Jay Levine proposed to the United State Fish and Wildlife Service (USFWS) plans to convert the research facility into a freshwater mussel propagation facility for purposes of producing native mussels for recovery efforts and experimentation. The Service generously responded with startup funds to convert the facility. In March 2000, I was hired by the University of North Carolina to make the necessary renovations, develop protocols, and direct propagation research.

The major objectives of this project were to modify the pre-existing facilities for the purposes of propagation of freshwater mussels, and to develop and test protocols for the artificial propagation of native freshwater mussel.

*Research laboratory*

The pre-existing wet laboratory was supplied with both treated municipal water and well water. However, the well water was not used because it contained high levels of copper (Cu~ 54 ppt). The original design of the wet-lab included 5 closed recirculating systems. Each system held a maximum of 1700 L of water and was composed of 2 stacked 500 L troughs, a 700 L reservoir/sump, a trickle filter and was temperature controlled with an Aqua Logic chiller unit (San Diego, CA 92111). Troughs, reservoirs, and trickle filters were constructed of fiberglass and were fabricated by Hulls Unlimited-East Inc. (Deltaville, VA). Water in each system was pumped via a ½ hp centrifugal pump through the chiller unit to the head of the trickle filter. Water then descended through the trickle filter, which contained bio-surface elements and was captured in the upper 500 L trough. Through a 38 mm diameter stand pipe, water was gravity fed from the upper trough to the lower 500 L trough and from the lower trough to the reservoir. From the reservoir, water flowed to the pump to complete the cycle. The stacked configuration of each system was supported on a frame constructed of fiberglass leg angle (Fibergate Composite Structures Inc., Addison, TX). All pumps and chiller units were housed outside of the main facility in adjacent sheds to eliminate excessive noise and heat production from the main laboratory space.

An air supply system was installed within the main facility to deliver air to various recirculating systems for holding captive fish, adult mussels, and cultured juvenile mussels. Air was distributed overhead by PVC pipe mounted to the ceiling. Air was generated with a 1 hp

Sweetwater regenerative blower that was housed externally to the main building. A wooden structure with a shingled roof was constructed to house the regenerative blower.

*Captive fish hosts*

A recirculating fish holding system was designed and constructed to maintain and grow-out commonly used fish hosts for mussel propagation purposes. This high volume system retained a total of 1400 L of water and consisted of two 378 L and two 189 L Rubbermaid tubs, a 167 L reservoir, and a trickle filter. Rubbermaid tubs were elevated and supported by a pressure-treated wooden structure so that the rims of all tubs were approximately 100 cm high. Water drained from each Rubbermaid tub through a standpipe (38 mm diameter), which maintained water volume at near maximum levels. From each tub, water drained into a central PVC pipe that ran underneath all 4 tubs and discharged into the reservoir. From the reservoir, water was pumped by a ¾ hp centrifugal pump up to the top of a 225 L tickle filter. The trickle filter was constructed of a conical-bottom tube (46 cm diameter) containing 125 L of bio-surface elements, which provided surface area for the colonization of nitrifying bacteria. From the bottom of the trickle filter, water was collected and gravity fed back to the Rubbermaid tubs through a return pipe (38 mm diameter PVC). Overflow water drained back to the reservoir via a junction in the return pipe. All components of this system were connected using threaded unions, which enabled us to disconnect components easily for routine maintenance and cleaning.

To conduct fish host studies, an existing closed recirculating system with 16 separate aquaria was used in a nearby fish research facility. Each 60 L aquarium was fed independently with water delivered through PVC pipe. A central standpipe (12 mm diameter) within each aquarium maintained water levels at near maximum levels. Out-flowing water drained down the standpipe into a central drainpipe, which emptied water into a 400 L sump which then emptied

into a 380 L reservoir. From the reservoir, water was pumped by a ½ hp centrifugal pump through a charcoal filter, an Aqua Logic chiller unit, and back up to each aquarium through a central distribution pipe (50 mm diameter PVC). The two stacked rows of 8 aquaria were supported above the sump and reservoir by a framed structure constructed of fiberglass leg angle.

*Juvenile culture system*

A prototype recirculating system was designed and constructed to culture juvenile freshwater mussels. Two additional systems were later constructed for future experiments and culture trials. Culture systems were designed to be low maintenance and provide uniform flow for juvenile mussels during grow out. Each juvenile recirculating system (JCS) was designed with 6 independently controlled channels. Each channel was constructed of a 150 cm long, 5 cm diameter PVC pipe cut in half longitudinally. A small sump was affixed to the head of each channel where the turbulence of in-flowing water was dampened before being released to the channel. A trap containing a 150 μm mesh screen was attached to the outflow of each channel to capture any juvenile mussels that migrated out of the channel. Out-flowing water was captured in a reservoir and pumped back to the head of the channels via a 1/25 hp magnetic pump. All 6 flow channels shared the same water source but were independently controlled for water flow. A 3 cm layer of various sized gravel was placed in the bottom of the reservoir to provide a substrate for nitrifying bacteria.

*Algae production*

A 73 $m^2$ Janco, camellia series greenhouse (6.1 x 3.7 m) was constructed (J.A. Nearing Co., Inc., Laurel, MD) adjacent to the main facility for the purposes of culturing algae as a food source for captive and artificially propagated freshwater mussels. The tempered glass of the greenhouse was supported by an aluminum frame, set on a 91 cm high cinderblock foundation.

The structure was supplied with electricity and was plumbed with a municipal water supply and a dechlorinated water supply which was conditioned and contained in a 4250 L reservoir tank (Hulls Unlimited-East Inc., Deltaville, VA) within the main laboratory. Temperature in the greenhouse was crudely regulated by a ¼ hp direct drive fan that was thermostat controlled. Six 250 L conical-bottom transparent culture tubes (45 cm diameter x 152 cm high) (Solar Components Corp., Manchester, NH) were supported by holding racks constructed of fiberglass leg angle (Fibergate Composite Structures Inc., Addison, TX) and were housed within the greenhouse on the south-facing wall. Two florescent lights were mounted parallel to each culture tube to supplement sub-optimal solar exposure during winter months. Pressurized air was distributed to each culture tube through PVC pipe and was generated by a Sweetwater linear-piston air pump (Aquatic Ecosystems Inc., Apopka, FL 32704) which was housed within the greenhouse. This air pump generated very little heat, was extremely quite, and was sufficient in supplying air to all 6 culture tubes.

## SECTION 2: Fish Host Identification

### INTRODUCTION

Attempts to identify fish hosts for any given mussel species usually involve the collection of wild fishes and gravid mussels from the same location. Wild fishes are typically held in a laboratory setting where they experience compounding stressors, namely poor water quality, the foreign captive environment, and compromised physiology as a result of glochidial infestation. One of the major pitfalls of identifying fish hosts is the difficulty of maintaining the life of wild fish long enough to complete the glochidia-to-juvenile metamorphosis period (the endpoint which ultimately confirms or rejects the fish as a host). Comprehensive fish host studies increase our understanding of the community ecology of native freshwater mussels and their associated fish hosts. Therefore collecting and testing native fishes for comprehensive studies may be necessary. However, collecting native fish as hosts for production purposes may be inefficient and may ultimately jeopardize particular native populations of fishes. In addition, collection techniques, such as electrofishing, are not target specific and may be potentially harmful to other aquatic vertebrate and invertebrate species. As an alternative to the exploitation of native fishes, the use of hatchery-reared fishes may be more effective in propagating numerous species of freshwater mussels. By using hatchery-reared fish, the potential negative impact of collecting wild fishes can be avoided. Furthermore, hatchery fish are typically more tolerant of captive environments, resulting in lower fish host mortality, thus increasing juvenile mussel productivity. Hatchery-fish alternatives are limited because many species of freshwater mussel require specific fish hosts that are difficult or impractical to culture under hatchery conditions with current knowledge and technology. However, for a vast number of mussel species, production of juveniles can be effective using hatchery reared fish.

We tested two common hatchery-reared fish, largemouth bass (*Micropterus salmoides*) and hybrid bluegill (*Lepomis macrochiru cyanelluss*), as potential hosts for several species of freshwater mussels.

## METHODS

Largemouth bass (7 - 25 cm in length) and hybrid bluegill (10 - 20 cm in length) were obtained from two suppliers (Bay's Fish and Merchants, Auror, NC; Foster Lake and Pond Management, Garner, NC). Fishes were transported to the freshwater mussel propagation facility, and held in the closed recirculating fish holding system described in section 1. Once fish had fully acclimated to the recirculating system (1 wk), fish were fed daily with pellet food. Weekly maintenance consisted of cleaning solid waste from the bottom of holding tanks, and 40% water changes 2 x/wk. City water was dechlorinated with sodium thiosulfate and was used for all fish host and propagation studies.

Fish were infested with glochidia using a procedure similar to the techniques outlined by Zale and Neves (1982b). Gravid female mussels were pried open slightly and held open by a small rubber stopper placed between the valves. A water-filled syringe with a hypodermic needle was inserted into one of the marsupial gill chambers and used to flush the gill chamber with water. Glochidia were liberated from the marsupium and contained in a petri dish. A small sample from this brood was placed into a separate petri dish and tested for viability by adding a few grains of salt. Glochidia that rapidly snapped shut when exposed to saline solution were considered viable

Fishes were collected from the holding tanks and placed into a small bucket containing just enough water to keep the fish buoyant. Glochidia extracted from mussels were immediately added to the bucket and continuously suspended by agitating the water by hand every 15 sec to

ensure infestation. A few fish were sampled and visually checked every 60 sec to confirm attachment of glochidia on the gills and/or fins. Once glochidial infestation was obvious, fish were immediately removed from the bucket and placed in 60 L aquaria sharing a central recirculating water source (described in section 1). If glochidial attachment was not evident after 15 min, fish were removed from the bucket and placed in aquaria. Largemouth bass and hybrid bluegill were held separately with 1 largemouth bass per aquarium and three or less hybrid bluegill per aquarium. After 1 wk, the aquaria were checked periodically for metamorphosed juvenile mussels by siphoning the bottom of each aquarium, capturing juveniles in a 120 μm nylon mesh screen. The contents collected from each aquarium were rinsed from the nylon mesh sieve into a labeled petri dish. With the aid of a stereozoom microscope, each petri dish was visually examined for juveniles. The presence of fully metamorphosed juveniles indicated a positive host. The following mussel species were tested: yellow lance [*Elliptio lanceolata* (I. Lea)] collected from the Tar R. in Vance and Franklin Co. and from Swift Cr. in Johnston Co., carolina creekshell [*Villosa vaughaniana* (I. Lea)] collected from Tom's Cr. in Randolf Co., notched rainbow [*Villosa constricta* (Conrad)] collected from the Dan R. in Stokes Co., eastern floater [*Pyganodon cataracta* (Say)] collected from Lake Tillery in Montgomery Co.

## RESULTS

*Elliptio lanceolata*—Three adults of *E. lanceolata* were collected from the Tar R. in late June. All were gravid, however, only one had a partially mature brood; the broods of the other two mussels were still in an obvious embryonic stage. On July 12, four gravid *E. lanceolata* were collected from Swift Cr. Johnston Co., all of which had immature broods. After being held in the laboratory for 16 d, two of the gravid mussels from Swift Cr. developed mature broods. Through the process of extracting glochidia from *E. lanceolata*, glochidia exited the marsupium

via the superbranchial chamber. The undeveloped embryos maintained a conglutinate cluster in the shape of the associated water tube within the marsupium. However, mature glochidia did not maintain the conglutinate structure and exited the superbranchial chamber suspended in a clear gelatinous matrix. Attempts to separate glochidia from the mucus were difficult. Visual confirmation of glochidia on the gills of fish hosts was negative after infestation. Infestation of fish with *E. lanceolata* collected from the Tar R. produced a total of 20 live juveniles, 7 from largemouth bass and 13 from hybrid bluegill, between 16 and 23 d post infestation (Table 1). The second infestation trial, using mussels collected from Swift Cr. (Nuese R. system), produced no live juveniles.

*Villosa vaughaniana*—One gravid *V. vaughaniana* was collected from Tom's Cr. in Randolf Co. on October 5. Upon preparing for the infestation procedure, glochidia were liberated from the marsupial gills through micro-pores located at the ventral edge of the gill. Glochidia were not released from the gill pores in conglutinate structures, but as individual mature glochidia. The initial infestation trial was light. However, visual examination of glochidial attachment on the gills of largemouth bass and hybrid bluegill was positive. Eight live juveniles were obtained from hybrid bluegill and 1 juvenile was collected from largemouth bass after 20 days post infestation. The juvenile collected from the largemouth bass aquarium, we suspect, was an artifact of contamination from the collecting devices after siphoning juveniles from hybrid bluegill aquaria. The second infestation trial was heavier and only hybrid bluegill were infested. Seven hundred and sixty six juveniles of *V. vaughaniana* were produced from 5 hybrid bluegill between 13 and 30 d post infestation, with the number of metamorphosed juveniles peaking at 19 days.

*Villosa constricta*—Glochidia were liberated from the marsupial gills of two gravid *V. constricta*. Glochidia exited the gills in a manner similar to that of *V. vaughaniana*, through pores on the ventral margin of the gill. After infesting largemouth bass and hybrid bluegill, glochidial attachment was visually apparent. No juveniles successfully metamorphosed over a 30 d period and encysted glochidia were no longer apparent on the gills of the fish.

*Pyganodon cataracta*—Ten non-gravid *P. cataracta* were collected from Lake Tillery in mid August and held in recirculating systems at the FMRF. Mussels were checked again in mid October for gravidity and several were fully gravid with mature broods. When attempting to extract glochidia from the marsupial gills, glochidia exited through the superbranchial chamber in a gelatinous matrix. As observed in gravid mussels of *E. lanceolata*, separating glochidia from the mucus was difficult. Examination of fish infested with glochidia of *P. cataracta* showed no apparent sign of successful glochidial attachment on the gills, fins or skin. Production of metamorphosed juveniles over 30 d was negative.

**Table 1.** Largemouth bass and hybrid bluegill were tested as potential hosts for four freshwater mussel species. Parasitic duration is the days from infestation of glochidia to the successful recovery of fully metamorphosed juvenile mussels.

| Mussel Species | Largemouth Bass | | Hybrid Bluegill | | Parasitic |
|---|---|---|---|---|---|
| | Number Infested | # Juveniles Recovered | Number Infested | # Juveniles Recovered | Duration (days) |
| *Elliptio lanceolata*[1] | 4 | 7 | 4 | 13 | 16 - 23 |
| *Elliptio lanceolata*[2] | 18 | 0 | 18 | 0 | --- |
| *Villosa vaughaniana*[a] | 3 | 1 | 3 | 8 | 20 |
| *Villosa vaughaniana*[b] | 0 | nt | 5 | 766 | 13 - 30 |
| *Villosa constricta* | 3 | 0 | 3 | 0 | --- |
| *Pyganodon cataracta* | 3 | 0 | 3 | 0 | --- |

[1] Gravid mussels collected from the Tar R. at Vance and Franklin Co. Line; [2] Gravid mussels collected from Swift Cr. Johnston Co.; [a] First trial; [b] second trial; nt = not tested

## DISCUSSION

It is clear that hybrid bluegill serve as a suitable host for *V. vaughaniana*, yielding high numbers of fully metamorphosed juveniles. Despite the close phylogenetic relationship to *V. vaughaniana*, metamorphosis of glochidia of *V. constricta* via hybrid bluegill was unsuccessful. Results of fish host studies may vary due to possible experimental error and unknown and uncontrollable influences. Some of these influences may include high stress levels experienced by the gravid mussel and host fish when maintained in captivity and subjected to artificial infestation procedures. Consequently, negative results in this fish host study are not definitive.

In this study, *P. cataracta* glochidia did not attach to fish and attachment of *E. lanceolata* glochidia was not evident. One possible explanation for this is that extracted glochidia may not have been mature enough to attach to fish gills or fins. Even though, prior to each infestation procedure, a sample from each brood tested positive for viability using the saline test described earlier. The reliability of the saline test has not been proven. Another potential reason why attachment was not evident may have resulted from possible inhibitory properties of the mucus secreted by the gravid *P. cataracta* and *E. lanceolata*. The excretion of mucus may be a stress response to manual extraction of glochidia from the marsupial gills. The origin and function of this mucus is not understood and its influence on glochidial attachment is purely speculative. Although both largemouth bass and hybrid bluegill were confirmed as hosts for *E. lanceolata* collected from the Tar R., the number that successfully metamorphosed from infected fish was low. Therefore, the suitability of largemouth bass and hybrid bluegill as hosts for *E. lanceolata* is unclear. Furthermore, transformation success using largemouth bass and hybrid bluegill was negative for glochidia obtained from *E. lanceolata* collected from Swift Cr. (Nuese R. system). This conflicting result raises questions about fish host suitability among conspecific mussels

collected from different drainages. Further investigation is needed to evaluate the variability of glochidia and their affinity to potential hosts, and the relationship between fish hosts and conspecific mussels from different drainage basins.