Final Report
Propagation of freshwater mussels for release into North Carolina waters

Submitted To:

North Carolina Department of Transportation
(Project Number: HWY-2005-07)
FHWA/NC/2006-37
May 2007

Prepared By:

Chris B. Eads[1]
Morgan E. Raley[1]
Erin K. Schubert[1]
Arthur E. Bogan[2]
Jay F. Levine[1]

[1]North Carolina State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606

[2]North Carolina Museum of Natural Sciences
Research Laboratory
4301 Reedy Creek Road
Raleigh, NC 27606

Technical Report Documentation Page

1. Report No. **FHWA/NC/2006-37**	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle **Propagation and Culture of Freshwater Mussels for Release into North Carolina Waters**		5. Report Date **May 2007** 6. Performing Organization Code
7. Author(s) **Chris B. Eads, Morgan E. Raley, Erin K. Schubert, Arthur E. Bogan, and Jay F. Levine**		8. Performing Organization Report No.
9. Performing Organization Name and Address **NC State University** **4700 Hillsborough Street** **Raleigh, NC 27606** **NC Museum of Natural Sciences** **4301 Reedy Creek Road** **Raleigh, NC 27607**		10. Work Unit No. (TRAIS) 11. Contract or Grant No.
12. Sponsoring Agency Name and Address **North Carolina Department of Transportation** **Research and Analysis Group** **1 South Wilmington Street** **Raleigh, North Carolina 27601**		13. Type of Report and Period Covered **Final Report** **July1, 2004-June 30, 2006** 14. Sponsoring Agency Code **2005-07**
Supplementary Notes:		

16. Abstract

Propagation and culture of freshwater mussels has been identified as important to their conservation. Though some propagation had been done previously in North Carolina, it was done on a small scale and for experimental purposes only. The purpose of this project was to further the science of mussel propagation and culture and to increase the available resources to facilitate growout. During the project, we increased our lab capabilities and propagated 10 mussel species native to North Carolina. New host fish were determined for several of these species. Raceways for growout were established and evaluated at two state hatcheries, and we were able to culture 4 species to a size suitable for stocking. Additionally, progress was made in maintaining survival of some rare species which previously proved difficult to maintain in captivity. We worked to identify genetic markers, called microsatellites, in one species of mussel to facilitate tracking of propagated animals post-release.

17. Key Words Endangered species, freshwater mussels, propagation, culture, fish, fish hatchery	18. Distribution Statement …		
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 87	22. Price

Form DOT F 1700.7 (8-72) **Reproduction of completed page authorized**

Disclaimer:

The contents of this report reflect the views of the author(s) and not necessarily the views of the University. The author(s) are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of either the North Carolina Department of Transportation or the Federal Highway Administration at the time of publication. This report does not constitute a standard, specification, or regulation.

Acknowledgements:

Support for this project was provided by the U.S. Department of Transportation and the North Carolina Department of Transportation through the Center for Transportation and the Environment, NC State University.

We thank Scott Van Horn and his staff of biologists in the aquatic non-game section of the North Carolina Wildlife Resources Commission for their assistance in the field and support throughout the project. We thank Carl Kittel, Gene Wilson, Rick Bradford and the rest of the hatchery staff at the Table Rock and McKinney Lake State Fish Hatcheries for their support and assistance. They went above and beyond the call of duty to help these culture efforts get off the ground. We thank John Barr for his invaluable work in maintaining fish and mussels in the lab in the early stages of these efforts. For assistance in the field collecting animals, we thank Chris Wood, Shad Mosher, Jason Meador, and Paul Hubert.

Executive Summary:

Freshwater mussels are a highly endangered fauna in North America. Invasive species, loss of water quality and habitat alterations dating back to the 19th century have been implicated in these declines. Rapid declines continue today as humans alter the landscape.

North Carolina has 7 species listed as federally endangered. When construction projects, such as roads and bridges occur near the habitats of these protected animals, a great deal of emphasis is placed on minimizing or avoiding impacts to these species. This often causes conflict between government agencies and the use of considerable financial resources by all parties involved to research the problem and agree upon solutions. Reviving populations of these rare species to sustainable levels to remove them from the endangered species list is the goal of conservation agencies. Delisting of these species is also beneficial to developers and transportation officials whose projects no longer face the full force of the Endangered Species Act.

Propagation and culture of these animals has been identified as one tool that can potentially aid in strengthening the populations of rare species and fighting against future listing of other species in decline. This technology is still in its early stages and much research is needed to improve the captive culture of these animals. Though some propagation had been done previously in North Carolina, it was done on a small scale and for experimental purposes only. The purpose of this project was to further the science of mussel propagation in the state and culture and to increase the available resources to facilitate growout of juvenile mussels.

Freshwater mussel larvae, called glochidia, must attach to the gills or fins of a host fish to complete their life cycle. While attached to the host, the larvae undergo a metamorphosis developing their foot and their internal organs. Often this mussel-fish relationship is species-specific with the mussel requiring a specific fish species or small group of related species. Unfortunately, the necessary hosts for several mussels on the Atlantic slope were unknown prior to this study. During this project, we were able to identify new hosts for eight mussel species, three of which had no hosts previously identified.

A considerable effort was placed into improving the indoor propagation and culture facilities as well as establishing raceway space in a hatchery setting to facilitate mussel growout. Indoors, we added additional aquaria, water chillers, and mussel troughs. We also constructed a recirculating system to be used in feeding trials. We designed, constructed and maintained a culture system at two state hatcheries in North Carolina. These facilities allowed us to grow 4 mussel species to sizes suitable for stocking in the wild.

Our total propagation effort over the project period produced approximately 60,000 juveniles representing 10 species. Survival in the lab varied widely across species and within species between separate propagation efforts. One subfamily of mussels

(Lampsilinae) have typically been the easiest to maintain in captivity among labs who conduct propagation and culture. This was the case here as we had the most success with *Villosa delumbis* and *Lampsilis radiata.* Other Lampsilines, such as *Villosa constricta,* were more difficult to grow. We made progress with 3 Anodontine species (*Alasmidonta viridis, A. varicosa,* and *Strophitus undulatus*) which previously proved difficult to maintain. At the end of this project and at the time of this writing, all of these species remained alive in the lab and have reached an age where long-term survival is expected. Providing cooler temperatures during excystment on the fish and during initial culture phases seemed to be the key in these successes.

We conducted experiments in 3 primary areas of early culture. We tested variability in production between broods from the same location and found significant variation in brood health of *Villosa constricta* from the North Fork Little River (upper Neuse). These results also suggested that the salt exposure test typically used to test brood viability is not precise enough to identify all broods in poor health. We tested multiple food sources and their effects on growth and survival of juvenile mussels. Results of those trials were mixed, and no statistically significant improvements could be made over current feeding practices. Lastly, we tested the long-term survival of juvenile *Lampsilis radiata* at different times to first feeding. Results of those trials suggested that having algae in the aquaria to allow mussels to feed immediately after dropping off the fish may improve the health and survival of juveniles in the long run.

Microsatellites, or short repeated DNA sequences present in all eukaryotic organisms, have been used in forensic, population and conservation genetic applications to measure and monitor demographic data for over a decade. Recent technological advances have made development of these genetic markers easier, though still expensive, for any group of organisms for which basic population genetic data are desired. We are developing these markers for a state endangered species, *Villosa vaughaniana,* to collect baseline population genetic data from which to base our proposed augmentation protocols. We are working to find markers to help researchers define particularly threatened populations (low effective population sizes, low levels of genetic variability, etc.) and to allow tracking of the integration and reproductive success of our artificially propagated stocks when released and monitored in a long-term recovery and management plan. To date, we have developed 15 genetic markers that we hope to test for variability in wild and propagated stocks over the next year. We anticipate generating an additional 100-150 markers in the coming months. In all, we expect to find 20 to 30 markers with informative levels of variation for our proposed genetic studies.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 5: Microsatellite Development

LIST OF FIGURES

Chapter 4: Mussel Culture Experiments

Chapter 5: Microsatellite Development

No Figures

Introduction

Over 55 species of freshwater mussels (Bivalvia: Unionidae) inhabit the surface waters of North Carolina (Bogan 2002). Freshwater mussels are an integral part of aquatic ecosystems. They provide food for a variety of terrestrial and aquatic species, and they filter algae, bacteria, and fine particulate organic matter from the water (McMahon and Bogan 2001). These living filters improve water quality and serve as indicators of pollution and habitat degradation (Goudreau et al. 1993; Foe and Knight, 1987). Unfortunately, the majority of species in North Carolina are considered endangered, threatened, or of special concern with six of them being federally endangered. Declines in mussel populations likely began with agriculture-based mass deforestation in the 1800s (Hughes and Parmalee 1999) and have continued due to stream impoundment, poor agricultural practices, industrial and municipal discharge and urban sprawl (Bogan 1993). Habitat protection is the preferred way to conserve these species, but in some cases, habitat protection alone will not bring mussel populations back to healthy levels. Biologists have recognized artificial propagation and culture of freshwater mussels as an important tool for augmenting and restoring some populations (NNMCC 1998).

Artificial propagation involves raising larval mussels through a required life-stage where they must attach to and parasitize the gills or fins of fish. Once attached, larval mussels, called glochidia, are encysted by the fish's epithelial cells. They remain encysted on their fish host for several days to several weeks, metamorphose into their juvenile form, and then drop off the host as juvenile mussels. This relationship is often very species specific, with a particular mussel species needing a particular fish species or small group of fish hosts. Unfortunately, the host fish species is unknown for many of North Carolina's resident mussel species (Bogan 2002). Research was needed to determine suitable host species for the state's fauna – especially those along the Atlantic Slope.

Mussel propagation for restocking was originally attempted back in the early 1900s as a way of replenishing stocks depleted by harvest for the pearl button industry (Coker et al. 1921; Lefevre and Curtis 1908; Lefevre and Curtis 1910). After harvest for the button industry subsided, little work was done on mussel propagation until the last decade. Several laboratories around the country, including ours at NCSU, have begun to experiment with propagation and culture of these species. This renewed interest in mussel propagation has brought about the use of indoor recirculating systems (Gatenby et al. 1997; Eads and Layzer 2002, Barnhart 2006) as well as hatchery raceways (Westbrook 1999; Hanlon 2000, Welke et al. 2000) for the rearing of juveniles. While some species, particularly those of the subfamily Lampsilinae, have faired well in captivity, other species are released into the wild at less than 1mm in length (Jones and Neves 1999) to avoid inevitable mortality in the laboratory. Releasing juveniles at a small size (<1 mm) likely results in high mortality and unknown contribution to wild populations. The reasons behind poor survival of some species in captivity are unknown but can likely be attributed to the use of immature glochidia, limited knowledge of species requirements, heightened sensitivity of some species, and inadequate diet and

water quality in a laboratory setting. Additional research is needed to continue to resolve culture techniques to grow mussels in captivity.

Hatchery settings, in which water is directly circulated through the rearing facility from adjacent streams or reservoirs, provide a more natural diet and have yielded better survival and growth of some species compared to laboratory systems (Hanlon 2000; Eads and Layzer 2002). Because of the increased growth and survival as well as physical space for growout, fish hatcheries can be a highly valuable asset to conservation programs seeking to stock mussels into the wild. With indoor systems, water quality is restricted to that provided by the municipal water source, and forage is often limited to those algae that can be easily cultured or purchased commercially.

As conservation agencies look toward potentially using hatchery stock to preserve species, some concerns over the preservation of genetic diversity arise. Advancements in genetic technologies during the 1980s and 1990s have aided wildlife managers in making informed decisions concerning the conservation of imperiled species (Avise and Hamrick 1996). Scientists have discovered that lack of foresight in past fish stocking programs has limited genetic diversity and fitness in some populations (Waples 1991). Now, genetic technologies previously used to help preserve forms of terrestrial wildlife are now being developed by our laboratory and others to address management issues involving freshwater mussels (Eackles and King, 2002; Geist, et al., 2003). Microsatellite analysis is one tool that has been recently developed that can address some of the questions posed by the stocking of artificially propagated animals. Microsatellites are short repetitive DNA sequences distributed across the nuclear genome of all organisms. They have been used to determine parentage, examine genetic diversity, and document severity and timing of bottleneck events in wild populations (Walker et al. 2002). While development of these markers is time-consuming and costly (screening the entire genome for these small 2, 4, or 6 base-pair repeat segments), they are essential for developing a propagation and stocking program that will support long-term conservation of these species.

The goal of this project was to conduct research to improve propagation and culture practices of North Carolina mussel species and to initiate a propagation and culture program that could produce mussels for stocking into the wild. Specific objectives included:

1. *Establishing locations for testing the augmentation and reintroduction of mussel species with captive-reared juveniles,*
2. *Identifying mussel species that represent a variety of taxonomic groups and ecological requirements for potential culture and stocking into the identified locations,*
3. *Enhancing existing laboratory facilities at the NCSU Mussel Propagation Facility for increased production and experimentation capabilities,*
4. *Establishing raceway space in North Carolina for the growout of the selected mussel species,*
5. *Propagating and culturing selected species,*

6. Conducting experiments to identify host fish species and determine effective protocols for maximizing long-term growth and survival of these species in captivity,

7. Developing genetic markers (microsatellites) of one selected species for use in assessing genetic diversity and parentage patterns,

8. Releasing cultured juveniles into identified waters.

Literature Cited:

Avise, JC, and JL Hamrick (eds.). 1996. Conservation Genetics: Case Histories from Nature. Chapman & Hall, New York 512 pp.

Barnhart, M. C. 2006. A compact system for rearing juvenile freshwater bivalves. Aquaculture. 254:227-233.

Bogan, AE. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. American Zoology. 33:599-609.

Bogan, AE. 2002. Workbook and key to the freshwater bivalves of North Carolina. North Carolina Museum of Natural Sciences, Raleigh, NC. 101 pp. 10 color plates.

Coker, RE, AF Shira, HW Clark, and AD Howard. 1921. Natural history and propagation of fresh-water mussels. Bulletin of the Bureau of Fisheries. 37:75-181

Eackles, MS, and TL King. 2002. Isolation and characterization of microsatellite loci in *Lampsilis abrupta* (Bivalvia: Unionidae) and cross-species amplification within the genus. Molecular Ecology Notes. 2:559-562.

Eads, CB. 2000. Chemical marking of juvenile freshwater mussels. MS Thesis. Tennessee Technological University, Cookeville, Tennessee. 46 pp.

Eads, CB, and JB Layzer. 2002. How to pick your mussels out of a crowd: using fluorescence to mark juvenile freshwater mussels. Journal of the North American Benthological Society. 21(3):476-486.

Foe, C, and A Knight. 1987. Assessment of the biological impact of point source discharges employing Asiatic clams. Archives of Environmental Contamination and Toxicology. 16:39-51.

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

Geist, J, O Rottmann, W. Schröder, and R Kühn. 2003. Development of microsatellite markers for the endangered freshwater pearl mussel *Margaritifera margaritifera* L. (Bivalvia: Unionoidea). Molecular Ecology Notes. 3:444-446.

Goudreau SE, RJ Neves, and RJ Sheehan. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia. 252: 211-230.

Hanlon, SD. 2000. Release of juvenile mussels into a fish hatchery raceway: a comparison of techniques. MS Thesis. Virginia Tech University, Blacksburg, Virginia.

Hansen, MM, DE Ruzzante, EE Nielsen, and K-LD Mensberg. 2001. Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. Ecological Applications. 11(1):148-160.

Hughes, MM, and PW Parmalee. 1999. Prehistoric and modern freshwater mussel (Mollusca: Bivalvia: Unionoidea) faunas of the Tennessee River: Alabama, Kentucky, and Tennessee. Regulated Rivers: Research and Management 15:25-42.

Johnson, CS. 2002. Hatchery enhancement: a geneticist reports on the risks and benefits. Aquaculture Magazine. 28(5):8-15.

Jones, JW, and RJ Neves. 1999. Life history and artificial culture of endangered mussels. Annual progress report 1998. Department of Fisheries and Wildlife Sciences, Virginia Tech University, Blacksburg, Virginia. Submitted to Tennessee Wildlife Resource Agency, Nashville, Tennessee.

Lefevre, G, and WC Curtis. 1908. Experiments in the artificial propagation of fresh-water mussels. Proceedings of the Fourth International Fisheries Congress. Washington, D.C. Bulletin of the Bureau of Fisheries. 28:613-628.

Lefevre, G, and WC Curtis. 1910. Studies on the reproduction and artificial propagation of fresh-water mussels. Bulletin of the Bureau of Fisheries. 30:105-201.

McMahon, RF and AE Bogan. 2001. Mollusca: Bivalvia. Pp. 331-429. IN: J.H. Thorpe and A.P. Covich. Ecology and classification of North American freshwater invertebrates. 2nd edtiion. Academic Press.

Neves, RJ, AE. Bogan, JD Williams, SA Ahlstedt and PD Hartfield. 1997 (March 1998). Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. pp. 43-86. IN: G.W. Benz and D.E. Collins (eds). Aquatic fauna in peril: The southeastern perspective. Special Publication No. 1, Southeast Aquatic

Research Institute, Lenz Design and Communications, Decatur, GA. 554 pp. [Published May 1998]

NNMCC (National Native Mussel Conservation Commission). 1998. National strategy for the conservation of native freshwater mussels. Journal of Shellfish Research. 17:1419-1428.

Stickney, RR. 1994. Use of hatchery fish in enhancement programs. Fisheries. 19(5):6-13.

van Snik Gray, E, WA Lellis, JC Cole, and CS Johnson. 2002. Host identification for *Strophitus undulatus* (Bivalvia: Unionidae), the creeper, in the Upper Susquehanna River basin, Pennsylvania. American Midland Naturalist. 147(1):153-161.

Walker, D, BA Porter, and JC Avise. 2002. Genetic parentage assessment in the crayfish Orconectes placidus, a high-fecundity invertebrate with extended maternal brood care. Molecular Ecology. 11:2115-2122.

Waples, RS. 1991. Genetic interactions between hatchery and wild salmonids—lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences. 47:968-976.

Watters, G.T., O'Dee, S.H., Chordas, S. and J. Rieger. 1999. Potential hosts for *Villosa constricta.* Triannual Unionid Report 17: 35.

Welke, K, T Turner, R Gordon, V. Hyde, and P Thiel. 2000. Propagation of the federally endangered Higgin's eye pearlymussel (*Lampsilis higginsi*) at the Genoa National Fish Hatchery as a survival strategy. Interim report to the US Fish and Wildlife Service. 6 pp.

Westbrook, OJ. 1999. Production of juvenile mussels in fish hatchery raceways. MS Thesis, Tennessee Technological University, Cookeville, Tennessee.

Williams, JD, ML Warren, KS Cummings, JL Harris, and RJ Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries. 18(9): 6-22.

Zale, A.V., and R.J. Neves. 1982. Identification of a fish host for Alasmidonta minor (Mollusca: Unionidae). American Midland Naturalist 107(2):386-388.

Chapter 1: Fish Host Identification

Introduction

Freshwater mussel reproduction is dependent on the sympatric presence of freshwater mussel species and the species needed for glochidial attachment. Glochidia, freshwater mussel larvae, must attach to the gills or fins of an appropriate fish species to complete their metamorphosis. For many mussel species in North Carolina, the host fish species is unknown, and this is especially true among Atlantic slope species (Bogan 2002). Fish hosts can be determined in multiple ways. One way is to collect a sufficient sample of all fish species in a stream at a site where the mussel in question is common during glochidial release. These studies are particularly intensive. The gills are then checked for the presence of glochidia, and attempts are made to identify the glochidia that have attached. Both genetic techniques and direct microscopy can be used to correctly identify attached species. This method has the advantage of documenting actual infestation of the fish in the wild, but it also has drawbacks. First, it typically requires a large sample of fish, and sacrifice of the fish is often necessary to fully assess the gills. Also, encystment by a particular mussel does not guarantee transformation, and although somewhat unlikely, you may be seeing attachment to a non-host fish species. Attachment to a particular fish at a given time does not necessarily mean that those glochidia will successfully metamorphose into a juvenile mussel. Non-host fish species will undergo an immune response and eventually slough glochidia. Lastly, glochidia can be difficult to identify to the species level. The work presented in this chapter represents our findings using an alternative method, exposure of fish to glochidia in the laboratory. Fish of multiple species were exposed to glochidia from the mussel species of interest then held in separate aquaria. Fish species that supported transformation were then determined to be suitable fish hosts. This method may not definitively reveal all fish hosts actually used by a mussel species in the wild, but since our focus was propagation, simple transformation was an adequate indicator of suitable hosts.

Methods

In all host trials performed during this research, gravid female mussels were collected from the wild and brought into the laboratory. Brooding females were stored in chilled tanks (6 – 14º C) and fed cultured green algae (*Franceia sp*.) or a mixture of cultured algae and two commercially produced algal diets of *Nannochloropsis, Isochrysis, Pavlova, Tetraselmis*, and *Thalassiosira weissflogii* (Reed Mariculture Inc., Campbell, CA). Glochidia were extracted from the gravid females by flushing their marsupia (the portion of the gills where glochidia are stored) with a water filled syringe. There are two primary methods used in the infestation (attachment of glochidia to the gills or fins) of fish. During this project, we infested fish both by hand and by using the batch infestation method.

To infest fish by hand, we anesthetized the fish with tricaine methanesulfonate (Finquel®; Argent Laboratories, Inc., Redmond, WA) and pipetted the glochidia on to the gills or fins. Batch infestations were done by placing fish in a small volume of water with the glochidia and aerating vigorously from 10 – 45 minutes. The volume of water varied with the number and size of fish being infested as well as with the number of

glochidia available. As the fish respired, suspended glochidia circulated over the gills and would attach on contact. Fish were monitored during batch infestations to determine when a sufficient number of larvae had attached. After infestation, fish were held in aquaria ranging from 6 – 380 L, and each species of fish was held in separate aquaria to ensure transformed glochidia could be attributed to a specific fish species. Two weeks after infestation, water from each aquarium was siphoned through a 150-µm-mesh sieve. The contents of the sieve were rinsed into a Petri dish and examined using a dissecting microscope. Live juveniles could be distinguished by the presence of adductor muscle scars or by foot movement.

Results and Discussion

We identified viable hosts previously undocumented for the following species:

Brook Floater	*Alasmidonta varicosa*	State Endangered
Paper Pond Shell	*Pyganodon cataracta*	Regionally abundant
Creeper	*Strophitus undulatus*	State Threatened
Notched Rainbow	*Villosa constricta*	State Special Concern
Eastern creekshell	*Villosa delumbis*	Significantly rare
Carolina creekshell	*Villosa vaughaniana*	State Endangered
Yellow lampmussel	*Lampsilis cariosa*	State Endangered
Undescribed species (Upper Tar, Neuse, and Cape Fear)	*Lampsilis sp.*	N/A

Alasmidonta varicosa

Previously published information on *Alasmidonta varicosa* hosts gave little information on host suitability (Wicklow and Richards 1995). We transformed juveniles of this species on 9 fish species from 3 families (Table 1.1). Success varied between infestation events and within fish species. Although our first trial with white shiners (*Luxilus albeolus*) and fantail darters (*Etheostoma flabellare*) produced a moderate number of juveniles, our second trial with these species was less successful. Our first trial with bluegill (*Lepomis macrochirus*) produced no juveniles while subsequent trials were quite productive. Wicklow, who conducted much of the early host work on this species in the Northeast, suggested we use johnny darters (Etheostoma nigrum) or the closely related tesselated darter (*E. olmstedi*); however, an infestation of 85 tessellated darters yielded no juveniles. A later infestation with johnny darters did produce juveniles. The reason behind this variability is unknown.

The greatest number of juveniles were produced by piedmont darters (*Percina crassa*) and bluegill. For future production of *A. varicosa* species in North Carolina we recommend these fish species for production. Because bluegill are larger and easier to obtain, this would likely be the best species to use for propagation of large numbers of juveniles. Given their physiological similarities, other sunfish species (*Lepomis spp.*) may be equally suitable. The variability in our results and the differences from previous work with the species shows that more host research is needed for *A. varicosa*.

Table 1.1 Results of host trials with *Alasmidonta varicosa*.

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles transformed
Denson's Creek (Yadkin-Pee Dee)	12/29/04	Redbreast sunfish	*Lepomis auritus*	11	16
		Bluegill	*Lepomis macrochirus*	1	0
Denson's Creek (Yadkin-Pee Dee)	2/9/05	Tesselated darter	*Etheostoma olmstedi*	85	0
		Piedmont darter	*Percina crassa*	10	12
		Margined madtom	*Noturus insignis*	10	0
		Highfin shiner	*Notropis altipinnis*	15	0
		Whitemouth shiner	*Notropis alborus*	2	0
		Blacknose dace	*Rhinichthys atratulus*	3	4
		Mottled sculpin	*Cottus bairdi*	Unknown-accidental	5
Mitchell River (Yadkin-Pee Dee)	3/17/05	White Shiner	*Luxilus albeolus*	5	13
		Fantail darter	*Etheostoma flabellare*	25	53
		Piedmont darter	*Percina crassa*	10	81
		Roanoke darter	*Percina roanoka*	18	27
		Johnny darter	*Etheostoma nigrum*	6	16
		Bluegill	*Lepomis machrochirus*	3	184
Mitchell River (Yadkin-Pee Dee)	3/17/06	White Shiner	*Luxilus albeolus*	24	11
		Fantail darter	*Etheostoma flabellare*	17	18
Mitchell River (Yadkin-Pee Dee)	4/8/06	Mixed sunfish spp	*Lepomis spp.*		207
		Bluegill	*L. macrochirus*	9	?
		Redbreast sunfish	*L. auritus*	44	?
		Green sunfish	*L. cyanellus*	2	?
		Pumpkinseed	*L. gibbosus*	1	?
		.			

Pyganodon cataracta

On 8 February 2005, we collected fish using seine and backpack electroshocker from Terrell's Creek in Chatham County, NC for use in propagation of *Alasmidonta varicosa.* Fish were infested the next day with *A. varicosa* glochidia and held at 14° C in 45-L aquaria. From the aquarium with highfin shiners (*Notropis altipinnis*), we recovered 3 live juveniles on 28 February and 2 live juveniles on 2 March 2005 that, upon visual examination, were obviously not *A. varicosa*, but rather were determined to be *Pyganodon cataracta.* This is a relatively common species at the fish collection site. Thus it is assumed that one or more of the highfin shiners collected had been infested in the wild before being captured for propagation. We then concluded one host of *P.*

cataracta is the highfin shiner. This is also documentation of another species of the subfamily Anodontinae releasing glochidia during the winter.

Strophitus undulatus

Strophitus undulatus has been shown to be a generalist in its use of different host species. It has previously been recorded transforming successfully on many fish species across several families and even on *Notophthalmus viridescens*, the red-spotted newt (van Snik Gray et al. 2002). Our research confirms this generalist behavior for Atlantic Slope North Carolina populations as we documented transformation of *S. undulatus* on 6 fish species (Table 1.2).

Table 1.2. Results of propagation trials with *Strophitus undulatus*

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles transformed
Little River (Yadkin-Pee Dee)	3/18/05	White shiner	*Luxilus albeolus*	34	177
		Fantail darter	*Etheostoma flabellare*	27	147
		Redbreast sunfish	*Lepomis auritus*	1	8
		Green sunfish	*Lepomis cyanellus*	1	10
		Bluegill	*Lepomis macrochirus*	6	16
Tar River	3/29/06	White shiner	*L. albeolus*	20	614
		Roanoke darter	*Percina roanoka*	3	3
North Fork Little River (Neuse)	3/30/06	Assorted Sunfish spp. (fish in same tank)	*Lepomis spp.*	15 total	748
		Warmouth	*L. gulosus*	1	?
		Bluegill	*L. macrochirus*	4	?
		Green sunfish	*L. cyanellus*	1	?
		Redbreast sunfish	*L. auritus*	9	?

Villosa constricta

Watters et al. (1999) used lab trials to report several potential hosts for *Villosa constricta*; however, a majority of those reported hosts do not co-occur with this species. Transformation success was limited with no more than 5 juveniles produced from any single fish species. We found that the fantail darter (*Etheostoma flabellare*) served as the best host (Table 1.3). Strangely, fantail darters are not present in all streams where *V. constricta* exist, yet we were unable to find an additional host in the lab. More host trials should be conducted with this species.

Table 1.3. Results of host trials with *Villosa constricta.*

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles transformed
North Fork Little River (Neuse)	4/14/05	Margined madtom	*Noturus insignis*	3	0
		Flat bullhead	*Ameiurus platycephalus*	1	0
		Northern hogsucker	*Hypentelium nigricans*	2	0
		Notchlip Redhorse	*Moxostoma collapsum*	4	0
		Swallowtail shiner	*Notropis procne*	1	0
		Satinfin shiner	*Cyprinella analostana*	5	0
		White shiner	*Luxilus albeolus*	4	0
		Bluehead chub	*Nocomis leptocephalus*	3	0
		Largemouth bass	*Micropterus salmoides*	1	0
		Bluegill	*Lepomis macrochirus*	2	0
		Green sunfish	*Lepomis cyanellus*	2	0
		Redbreast sunfish	*Lepomis auritus*	1	0
		Warmouth	*Lepomis gulosus*	1	0
		Fantail darter	*Etheostoma flabellare*	4	14
		Glassy darter	*Etheostoma vitreum*	1	0
		Roanoke darter	*Percina roanoka*	1	0
North Fork Little River (Neuse)	5/18/05	Fantail darter	*Etheostoma flabellare*	123	192
		Roanoke darter	*Percina roanoka*	6	1
North Fork Little River (Neuse)	4/17/06	Fantail darter	*Etheostoma flabellare*	45	72
		Johnny darter	*Etheostoma nigrum*	5	1

Villosa delumbis

Any host fish species for *Villosa delumbis* were unknown prior to this study. Through multiple infestations with this species, we found that at least 5 sunfish species (*Lepomis* spp.) will serve as viable hosts for this mussel (Table 1.4). Bluegill, redbreast sunfish, green sunfish, warmouth, and redear sunfish all were viable hosts in the lab.

Table 1.4. Results of host trials and propagation efforts with *Villosa delumbis*

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles transformed
Little River (Yadkin-Pee Dee)	4/7/04	Largemouth bass	*Micropterus salmoides*	3	0
		Bluegill	*Lepomis macrochirus*	1	4
		Redbreast sunfish	*Lepomis auritus*	10	162
		Green sunfish	*Lepomis cyanellus*	6	110
		Tessellated darter	*Etheostoma olmstedi*	15	0
		Highfin shiner	*Notropis altipinnis*	3	0
		Whitemouth shiner	*Notropis alborus*	2	0
		White shiner	*Luxilus albeolus*	6	0
		Bluehead chub	*Nocomis leptocephalus*	15	0
		Margined madtom	*Noturus insignis*	8	0
		Notchlip Redhorse	*Moxostoma collapsum*	2	0
		Pirate perch	*Aphredoderus sayanus*	1	0
Little River (Yadkin-Pee Dee)	5/12/04	Hybrid bluegill	*L. macrochirus x cyanellus*	7	40
		Bluegill	*L. macrochirus*	8	29
		Redbreast sunfish	*L. auritus*	1	104
		Green sunfish	*L. cyanellus*	3	107
West Fork Little R. (Yadkin–Pee Dee)	11/1/04	Redbreast sunfish	*L. auritus*	20	1521
Denson's Creek (Yadkin-Pee Dee)	11/1/04	Redbreast sunfish	*L. auritus*	20	370
Little River (Yadkin-Pee Dee)	3/8/05	Redbreast sunfish	*L. auritus*	12	765
Deep River (Cape Fear)	9/23/05	Bluegill	*L. macrochirus*	1	151
		Redbreast sunfish	*L. auritus*	7	1531
Little River (Yadkin-Pee Dee)	11/28/05	Redbreast sunfish	*L. auritus*	11	1787
Denson's Creek (Yadkin-Pee Dee)	1/20/06	Redbreast sunfish	*L. auritus*	10	674

Table 1.4 continued.

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles transformed
Upper Creek (Catawba)	3/17/06	Mixed sunfish spp.	*Lepomis spp.*	11	1739
		Redbreast sunfish	*L. auritus*		
		Green sunfish	*L. cyanellus*		
		Redear sunfish	*L. microlophus*		
		Warmouth	*L. gulosus*	1	80
Upper Creek (Catawba)	4/17/06	Bluegill	*L. macrochirus*	1	80
		Warmouth	*L. gulosus*	1	70
		Redear sunfish	*L. microlophus*	2	203

Villosa vaughaniana

Hanlon (2001) tested largemouth bass (*Micropterus salmoides*) and hybrid bluegill (*Lepomis macrochirus* x cyanellus) as potential hosts for *Villosa vaughaniana* and found that hybrid bluegill served as a viable host for this species. During this project, we found that multiple sunfish species (*Lepomis spp*.) will serve as hosts for V. vaughaniana (Table 1.5).

Table 1.5. Results of host trials and propagation efforts with *Villosa vaughaniana*.

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles produced
West Fork Little R. (Yadkin-Pee Dee)	11/1/04	Hybrid bluegill	*L. macrochirus x cyanellus*	85	391
		Bluegill	*L. macrochirus*	26	226
		Redbreast sunfish	*L. auritus*	10	460
		Green sunfish	*L. cyanellus*	6	1283
		Pumpkinseed	*L. gibbosus*	1	596
Little River (Yadkin-Pee Dee)	11/28/05	Redbreast sunfish	*L. auritus*	10	3573
Denson's Creek (Yadkin-Pee Dee)	1/20/06	Redbreast sunfish	*L. auritus*	8	680

Lampsilis cariosa

An infestation of 40 largemouth bass yielded 21,853 live juveniles of *Lampsilis cariosa*. Although this host species had not been documented previously, it is no surprise that largemouth bass serve as a host for this species as largemouth bass serve as host for several other *Lampsilis* species (Parmalee and Bogan 1998).

Lampsilis sp.

This species of *Lampsilis* is thought to be an undescribed species that occurs primarily in the upper Neuse and Tar River basins. It is also known to occur in one stream (New Hope Creek, Orange County, NC) in the Cape Fear River Basin. We conducted two host trials to identify the host fish of this species and found that largemouth bass (*Micropterus salmoides*), roanoke bass (*Ambloplites cavifrons*), and redfin pickerel (*Esox americanus*) serve as suitable hosts (Table 1.6). A very small number of juveniles transformed on redbreast sunfish (*Lepomis auritus*), bluegill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*) and eastern mosquitofish (*Gambusia holbrooki*), but these acted as poor hosts at best. We believe that our first trial produced few juveniles because it was held too early in the brooding period for this animal. Although we had success producing other mussels from the same same subfamily (Lampsilinae) in early November, we would recommend this species be produced in the spring rather than in the fall. Because largemouth bass are much easier to acquire than roanoke bass or redfin pickerel, they would serve as a better host for propagation.

Table 1.6. Results of host trials for Lampsilis sp. (Upper Tar/Neuse River Basins)

Source stream	Date of infestation	Fish Species Common Name	Fish Species Scientific Name	Total Number of fish infested	Total Number of juveniles produced
Tar River	11/4/05	Fantail darter	*Etheostoma flabellare*	5	0
		Johnny darter	*Etheostoma nigrum*	9	0
		Roanoke darter	*Percina roanoka*	5	0
		Margined madtom	*Noturus insignis*	1	0
		Flat bullhead	*Ameiurus platycephalus*	1	0
		Creek chub	*Semotilus atromaculatus*	1	0
		Creek chubsucker	*Erimyzon oblongus*	6	0
		Bluehead chub	*Nocomis leptocephalus*	2	0
		Bull chub	*Nocomis raneyi*	6	0
		Rosyside dace	*Clinostomus funduloides*	1	0
		Swallowtail shiner	*Notropis procne*	8	0
		White shiner	*Luxilus albeolus*	5	0
		Golden shiner	*Notemigonus chrysoleucas*	5	0
		Eastern mosquitofish	*Gambusia holbrooki*	2	4
		Redbreast sunfish	*Lepomis auritus*	9	1
		Green sunfish	*Lepomis cyanellus*	2	0
		Warmouth	*Lepomis gulosus*	1	0
		Pumpkinseed	*Lepomis gibbosus*	1	0
		Bluegill	*Lepomis macrochirus*	6	0
		Largemouth bass	*Micropterus salmoides*	4	55
Tar River	4/26/06	Roanoke darter	*Percina roanoka*	6	0
		Chainback darter	*Percina nevisense*	1	0
		Johnny darter	*Etheostoma nigrum*	1	0
		Black jumprock	*Moxostoma cervinum*	3	0
		Northern hogsucker	*Hypentelium nigricans*	1	0
		Pirate perch	*Aphredoderus sayanus*	3	0
		Eastern mosquitofish	*Gambusia holbrooki*	3	0
		Bluehead chub	*Nocomis leptocephalus*	1	0
		Pinewoods shiner	*Lythrurus matutinus*	4	0
		White shiner	*Luxilus albeolus*	4	0
		Swallowtail shiner	*Notropis procne*	1	0
		Roanoke bass	*Ambloplites cavifrons*	3	65
		Bluespotted sunfish	*Enneacanthus obesus*	1	0
		Bluegill	*Lepomis macrochirus*	2	1
		Redbreast sunfish	*Lepomis auritus*	4	0
		Pumpkinseed	*Lepomis gibossus*	1	1
		Largemouth bass	*Micropterus salmoides*	4	1146
		Redfin pickerel	*Esox americanus*	2	479

Conclusions

Overall, species in the subfamily Anodontinae (*Alasmidonta varicosa* and *Strophitus undulatus*) seem to be able to use a wide variety of hosts. *Villosa constricta* can use the fantail darter as a host, but there are likely other hosts that could be discovered with additional trials. Overall, we currently recommend the following hosts be used in North Carolina to maximize production:

Mussel Species	Fish Hosts
Alasmidonta varicosa	Bluegill (*Lepomis macrochirus*)
	Piedmont darter (*Percina crassa*)
Strophitus undulatus	Sunfish species (*Lepomis* spp.)
	White Shiner (*Luxilus albeolus*)
Villosa constricta	Fantail darter (*Etheostoma flabellare*)
Villosa delumbis	Sunfish species (*Lepomis* spp.)
Villosa vaughaniana	Sunfish species (*Lepomis* spp.)
Lampsilis cariosa	Largemouth bass (*Micropterus salmoides*)
Lampsilis sp. (undescribed)	Largemouth bass (*Micropterus salmoides*)

Literature Cited

Bogan, AE. 2002. Workbook and key to the freshwater bivalves of North Carolina. North Carolina Museum of Natural Sciences, Raleigh, NC. 101 pp. 10 color plates.

Parmalee, PW, and AE Bogan. 1998. Freshwater mussels of Tennessee. University of Tennessee Press, Knoxville.

Wicklow, BJ, and LD Richards. 1995. Determination of host fish species for glochidia of the endangered freshwater mussel *Alasmidonta varicosa*. Fifth Annual Northeastern Freshwater Mussel Meeting. US Fish and Wildlife Service. Concord New Hampshire.

Chapter 2: Enhancement of Propagation and Culture Facilities and Establishment of Growout Space in a Hatchery Setting

Introduction

Prior to this project, our laboratory had conducted some propagation and culture on a small scale for experimental purposes. To be able to produce enough individuals and grow them to a suitable size for stocking, significant upgrades were necessary. More systems were needed for holding fish in the lab, and more space was needed for the growout of mussels. For mussel growout, we sought to not only improve resources indoors, but also to establish raceway space in an outdoor hatchery setting. Outdoor hatchery settings have been shown to yield significantly better growth than indoor culture systems (Hanlon 2000; Eads and Layzer 2002).

Freshwater Mussel Propagation Laboratory at NC State University

Located at the College of Veterinary Medicine at NC State University, the Freshwater Mussel Propagation Laboratory was the site where most of the research was done for this project. The facility used conditioned municipal water from Raleigh, NC treated with a carbon filter, sodium thiosulfate, Ammo-Lock®. A 1-hp air pump is used to distribute air through a centralized air system to all parts of the facility.

A greenhouse (6.1 x 3.7 m) adjacent to the laboratory is used to culture algae feeding adult and juvenile mussels. Six 250-L conical-bottom transparent culture tubes (45 cm diameter x 152 cm high) were housed within the greenhouse on the south-facing wall. Tubes are well aerated, and an F/2 algal solution (Kent Marine) was used for production of algae (*Scenedesmus* and *Franceia sp.*). All juvenile and adult mussels were fed an even mixture (by approximate cell count) of cultured algae and two commercially available shellfish diets of *Nannochloropsis* , *Isochrysis, Pavlova, Tetraselmis,* and *Thalassiosira weissflogii* (Reed Mariculture Inc.). Mussels were fed 5 times per week to a concentration of 50,000 - 100,000 cells/mL in the culture system. Feeding protocol varied over the period of this project. Initially, only cultured algae was used. Various feeding trials were conducted with detritus and other types of commercial algae, but the algal mixtures from Reed Mariculture were adopted in March 2006 based on success with this feed in other labs culturing freshwater mussels (Barnhart 2006).

Fish holding systems

Host fish were held in various static and recirculating systems in the lab to propagate juvenile mussels. One high volume recirculating system was used for mass production of juveniles (Fig. 2.1) when host fish were large or when we needed to hold a large number of host fish. The system held 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 125-L trickle biofilter. Rubbermaid tubs were elevated and supported by a pressure-treated wooden structure so that the rims of all tubs were approximately 100 cm high.

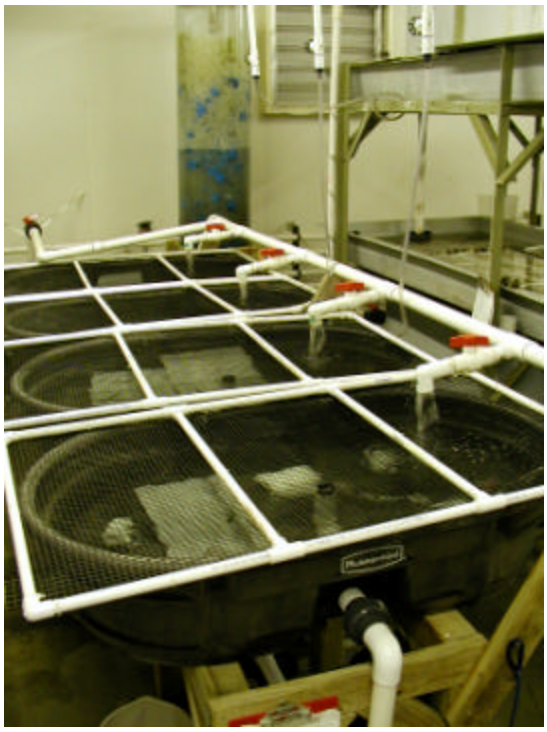

Figure 2.1 High volume recirculating system for mass propagation efforts.

Two identical recirculating systems were used for host trials as well as mass propagation (Fig. 2.2). In each system twelve 45-L aquaria were used to hold fish. Water returned through a standpipe to a submerged filter which also served as the reservoir. Submersible pumps were used to pump water back to the aquaria, and valves on each tank allowed flow to be adjusted.

Figure 2.2. 12-tank recirculating system with submerged biofilter used in host trials and mass propagation efforts

We constructed an additional recirculating system for use with small fishes in host trials. Eight 4-L, four 10-L, and two 75-L aquaria were connected to a 75-L submerged biofilter and 75-L sump where submersible pumps moved water back to the aquaria. Lastly, one 114-L, and one 189-L aquarium, and sixteen 38-L aquaria were used as static tanks with foam biofilters to hold fishes.

Indoor Juvenile Mussel Culture Systems

Juvenile Culture Troughs

Two recirculating systems were used to culture juvenile freshwater mussels (Fig. 2.3). Each system was constructed of six 150-cm-long troughs made from 5-cm PVC pipe cut longitudinally and bracketed on each end by pipe fittings. Mesh traps (150 µm) were fitted on the end of the troughs to catch escaping juveniles. All 6 flow channels shared the same water source but were independently controlled for water flow. A layer of gravel and filter mesh were placed in the reservoirs to provide a substrate for nitrifying bacteria. Water was changed weekly.

Figure 2.3. Recirculating juvenile mussel culture system

Juvenile Mussel Feeding Trial Unit

To conduct feeding trials we constructed a system of 6 troughs with each trough fed by a separate reservoir (Figure 2.4). Troughs were constructed of 5-cm PVC pipe cut longitudinally and bracketed on each end by pipe fittings. Mesh traps were fitted on the end of the troughs to catch escaping juveniles, and water was returned to the corresponding reservoir (38-L aquarium) by way of vinyl guttering. Each reservoir was outfitted with an airstone and small submersible pump to return the water to the head of the trough (approx. 0.5 L/min). A thin (approx. 5 mm) layer of sediment was placed in each trough to provide substrate for the mussels. Water was changed weekly.

Figure 2.4. System used for conducting juvenile mussel feeding trials.

Static Juvenile Tanks

To culture some species below 20°C (room temperature), we constructed a chilled water bath to hold multiple static tanks for holding newly transformed juvenile mussels. A ½ hp chiller with drop-in coil was used to chill water in a 250-L trough (2.4 x 0.6 m). Water was chilled as low as 12°C. Multiple 8-L glass aquaria were placed in the water bath to hold juvenile mussels. Tanks were well-aerated to create flow within each aquarium, and although there was no biofiltration, a 90% water change was done weekly.

Second Stage Indoor Culture System

An existing large trough in the laboratory was retrofitted to facilitate two additional methods of growout of juvenile mussels. The system totaled 1100 L in volume and consisted of a large reservoir, pump, trickle biofilter, settling basin and mussel holding basin. We used vinyl house gutters cut to 1.5 meters and fitted with end caps to divide the holding basin into small troughs (Fig. 2.5). A PVC valve manifold was constructed to direct flow (approx. 6 L/min) separately into each gutter. Holes were drilled in the endcap opposite the valves, and a 1-mm mesh screen was adhered over the holes. These troughs were used to culture mussels indoors once they reached at least 2 mm in length.

At the end of the holding basin, a mesh cage system was used to culture newly transformed juvenile mussels. Mesh cages (250-µm stainless steel) were 100 x 150 mm in size extending above the water line. We installed additional plumbing and 16 plastic valves (Figs. 2.6 and 2.7) and fitted the valves with 4-mm inside-diameter plastic tubing to deliver water flow (100 mL/min) to each mesh cage.

Figure 2.5. Vinyl gutter troughs used for indoor mussel growout

Figure 2.6 Troughs and plumbing for small cages

Figure 2.7. Valves and mesh cages used for indoor mussel growout

Establishment of Hatchery Growout Space

In the fall of 2004, discussions began with the NC Wildlife Resources Commission about the use of space at their fish hatcheries for growing freshwater mussels. After touring 4 hatcheries and discussing options with the manager of a 5th hatchery, we decided to attempt mussel culture at the Table Rock Fish Hatchery near Morganton, NC and the McKinney Lake Fish Hatchery near Hoffman, NC. Both hatcheries had existing raceway space unused by normal operations that was available for use.

Containment of all juvenile mussels was of high priority because we were culturing species not native to the basin where the hatcheries were located. Using existing raceway space, we designed and constructed a system to contain all cultured mussels (Fig. 2.8). At both hatcheries, two troughs were placed on top of an existing concrete raceway and supported by cross-beams. The system was plumbed to deliver water at multiple points along the length of the troughs using a PVC valve-manifold system. Mussels were cultured in mesh cages (20 x 20 x 20 cm) with mesh fine enough to contain the smallest mussel cultured. The tops of the cages extending well above the water line. By directing flow (2-4 L/min) from the valve manifold system directly into each cage, water exchange in the cage was maximized. A frame with a mesh cover was placed over the troughs and secured with padlocks to prevent hatchery visitors from disturbing the system. Water flowed through the mesh cover into the cages and then flowed out into the trough. A standpipe on one end of the trough sent water to the raceway below. The raceways were outfitted with 10-cm diameter standpipes that allowed for near maximum water volume. In the raceway, a 2/3 hp submersible pump was outfitted with a float switch. Before the raceway filled to the top of the standpipe, the float switch triggered the pump to empty the raceway. The pump was slightly elevated off the raceway bottom so it wasn't directly removing objects from the bottom. Water was pumped through a 76-cm diameter fiberglass sand filter then back down the exit drain of the raceway. Sandfilters were filled with artificial media designed to filter particles down to the 20-40 µm range. Since the sandfilter would be bypassed in the event of a power outage, the standpipe was fitted with 250-µm stainless steel mesh. The top of this mesh screen extended above the level of the raceway so that if it were in use and became clogged, water would spill out onto the ground rather than go down the drain. Before mussels were put in the system, this multi-level containment system was examined by NCWRC hatchery personnel and deemed to be effective. Presumably, no juveniles mussels could escape this system.

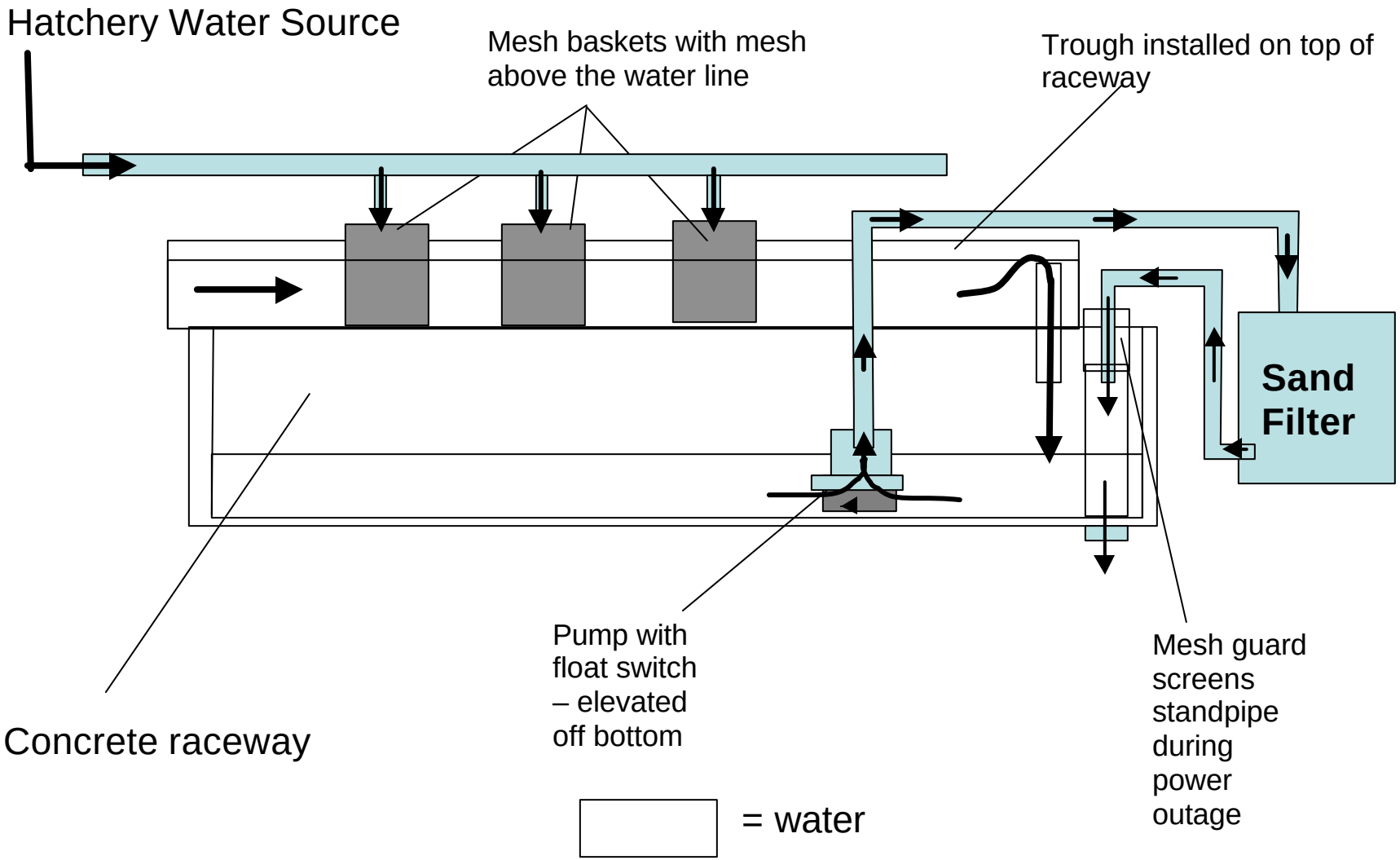

Figure 2.8. Diagram of juvenile mussel containment system employed at each hatchery.

Table Rock Fish Hatchery

The Table Rock Fish Hatchery (TRH) is located near Morganton, NC on Irish Creek in the Catawba River basin. The main water supply is a small (1.4 hectare) reservoir on the creek. Construction and evaluation of the culture system was completed in January 2005 (Figs. 2.9-2.11). A small number of mussels were placed in the trough at Table Rock in January to monitor early survival. In late June, we determined that the main water supply was too cold and infertile to support mussel growth during this part of the year. We began to supplement water from the main reservoir (Fig. 2.12) with water from the warmer, more nutrient-rich ponds (0.4 hectare) where the hatchery grows muskellunge (*Esox masquinongy*) (Fig. 2.13). Initially, pond water was delivered by siphon/gravity to the mussel trough, but flow was intermittent and growth results for 2005 were suboptimal. In 2006, we added a pump to the pond water line, but flow was still intermittent. Hatchery staff determined that air was somehow entering the old line and causing the pipe to lose its prime. We then installed a new pipeline with the help of hatchery staff, and this created consistent flow from the pond to the trough after this. During the heat of the summer of 2006, we used a mix water from the main reservoir and water from the muskellunge culture pond to control temperatures in an optimum range (approx. 22-27°C).

Figure 2.9. Mussel trough at Table Rock Fish Hatchery

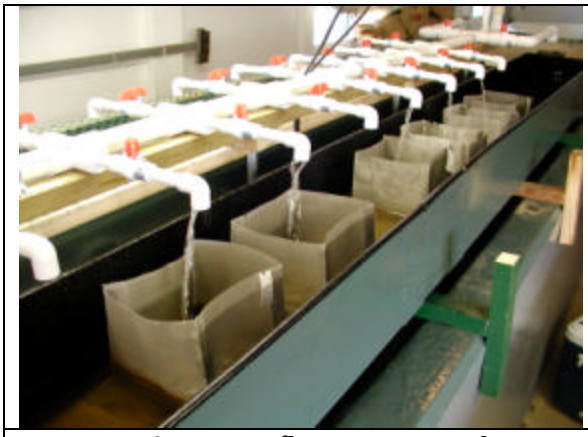

Figure 2.10. Water flow into juvenile mussel cages

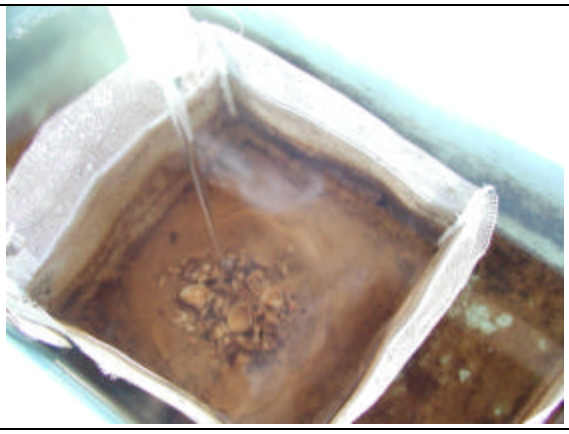

Figure 2.11. Crushed clam shell added to sediment as source of calcium

Figure 2.12. Main reservoir for Table Rock Fish Hatchery

Figure 2.13. Muskie Pond and Flumeline and Table Rock Fish Hatchery

McKinney Lake Fish Hatchery

McKinney Lake is a reservoir (Figs. 2.14-2.15) located near Hoffman, NC on Hitchcock Creek (Fig. 2.13) in the Yadkin-Pee Dee River Basin. The hatchery it supports has more than ample water supply for mussel culture, but because of its location in the Sandhills Ecoregion, the water is tannic, and has a low hardness (approx. 4 mg/L) and pH (4.5-5.5). Despite less than ideal water chemistry, 8 mussel species had been previously documented in Hitchcock Creek (NCWRC aquatics database) indicating that mussels could survive in this water. Our system (Fig. 2.14) was constructed in early May 2005 and mussels were placed there on 24 May 2005.

Figure 2.14. McKinney Lake

Figure 2.15. McKinney Lake Dam

Figure 2.16. Hitchcock Creek below McKinney Lake

Figure 2.17. Mussel culture trough at McKinney Lake Fish Hatchery

Literature Cited

Barnhart, M. C. 2006. A compact system for rearing juvenile freshwater bivalves. Aquaculture 254:227-233.

Eads, CB, and JB Layzer. 2002. How to pick your mussels out of a crowd: using fluorescence to mark juvenile freshwater mussels. Journal of the North American Benthological Society. 21(3):476-486.

Hanlon, SD. 2000. Release of juvenile mussels into a fish hatchery raceway: a comparison of techniques. MS Thesis. Virginia Tech University, Blacksburg, Virginia.

Chapter 3: Propagation and Culture Results/Production

Introduction

One of the goals of this project was to grow mussels to a size that could survive when released in the wild. First we had identified species we could grow (subfamily Lampsilinae) and species that we believed would be difficult to keep alive in captivity (subfamily Anodontinae). Relatively little work has been done with culturing any of the Anodontines. In our lab's initial attempt to rear one of these species (*Lasmigona subviridis*), survival was limited to 2 months (Hanlon 2001). Under the guidance of North Carolina Wildlife Resources Commission (NCWRC) biologists, we selected the appropriate species as well as locations that might be good candidates for receiving artificially produced individuals.

Methods

Propagation

Gravid female mussels of 9 species were collected from the wild from July 2004-March 2006 and transported to the Freshwater Mussel Propagation Laboratory at NC State University where we attempted to propagate juvenile mussels. In most cases, glochidia were extracted from the female with a water-filled syringe as described in Chapter 1. On two occasions (with *Alasmidonta viridis* and *Alasmidonta varicosa* in 2006), we simply caged the gravid mussels with their host fish in the hatchery mussel troughs. Lab propagation was done by infesting fish either by hand or by using a batch infestation method (Chapter 1). Fish were held in tanks of various sizes and transformed glochidia were collected by siphoning the tanks through a 150-µm sieve. Juveniles were counted using a stereomicroscope and considered viable if they had adductor muscle scars or showed foot movement.

Culture/Growout

Once collected and counted, newly transformed juveniles were immediately transferred to one of the culture systems described in Chapter 2. Periodically, we evaluated growth and survival of mussels from all cohorts of juveniles produced. Mussels were sieved from the sediment, counted and measured using either calipers or a 2-mm or 10-mm stage micrometer. Mussels larger than 10 mm were measured using calipers. Often, mussels from the same cohort were split between culture systems or between the lab and the hatchery to determine optimal conditions for growth and survival of a given species.

Results and Discussion

With laboratory infestations, we successfully produced a total of 59,623 juvenile mussels representing 9 species and 7 river basins across North Carolina (Table 3.1).

Table 3.1. Number of mussels propagated in the laboratory by species and location during this project

Species	Basin (Sub-basin)	Date of Infestation	Number of adult females	Number of Juveniles produced
Alasmidonta varicosa	Yadkin-Pee Dee (Little River)	12/29/2004	1	16
Alasmidonta varicosa	Yadkin-Pee Dee (Little River)	2/9/2005	3	12
Alasmidonta varicosa	Yadkin-Pee Dee (Mitchell River)	3/17/2005	6	374
Alasmidonta varicosa	Yadkin-Pee Dee (Mitchell River)	4/8/2005	6	207
Alasmidonta varicosa	Catawba (Upper Creek)	3/17/06	1	23
Alasmidonta viridis	French Broad (Mills River)	1/14/2005	8	819
Lampsilis cariosa	Neuse (Eno River)	3/30/06	2	21,853
Lampsilis fasciola	North Toe River	5/11/2005	1	38
Lampsilis fasciola	Pigeon River	5/11/2005	4	517
Lampsilis radiata	Neuse (Flat River)	5/11/2005	5	3,854
Lampsilis radiata	Neuse (Eno River)	10/26/05	2	5,586
Lampsilis radiata	Neuse (Eno River)	3/30/06	3	1,812
Lampsilis sp.	Tar River (Person Co.)	11/4/05	1	61
Lampsilis sp.	Tar River	4/26/06	2	1,692
Strophitus undulatus	Yadkin-Pee Dee (Little River)	3/18/2005	3	358
Strophitus undulatus	Tar River	3/29/06	1	792
Strophitus undulatus	Neuse (Little River – Orange Co.)	3/29/06	3	705
Villosa constricta	Neuse (Little River – Orange Co.)	5/18/2005	6	192
Villosa constricta	Yadkin-Pee Dee (Little River)	1/18/06	1	436
Villosa constricta	Neuse (Little River – Orange Co.)	3/29/06	6	72
Villosa delumbis	Yadkin-Pee Dee (Little River)	11/1/2004	5	1,891
Villosa delumbis	Yadkin-Pee Dee (Little River)	3/8/2005	1	765
Villosa delumbis	Cape Fear (Deep River)	9/23/05	1	1,682
Villosa delumbis.	Cape Fear (Haw River)	9/23/05	1	179
Villosa delumbis	Yadkin-Pee Dee (Little River)	11/28/05	1	1,787
Villosa delumbis	Yadkin-Pee Dee (Little River)	1/20/06	1	674
Villosa delumbis	Catawba (Upper Creek)	3/16/06	3	1,819
Villosa delumbis	Catawba (Upper Creek)	4/17/06	1	353
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	11/1/2004	11	6,160
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	3/16/2005	1	184
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	11/28/05	1	3,573
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	12/1/05	1	199
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	1/20/06	1	680
Villosa vaughaniana	Yadkin-Pee Dee (Little River)	3/24/06	1	240
Total # of Species: 9	Total River Basins: 7		Total # of Juveniles Produced:	59,605

The following is a summary of the growth and survival assessments of each of the species produced. Individual groups (cohorts), information on their propagation and culture conditions are separated. Cohorts are grouped by species.

Alasmidonta varicosa

Cohort: AvarDensons122904

Gravid Adult Collected: 27 October 2004
Gravid Adult Location: Denson's Creek, Little River Subbasin, Yadkin-Pee Dee
Date Infested: 29 December 2004
Fish Infested: 11 redbreast sunfish
1 bluegill
Fish held at (Temperature): 21
Total Juveniles produced: 16
Days to transformation: 12-17
Culture System: Immediately taken to Table Rock Hatchery (TRH)
Results: There were no survivors found after 3 ½ months (3 May 2005)

Cohort: AvarDensons020905

Gravid Adult Collected: 27 October 2004
Gravid Adult Location: Denson's Creek, Little River Subbasin, Yadkin-Pee Dee
Date Infested: 9 February 2005
Fish Infested: 85 tessellated darters
10 piedmont darters
10 margined madtoms
15 highfin shiners
2 whitemouth shiners
3 blacknose dace
Fish held at (Temperature): 21
Total Juveniles produced: 28
Days to transformation: 21 - 33
Culture System: Chilled static tank (14°C)
TRH (after 2 months)
Results: There were 25 individuals alive in the static tank after 2 months (89.3%). On 3 May 2005, the 25 survivors were taken to the trough at TRH. No survivors were found in the cage on 22 September 2005.

Cohort: AvarMitchell031705

Gravid Adult Collected: 05 March 2005
Gravid Adult Location: Mitchell River, Yadkin-Pee Dee
Date Infested: 17 March 2005
Fish Infested: 10 piedmont darter
18 roanoke darter
25 fantail darter
6 johnny darter
25 white shiner
3 bluegill
Fish held at (Temperature): most at 14°C
Bluegill at 21°C (184 of 374 juveniles)
Total Juveniles produced: 374
Days to transformation: at 14°C: 17 – 33
At 21°C : 11 – 13
Culture System: Indoor juvenile recirculation system: 261 individuals
TRH: 113 individuals
Results: From late April to early May, we added a total of 207 individuals from another infestation (cohort AvarMitchell040805 – See below) to the indoor trough containing this group for a total of 468 mussels in the trough. On 31 August 2005 (almost 5 months old), there were 6 surviving individuals in the indoor recirculating culture system. Length ranged from 0.5 – 1.2 mm. Original length after transformation was 0.3 mm. This group was assessed again 30 November 2005 (8 months old), and only dead shells were found. Shells ranged in length from 1.2 to 1.5 mm. The group taken to TRH after metamorphosis were assessed on 22 September 2005, and no survivors were found.

Cohort: AvarMitchell040805

Gravid Adult Collected: 05 March 2005
Gravid Adult Location: Mitchell River, Yadkin-Pee Dee
Date Infested: 8 April March 2005
Fish Infested: 9 bluegill
44 redbreast sunfish
2 green sunfish
1 pumpkinseed
Fish held at (Temperature): 21°C
Total Juveniles produced: 207
Days to transformation: 14 - 27
Culture System: Indoor juvenile recirculation system – This cohort was added to the trough that contained those *A. varicosa* from the same site from the last infestation (cohort AvarMitchell031705).
Results: See above (AvarMitchell031705).

Cohort: AvarUpper031706

Gravid Adult Collected: 09 March 2006
Gravid Adult Location: Upper Creek, John's River sub-basin, Catawba River Basin
Date Infested: 17 March 2006
Fish Infested: 24 white shiners
17 fantail darters
Fish held at (Temperature): 15°C
Total Juveniles produced: 29
Days to transformation: 20 - 30
Culture System: Chilled Static Tank (15°C)
Results: After 3 months, no survivors were found.

Discussion – Alasmidonta varicosa

As stated in Chapter 2, *Alasmidonta varicosa* is considered a host generalist; however, we found that juvenile production in this species varied from one infestation to the next. Exact reasons for this are unknown, but using fish from the same river basin that the mussel is from may be particularly important in this species. Perhaps the actual host fish varies between basins. Other studies have shown that transformation rates and host species can vary across river basins (Watters et al. 1998, Bigham 2002). The longest we could maintain survival was when both the host fish and newly transformed juveniles were chilled well below room temperature (12 - 14°C). In fact the 2-month survival rate of 89.3% that we had in 1 cohort is extremely high relative to other culture attempts of any species (Hanlon and Levine 2001). Since we found that this species is releasing their glochidia in winter in North Carolina, using lower temperatures in their propagation and culture is likely the best strategy to mimic the natural setting.

With the cohort that survived to 6 months, survival was severely hampered by catastrophic failure of the cooling system in the laboratory during the summer of 2005. Water temperatures in the lab climbed to over 30°C, and subsequent assessments showed a substantial drop in survival of all species in the lab after this event.

Complete mortality was seen in those mussels moved to the Table Rock Hatchery (TRH). While the exact reasons for this are unknown, we were unable to maintain newly transformed juveniles of any species at either hatchery. We suspect predation and extreme water temperatures may play a role in this.

Alasmidonta viridis

Cohort: AvirMills011405

Gravid Adult Collected: 11 January 2005
Gravid Adult Location: Mills River, French Broad River Basin
Date Infested: 14 January 2005
Fish Infested: 75 mottled sculpin
Fish held at (Temperature): 13°C
Total Juveniles produced: 819
Days to transformation: 38 - 106
Culture System: Chilled Static Tank (13°C) – 628 individuals
and TRH – 191 individuals
Results: There was an exceptionally long encystment period on the fish. The last juveniles were recovered from this tank over 3 months from the infestation date, and the peak transformation time occurred around 67 – 70 days. On 3 May 2005, we found only 1 surviving individual in the chilled static tank at the laboratory. That individual had roughly doubled in size (600 µm), and it was taken to TRH. On 12 September 2005, we assessed the cage of *A. viridis* at the hatchery and found no survivors. We found no survivors but only 2 dead shells that had grown to 750 and 850 µm respectively.

Cohort: AvirMills021706

Gravid Adult Collected: 16 February 2006
Gravid Adult Location: Mills River, French Broad River Basin
Infestation: Host fish were divided into eight 250-µm-mesh cages (20 x 20 cm) at MLH on 17 February 2006. In each cage, we also placed 1 gravid *A. viridis*. Mussels were checked for gravidity 2 weeks later, and 4 of the 8 had fully released their glochidia and 1 additional mussel had partially released. At the 3 week mark (8 March 2006), all mussels had released glochidia in the cage.
Infested fish: 76 mottled sculpin
Fish held at (Temperature): Temperature of the hatchery raceway ranged from 11-20 °C
Total Juveniles produced: Fish from 4 of the 8 cages were brought back to the lab and held in tanks to estimate total juvenile production using this method of infestation. The number of juveniles transformed per cage ranged from 20-137; however, production would have been higher had we not experienced some mortality of the host fish before excystment. We estimate that we produced between 400 and 800 juveniles using this method.
Days to transformation: Based on lab transformations, we estimate that all juveniles had finished transformation between 20 April and 30 April 2006 (maximum of 60 days)

Culture System:

TRH (transferred 2 cages of sediment from MLH to TRH) – unknown number
Chilled Static Tank (15°C) - transformed in lab at 20°C = 202 individuals
Chilled Static Tank (15°C) - sediment from 2 MLH cages – unknown number

Results: Sediment (with transformed juveniles) transferred to TRH soon after transformation yielded no live juveniles upon examination roughly 3 ½ months post-transformation (27 July 2006). Of the 202 mussels transformed in the lab, there were no survivors when checked at approximately 3 1/2 months post-transformation (2 August 2006). We also found no shells at that time. In sediment transferred directly from MLH to the lab and maintained at 15°C, we found 24 live juveniles at approximately 4 months post-transformation ranging in length from 1.2-2.0 mm (Figs. 3.1 and 3.2).

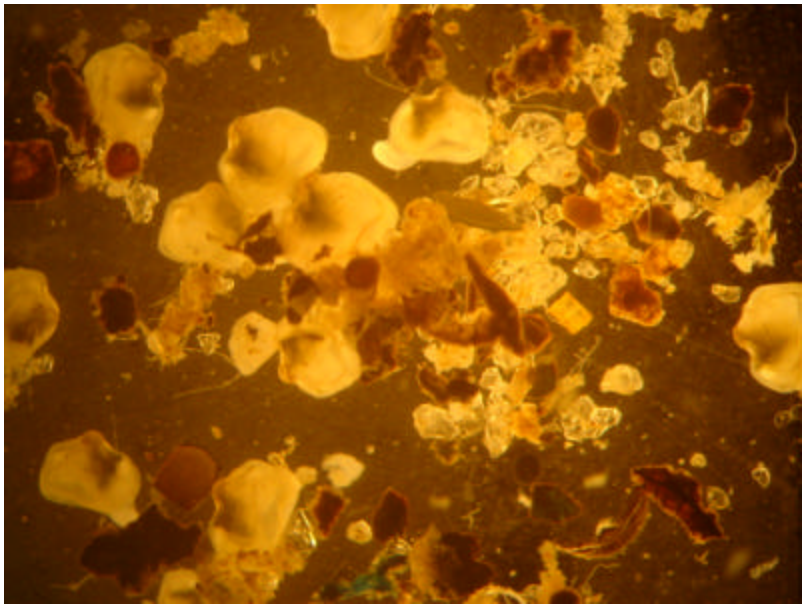

Figure. 3.1. *Alasmidonta viridis* (4-months old) cultured in the laboratory

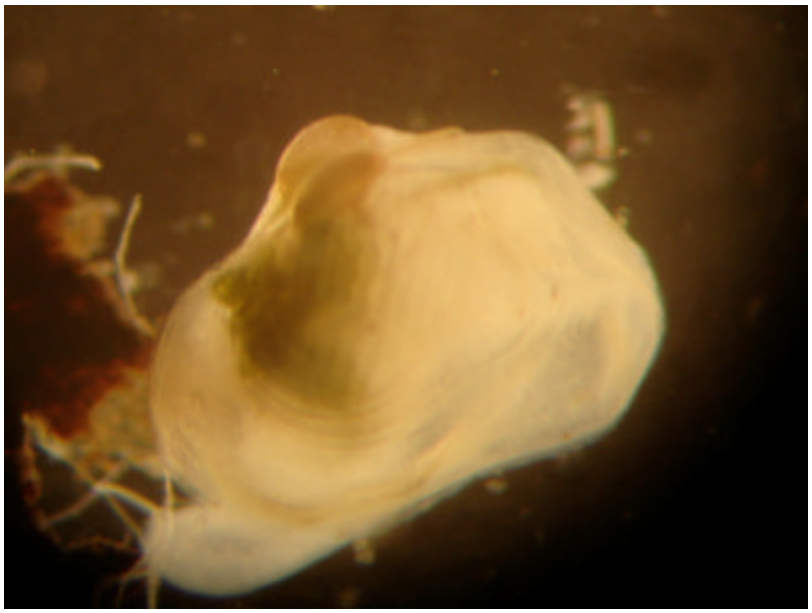
Figure. 3.2. Close-up of *Alasmidonta viridis* (4-months old) cultured in the laboratory

Discussion – Alasmidonta viridis

On 5 March 2004, we examined 140 adult *A. viridis* from the Mills River and found that there were no gravid animals at that time. We then took a small sample of fish from the site and found a mottled sculpin (*Cottus bairdi*) and a fantail darter (*Etheostoma flabellare*) with *A. viridis* glochidia attached. Like *A. varicosa*, this species seems to be releasing glochidia in February. Consequently, cooler conditions during propagation and culture would be best for mimicking the process in the wild setting. The exact reasons for such poor survival of the 2005 cohort in the lab is unknown. There was an outbreak of daphnids in the culture chamber, but we cannot be certain what impact this had on the juveniles. Perhaps the cultured algae fed to this cohort was unsuitable for their dietary needs. Alternatively, perhaps glochidia were not entirely mature when they were used in 2005. In 2006, we allowed the gravid mussels to release glochidia when they were ready to do so. This, along with conducting the infestation later in the year, may have allowed further development of the glochidia and subsequent higher survival in the lab.

As with *A. varicosa* and *S. undulatus*, there was complete mortality of mussels in the lab transformed at ambient lab temperature (approx. 21°C). Conversely, when sediment (containing newly transformed juveniles) was moved directly from the hatchery to the culture tank after transformation, 24 juveniles survived to 4 months. We are unsure what survival rate represents since there was an unknown number of juveniles to begin with; however, culture of this species to 4 months and an average length of 1.6 mm is very encouraging. Even though *A. viridis* is a small mussel (and likely exhibits naturally slow growth) and was cultured at low temperatures, growth rates of the 2006 cohort rivaled those of other faster-growing species produced in the lab.

Strophitus undulatus

Cohort: SundLittle031805

Gravid Adult Collected: 3 March 2005
Gravid Adult Location: Little River, Yadkin – Pee Dee River Basin
Date Infested: 18 March 2005
Fish Infested: 34 white shiners
27 fantail darters
6 bluegill
1 redbreast sunfish
1 green sunfish
Fish held at (Temperature): 14°C (shiners and darters)
21°C (sunfish species)
Total Juveniles produced: shiners and darters: 324
Sunfish: 34
Days to transformation: At 14°C: 18 - 39
At 21°C: 12 - 20
Culture System: Juvenile Recirculating: 40
TRH: 318
Results: The indoor juvenile recirculating system was checked on 1 September 2005, and no survivors were found. We checked the 2 cages at TRH on 13 September 2005 and also found no survivors.

Cohort: SundTar032906

Gravid Adult Collected: 27 March 2006
Gravid Adult Location: Tar River (Person County)
Date Infested: 29 March 2006
Fish Infested: 20 white shiners
3 roanoke darters
22 bluegill
15 redbreast sunfish
1 green sunfish
1 pumpkinseed
Fish held at (Temperature): 15°C
Total Juveniles produced: 792
Days to transformation: 17 - 29
Culture System: Chilled Static tank (15°C)
Results: We checked growth and survival at approximately 2 months post-metamorphosis (14 June 2006) and found 272 survivors (34.3%). Length ranged from 590 – 980 µm. There were 143 survivors (18.1%) at 4 months (7 August 2006), and length ranged from 1.55 – 2.1 mm. This was the last assessment made of this cohort for this project period.

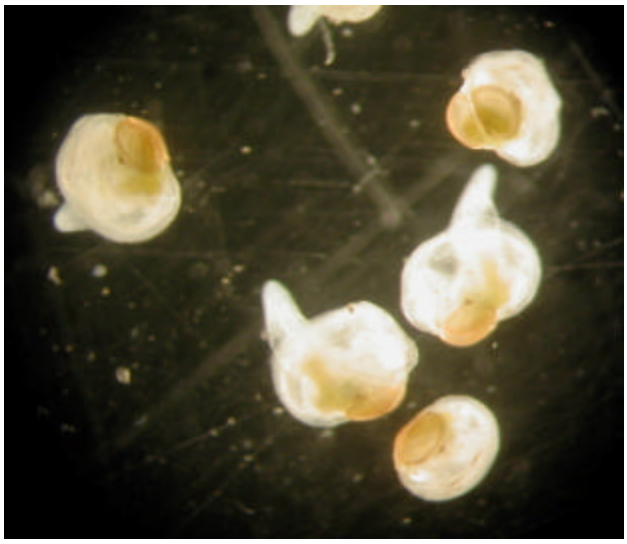

Figure. 3.3. *Strophitus undulatus* (2-months old) cultured in the laboratory

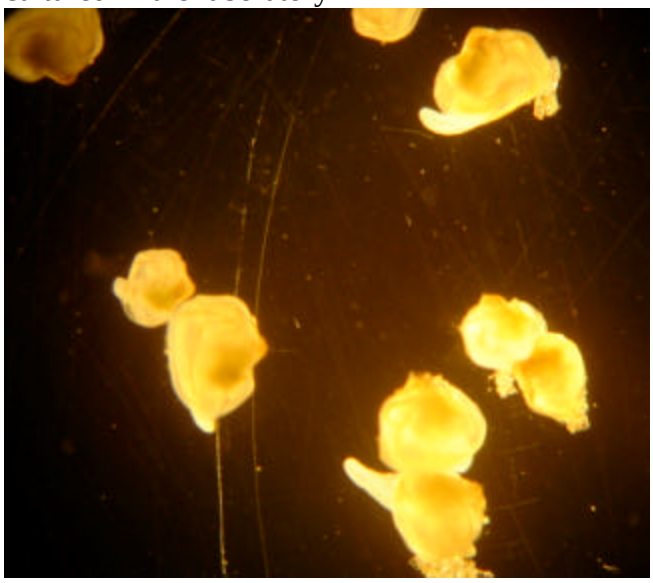

Figure. 3.4. *Strophitus undulatus* (4-months old) cultured in the laboratory

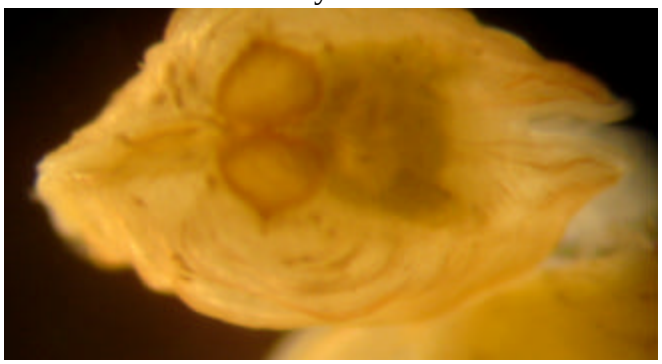

Figure. 3.5. Close-up of *Strophitus undulatus* (4-months old) cultured in the laboratory

Cohort: SundTar033006

Gravid Adult Collected: 27 March 2006
Gravid Adult Location: North Fork Little River, Neuse River Basin
Date Infested: 30 March 2006
Fish Infested: 1 warmouth
4 bluegill
1 green sunfish
9 redbreast sunfish
Fish held at (Temperature): 15°C
Total Juveniles produced: 748
Days to transformation: 17 - 34
Culture System: Chilled Static tank (15°C)
Results: We checked growth and survival at approximately 3 months post-metamorphosis (7 July 2006) and found 211 survivors (28.2%). This was the last assessment made of this cohort for this project period.

Discussion – Strophitus undulatus

As with *A. varicosa*, *Strophitus undulatus* is a host generalist, and we had success in producing juveniles with several fish species. Others have had success using several hosts with this species (Watter et al. 1998, van Snik Gray et al. 2001). Also like *A. varicosa*, our greatest success came through maintaining lower temperatures through propagation and early culture. Maintenance of roughly 300 *S. undulatus* to over 4 months of age and almost 2 mm in length represents our greatest success to date with a species other than a Lampsiline.

Lampsilis cariosa

Cohort: LcarEno033006

Gravid Adult Collected: 27 March 2006
Gravid Adult Location: Eno River, Neuse River Basin
Date Infested: 30 March 2006
Fish Infested: 40 largemouth bass
Fish held at (Temperature): 21°C
Total Juveniles produced: 21,853
Days to transformation: 19-40
Culture System: Juvenile Recirculating System – 18,821 juveniles
TRH – 3,032
Results: After approximately 3 ½ months, only 378 juveniles remained alive (2.0%) in the juvenile recirculating system. They ranged in length from 0.6 – 1.8 mm. When the group at TRH was checked on 27 July 2006 (3 months old), there were no survivors found. This was the last assessment of this cohort during the project period

Discussion - Lampsilis cariosa

This infestation produced, by far, the largest number of juveniles produced of all the infestations done for this project. We used the batch infestation method and immersed the fish in a very high concentration of glochidia (approximately 16,000 glochidia/L). Fish were heavily infested after only 8 minutes of exposure and were removed from the glochidial bath. Despite heavy infestations, largemouth bass remained healthy throughout the encystment period. Unfortunately, despite the large number of juveniles produced, survival was quite low in the lab, and there was complete mortality at the hatchery. In the lab, there was an outbreak of predacious flatworms in the fish tanks as well as the in the culture systems that seemed to heavily impact survival of mussels placed in the recirculating system. Flatworms have been observed in other mussel culture labs to be voracious predators (Zimmerman et al. 2003), and in fact they were seen eating newly transformed juvenile mussels in our laboratory. We recommend all fish be quarantined and treated with formalin prior to their use in propagation following recommendations made by Zimmerman and co-workers (2003).

At the hatchery, we have had no success maintaining newly transformed juveniles of any species. The reasons for this are unknown, but may be related to high temperatures and/or predation.

Lampsilis fasciola

Cohort: LfasLTN071904

Gravid Adult Collected: 13 July 2004
Gravid Adult Location: Little Tennessee River (Swain County)
Date Infested: 19 July 2004
Fish Infested: 98 largemouth bass
Fish held at (Temperature): 22°C
Total Juveniles produced: 3800
Days to transformation: 14 - 57
Culture System: Juvenile Recirculating System – 3007 juveniles
Static Culture Tanks (20-21°C) – 117 juveniles
TRH (beginning at 6-months old)

Results: Of the 3800 juveniles produced, 676 were used in toxicity tests, and the remaining mussels were cultured initially in the laboratory. On 8 November 2004 (2-3 months post infestation), we assessed survival in the 2 static tanks that had originally held 117 juveniles and found 71 survivors (60.6%). We then moved these juveniles into the recirculating system with the other juveniles to allow for easier maintenance. On 26 January 2005 (approx. 5 months post-transformation) we checked all troughs and found only 94 live juveniles (3.1% survival) in the troughs where juveniles had been placed from the beginning. We immediately took 30 of these individuals to TRH. In the trough that held mussels originally placed in the static tanks, we still had 71 juveniles remaining on 14 February 2005 (100% survival since 8 November 2004). Length in that group ranged from 1.5 –

3 mm. At this point, we had 133 juveniles in the lab. Approximately 3 months later (3 May 2005), there were 55 survivors. At that point, we took those 55 survivors to TRH.

TRH year 1: Of the 30 mussels placed at TRH on 27 January 2005, there were only 3 survivors on 27 June 2005, and there had been essentially no growth. At the end of the 2005 hatchery growing season (6 October 2005), all of these mussels were dead. Of the 55 placed at TRH on 3 May 2005, 44 remained alive in October and their length ranged from 5 – 8 mm.

Over-wintering Trial: We moved 34 of these survivors back to secondary growout system in the lab at NCSU for the winter. Over the winter, the mussels faired moderately well in the lab. On 3 May 2006, there were 28 of the original 34 surviving (82.4%), and they had grown to lengths ranging from 7-11 mm.

TRH Year 2: The 28 survivors were then taken back to TRH and the last assessment of the funding period was done on 27 July 2006. There were 26 survivors at that point, and they had grown to lengths ranging from 9 to 18 mm. Of the 10 mussels that remained at the hatchery throughout the winter, all survived through the winter until the last assessment on 27 July 2006. At that point, they had reached lengths ranging from 13-17 mm.

Figure. 3.6. *Lampsilis fasciola* (5-months old) cultured in the laboratory

Figure. 3.7. *Lampsilis fasciola* (2-years old) cultured at the Table Rock Hatchery since 5-months of age

Cohort: LfasToe051105

Gravid Adult Collected: 10 May 2005
Gravid Adult Location: North Toe River, French Broad River Basin
Date Infested: 11 May 2005
Fish Infested: 17 largemouth bass
Fish held at (Temperature): 22°C
Total Juveniles produced: 38
Days to transformation: 16 - 24
Culture System: Juvenile Recirculating System
Results: After 3.5 months, there was only 1 survivor of the original 38 (2.6%). This mussel measured 1.1 mm in length. At 6 months this mussel was dead, and the shell measured 2.7 mm in length.

Cohort: LfasPigeon051105

Gravid Adult Collected: 10 May 2005
Gravid Adult Location: Pigeon River, French Broad River Basin
Date Infested: 11 May 2005
Fish Infested: 40 largemouth bass
Fish held at (Temperature): 21°C
Total Juveniles produced: 517 juveniles
Days to transformation: 16 - 28
Culture System: Juvenile Recirculating System
Results: After 3 months, there were only 13 survivors of the original 517 (2.5%). Length ranged from 1.5 – 4.4 mm. At 9 months (21 March 2006), 11 of the 13 remained, and length ranged from 2.2 – 5.0 mm. At this point, the cohort was moved to the secondary growout system, and at 1 year post-metamorphosis (6 June 2006), 9 remained alive, ranging in length from 3 – 8 mm. This group was

then taken to TRH. After 45 days at TRH, 8 of the 9 remained, and length ranged from 9 – 14 mm.

Discussion – Lampsilis fasciola

With the 2004 cohort, we had relatively good survival at 2-3 months using a static tank. Juveniles in the recirculating system faired much worse even though sediment characteristics, feeding, and water change regime were identical between the systems. Survival of this cohort after 2-3 months was good.

Both of the 2005 cohorts produced an unusually low number of live juveniles compared to what would be expected with the number of glochidia and fish used. This could be related to collection of glochidia. For these cohorts, we collected glochidia in the field, leaving the female donor mussels in the river. Glochidia were then transported overnight to the laboratory and used for infestation approximately 24 hours after collection. When checked for viability using an NaCl solution (Zale and Neves 1982), 100% of the glochidia tested snapped shut, and the brood was deemed viable. Glochidia of this species have been shown to respond to this viability test for over a week after removal from the female (Greg Cope, NCSU, pers. comm.), but perhaps the salt test is an inaccurate predictor of transformation success. We recommend minimizing the length of time between extraction of glochidia and infestation of the fish. We do believe that the failure of the laboratory's cooling system in late May 2005 and resultant significant rise in water temperatures during transformation and excystment also contributed to the lower number of live juveniles produced and the low survival of the juveniles.

<u>Lampsilis radiata</u>

Cohort: LradFlat051105

Gravid Adult Collected: 05 May 2005
Gravid Adult Location: Flat River, Neuse River Basin
Date Infested: 11 May 2005
Fish Infested: 21 largemouth bass
Fish held at (Temperature): 21°C
Total Juveniles produced: 3,841
Days to transformation: 16 - 33
Culture System: Juvenile Recirculating System – 3575 individuals
MLH – 266 individuals

Results: At MLH, there were no survivors or shells found 2 ½ months after their placement in the raceway. Of the 3,575 individuals placed in the juvenile recirculating system in the laboratory, there were only 25 survivors (0.6%) after 3 months (21 September 2005), ranging in length from 0.5 – 0.8 mm. On 21 March 2006 (9 months old), there were 14 remaining alive in the lab, and length ranged from 1.4-2.8 mm. This was the last time this cohort was assessed.

Cohort: LradEno102605

Gravid Adult Collected: 21 October 2005
Gravid Adult Location: Eno River, Neuse River Basin
Date Infested: 26 October 2005
Fish Infested: 51 largemouth bass
Fish held at (Temperature): 21°C
Total Juveniles produced: 5,586
Days to transformation: 16 - 26
Culture System: Juvenile Recirculating System –2,700 individuals
Secondary Growout System (cages) – 2,886 individiuals
Veterinary School Pond – 34 individuals at 4 months old
MLH (beginning at 5 months old)
TRH (beginning at 7 months old)

Results: Of the 2,700 juveniles placed in the recirculating system, there were 240 survivors (8.8%) after almost 4 months (10 March 2006). Length ranged from 0.4 – 0.8 mm. Of the 2,886 placed in cages in the secondary growout system, growth and survival was as follows:

Age	Total number of mussels alive	Total Survival (%)	Length Range (mm)
50 days	1,045	36.2	0.48-1.12
110 days	700	24.3	0.65-3.77

At 4 months, 34 individuals were placed in a floating cage in sediment in the pond at the NCSU College of Veterinary Medicine. Three months later (26 July 2006), the cage was examined and no survivors were found.

At 5 months (20 April 2006), 57 individuals ranging in length from 3-7 mm were placed in a cage of sediment at MLH. On 6 July 2006 (2 ½ months later), this cage was examined, and only 23 survivors were found. Very little growth had occurred with mussels ranging from 4-8.5 mm in length. The surviving individuals had very soft shells that appeared eroded and were easily crushed. This was the final examination of this group.

At approximately 7 months of age (9 June 2006), the remaining survivors in the lab (ranging in length from 6-14 mm) were split into two groups with 260 going to TRH and 210 staying in the laboratory. On 27 July 2006 (48 days later), there were 245 alive at TRH (94.2%) ranging in length from 10-23 mm (Fig. 3.8) and 203 in the laboratory ranging from 6-16 mm. This was the last assessment of this cohort during the project period.

Figure 3.8. *Lampsilis radiata* (8-months old) cultured indoors for 7 months and moved to the Table Rock Hatchery during the 8th month.

Cohort: LradEno033006

Gravid Adult Collected: 27 March 2006
Gravid Adult Location: Eno River, Neuse River Basin
Date Infested: 30 March 2006
Fish Infested: 52 largemouth bass
Fish held at (Temperature): 23°C
Total Juveniles produced: 1,812
Days to transformation: 16 - 25
Culture System: Juvenile Recirculating System
Results: On 28 September 2006 (5 months old), there were 94 survivors ranging in length from 0.8-3.8 mm.

Discussion – Lampsilis radiata

The cohort produced in May 2005 was likely greatly affected by a failure of the laboratory cooling system and the resulting significant increase in water temperatures (over 30°C) over 2-3 days. This event happened during the excystment of this cohort. Significant mortality was seen across all species immediately after this event occurred. Still, all 3 groups of *L. radiata* produced during this project faired poorly in the juvenile recirculating system. Hanlon and Levine (2001) also saw relatively low survival (4.3-13.0%) after 2 months in this system. Hanlon and Levine (2001) used coarse sand (1-2 mm) with a dusting of silt (<100 µm) for substrate, whereas we used only a fine sand and silt mixture (<300 µm). We used identical substrate in our cages in the secondary growout system, but we had much greater survival there. *Lampsilis radiata* is a fast

growing animal, and within 1 year has reached sizes (>20 mm) at TRH that are likely suitable for survival if stocked into the wild.

Villosa constricta

Cohort: VconNFLittle051805

Gravid Adult Collected: 1 April 2005
Gravid Adult Location: North Fork Little River, Neuse River Basin
Date Infested: 18 May 2006
Fish Infested: 123 fantail darters
6 roanoke darters
Fish held at (Temperature): 18°C
Total Juveniles produced: 192
Days to transformation: 22 - 41
Culture System: Juvenile Recirculating System
Results: After 3 months in the recirculating system, there was only 1 survivor remaining. That individual was only 0.5 mm in length.

Cohort: VconNFLittle041705

Gravid Adult Collected: 27 March 2006
Gravid Adult Location: North Fork Little River, Neuse River Basin
Date Infested: 17 April 2006
Fish Infested: 120 fantail darters
Fish held at (Temperature): 16°C
Total Juveniles produced: 72
Days to transformation: 30 - 38
Culture System: Chilled Static Tank (15°C)
Results: We had significant mortality in the host fish around the time of encystment, so this reduced the total number of juveniles produced. After 2.5 months, there were 41 juveniles remaining (56.9% survival), ranging in length from 490-710µm. This was the last assessment of this cohort during the project period.

Discussion – Villosa constricta

As with other species, *V. constricta* faired better in a static system that was well aerated and slightly chilled than in the juvenile recirculating system at 21°C. Survival greater than 50% after 2 ½ months is favorable. Although growth was somewhat slow in the 2006 cohort, newly metamorphosed juveniles are somewhat small (200 µm), and the species grows slowly on the whole. Perhaps a slightly higher temperature would increase growth rates.

Villosa delumbis

Cohort: VdelWFLittle110104

Gravid Adult Collected: October 2004
Gravid Adult Location: West Fork Little River, Yadkin-Pee Dee River Basin
Date Infested: 1 November 2006
Fish Infested: 20 redbreast sunfish
Fish held at (Temperature): 21°C
Total Juveniles produced: 1,521
Days to transformation: 17 - 25
Culture System: Juvenile Recirculating System
TRH

Results: After 2 months, one trough contained 777 of the 933 juveniles originally placed in that trough (83.3% survival). Survival in the other trough of this cohort was not assessed at 2 months. At that point (27 January 2005), this cohort was divided into multiple groups:

Initial TRH stocking: On 27 January 2005, 45 individuals were taken to TRH to assess how they would survive the winter. Of those 45, only 5 survived to the end of the 2005 growing season (7 October 2005), ranging in length from 3.7-5.6 mm.

Feeding Trial: On 30 January 2005, 300 individuals were used to conduct a feeding trial with cultured algae, commercial algae and detritus (See Chapter 4). After 2 more months, 230 of the 300 remained alive, ranging in length from 1.0-3.7 mm. The feeding trial concluded, and these 230 were returned to the trough they came from originally.

On 20 April, 2005, there were 794 individuals of this cohort remaining alive in the juvenile recirculating system. When the original number was adjusted by the number taken to TRH, this results in a 5 month survival of 53.8%

Second TRH stocking: On 20 April 2005, 400 individuals were taken to TRH. After spending the growing season at TRH, there were only 181 alive on 13 September 2005. Length ranged from 3.4-6.9 mm at that time.

MLH stocking: On 24 June 2005, 30 individuals were taken to MLH ranging in length from 1.2-4.1 mm. On 14 November 2005, there were 10 survivors ranging from 7-10 mm. On 7 July 2006, only 5 survivors remained (9-14 mm long).

Over-wintering trial: On 7 October 2005, we moved 147 individuals (4.2-7 mm long) from TRH back to the secondary growout system in the laboratory to test growth and survival of these larger juveniles over the winter indoors. On 17 April 2006, 138 of these individuals remained alive, ranging in length from 7-13 mm. Of the 34 that remained at TRH over the winter, all survived, but no growth was observed over the winter period.

Final observations: On 3 May 2006, 60 individuals that over-wintered at the lab were returned to TRH. At the final observation (27 July 2006), 58 of those remained alive, ranging in length from 12-21 mm. Mussels that overwintered in the lab and remained there during the spring/summer 2006 ranged from only 9-16 mm in length at the end of the project (7 July 2006) (Fig. 3.9).

Figure 3.9. *Villosa delumbis* (1 ½ years old) cultured at TRH.

Cohort: VdelLittle030805

Gravid Adult Collected: March 2005
Gravid Adult Location: Little River, Yadkin-Pee Dee River Basin
Date Infested: 8 March 2005
Fish Infested: 12 redbreast sunfish
Fish held at (Temperature): 21°C
Total Juveniles produced: 765
Days to transformation: 17 - 29
Culture System: Juvenile Recirculating System
MLH

Results: At 2 ½ months (20 June 2005), only 13 of the original 765 remained alive (1.7% survival). These mussels ranged in length from 0.5-0.9 mm. These 13 individuals were then taken to MLH and placed in the raceway. When the cage was checked 3 months later (15 September 2005), there were no survivors.

Cohort: VdelDeep092305

Gravid Adult Collected: June 2005
Gravid Adult Location: Deep River, Cape Fear River Basin
Date Infested: 23 September 2005
Fish Infested: 7 redbreast sunfish
1 bluegill
Fish held at (Temperature): 21°C
Total Juveniles produced: 1,682
Days to transformation: 17 - 29
Culture System: Juvenile Recirculating System
TRH
Results: This group of mussels was used in a 4-month feeding trial to assess cultured algae, commercial algae, and detritus (see Chapter 4). Growth and survival across all treatments was as follows:

Age	Total number of mussels alive	Total Survival (%)	Length Range (mm)
1 month	908	54.0	0.52-0.99
2 months	474	28.2	0.74-1.6
4 months	385	22.9	0.8-2.5

At 5 months, this cohort was moved to the secondary growout system in the lab. At 6 ½ months (7 June 2006), there were 330 survivors ranging from 7-9 mm. At that time we moved 154 of them to TRH (although 47 were accidentally crushed during transport) and checked growth and survival of the lab and hatchery groups on respectively on 24 and 27 July 2006. All of those in the lab remained alive and ranged from 8-11 mm in length. Of the 107 that were successfully placed at the hatchery, all remained alive, and they had grown to a length of 9-15 mm (Fig. 3.10). This was the last assessment of this cohort.

Figure 3.10. *Villosa delumbis* (10 months old) cultured in laboratory for 6 ½ months and 3 ½ months at TRH.

Cohort: VdelNewHope092305

Gravid Adult Collected: June 2005
Gravid Adult Location: New Hope Creek, Cape Fear River Basin
Date Infested: 23 September 2005
Fish Infested: 2 redbreast sunfish
Fish held at (Temperature): 21°C
Total Juveniles produced: 179
Days to transformation: 18 - 28
Culture System: Juvenile Recirculating System
Results: At 3 ½ months, 32 individuals remained alive (17.9%), ranging in length from 0.6-1.1 mm. At 6 months, there were 21 survivors (11.7%) ranging from 0.72-1.34 mm.

Cohort: VdelLittle112805

Gravid Adult Collected: 9 November 2005
Gravid Adult Location: Little River, Yadkin-Pee Dee River Basin
Date Infested: 28 November 2005
Fish Infested: 11 redbreast sunfish
Fish held at (Temperature): 19°C
Total Juveniles produced: 1,787
Days to transformation: 25-31
Culture System: Juvenile Recirculating System

TRH

Results: 4 months (17 April 2006), there were 470 survivors (26.3%). Length ranged from 0.6-1.2 mm. On 5 June 2006, length ranged from 0.9-1.5 mm, and 105 individuals were taken to TRH. On 27 July 2006, there were 85 remaining at TRH, with a length of 2.0-5.5 mm. Those individuals that remained at the laboratory were not assessed again during the project period.

Cohort: VdelUpper031706

Gravid Adult Collected: March 2006
Gravid Adult Location: Upper Creek, Catawba River Basin
Date Infested: 17 March 2006
Fish Infested: 24 redbreast sunfish
Fish held at (Temperature): 21°C
Total Juveniles produced: 1,819
Days to transformation: 18 - 32
Culture System: Juvenile Recirculating System
Results: At 1 month, there were only 13 survivors (0.7%), and they had grown very little. At 2 months, there were no survivors

Cohort: VdelUpper041706

Gravid Adult Collected: March 2006
Gravid Adult Location: Upper Creek, Catawba River Basin
Date Infested: 17 April 2006
Fish Infested: 2 redear sunfish
1 bluegill
1 warmouth
Fish held at (Temperature): 21°C
Total Juveniles produced: 353
Days to transformation: 17 - 32
Culture System: Juvenile Recirculating System
Results: At 2 months (5 July 2006), there were 31 survivors (8.8%). Length ranged from 0.71 – 1.38 mm.

Discussion – Villosa delumbis

Overall, *V. delumbis* maintained the highest survival across multiple cohorts of all species cultured. The first cohort produced a very high rate of survival with 83.3% being alive after 2 months. As with other species, survival after the 2-month point was good. While significant mortality was seen at TRH during 2005, the improvements in the temperature and flow regime in 2006 yielded good growth results for this species. We recommend not exposing mussels under 5 mm to the harsh winter conditions of the hatchery. We saw poor survival of the initial group that was taken to the hatchery in January. Over-wintering the mussels in the lab allowed for extended growth as they

almost doubled in size during the time when there would be no growth at the hatchery setting because of cold winter temperatures. They also experienced little mortality in the lab at this time. The juveniles taken to MLH experienced greater mortality and slower growth than mussels at TRH. While there are mussels in Hitchcock Creek, which McKinney Lake feeds, the higher water temperatures and lower water pH and hardness at MLH appear less than ideal for this mussel species.

We were surprised by the complete mortality experienced by the group produced in March 2006. While culture conditions were identical to those used when good survival was observed, there were a few differences in the propagation process. Of the 3 individuals to be used for that infestation, 1 had an immature brood (glochidia still encased in an egg) and was saved and used for the April infestation. Perhaps those other 2 broods were mature enough to transform into the juvenile stage but not mature enough to maintain long-term survival after transformation. The mussel culture lab at Virginia Tech has observed differences in juvenile survival based on glochidial maturity (how closely they were harvested to the end of the brooding period) (Jess Jones, Virginia Tech, pers. comm.). Although the brood of the 3rd mussel was immature, by holding the mussel at 23°C for 1 month, the glochidia matured, and the juvenile mussels survived past 2 months.

Villosa vaughaniana

Cohort: VvauWestFork110104

Gravid Adult Collected: October 2004
Gravid Adult Location: West Fork Little River, Yadkin-Pee Dee River Basin
Date Infested: 1 November 2004
Fish Infested: 85 hybrid bluegill
26 bluegill
10 redbreast sunfish
6 green sunfish
1 pumpkinseed
Fish held at (Temperature): 21°C
Total Juveniles produced: 6,160
Days to transformation: 16 - 36
Culture System: Juvenile Recirculating System
TRH (beginning at 5 months)
MLH (beginning at 7 months)
Results: In one trough, we originally placed 3,087 juveniles, and at 5 months (18 April 2005), there were 863 survivors (30.0%). Length ranged from 0.9-2.3 mm. Of those 846, we took 617 to TRH. Survival in the other trough was checked only at 6 months (23 May 2006), and 535 of the original 2,926 juveniles placed had survived (18.3%). Length ranged from 0.9-1.7 mm. One week later, we experienced the failure of the lab cooling system, and many dead juveniles were seen on top of the substrate after this event. Both troughs were assessed on 20 June 2005, and only 22 live individuals remained in the laboratory.

TRH initial stocking: On 20 April 2005, we placed 617 juveniles in raceway cages at TRH. On 12 September 2005, there were 178 individuals remaining. They ranged in length from 2.5-6.1 mm. On 7 October 2005, we took 102 of those survivors back to the lab to assess the best location for over-wintering of this age of juvenile.

Over-wintering in the lab: In October 2005, the 102 juveniles were placed into the secondary growout system in the lab. On 29 March 2006, only 52 remained alive. Survivors grew to lengths ranging from 4.9-8.3 mm. On 3 May 2006, 24 of these survivors were taken back to TRH. All 24 survived to the last assessment on 27 July 2006, and they ranged in length from 10-16 mm.

Over-wintering at TRH: Of the 85 individuals that remained at the hatchery over the winter, 75 survived through the winter and to the last assessment of the project period on 27 July 2006. They ranged in length from 8-15 mm.

Growth and Survival at MLH: On 24 June 2005, we placed 22 juveniles at MLH ranging from 1.4-2.9 mm in length. On 15 September 2005, only 1 of those juveniles remained alive (4.3 mm long). That individual remained alive through the following winter up to the last assessment of the cohort at MLH on 6 July 2006. At that time it measured 9 mm in length.

Cohort: VvauLittle112805

Gravid Adult Collected: October 2005
Gravid Adult Location: Little River, Yadkin-Pee Dee River Basin
Date Infested: 28 November 2005
Fish Infested: 10 redbreast sunfish
Fish held at (Temperature): 21°C
Total Juveniles produced: 3,573
Days to transformation: 14 - 32
Culture System: Juvenile Recirculating System
Results: Juveniles were divided into 2 troughs, each containing approximately half of the individuals propagated respectively. At 3 ½ months (19 April 2006), there were 437 out of the original 1,849 surviving (23.6%). They ranged in length from 0.3-0.9 mm. In the other trough, there were only 3 survivors (0.2%). The trough that experienced the heavy mortality had an unusually large number of small snails (Physidae) in the trough. A month and a half later (5 June 2006), the other trough also had few survivors with only 31 live mussels found. This was the last assessment of this cohort during the project period

Cohort: VvauWestFork120105

Gravid Adult Collected: November 2005

Gravid Adult Location: West Fork Little River, Yadkin-Pee Dee River Basin
Date Infested: 1 December 2005
Fish Infested: 22 redbreast sunfish
1 redear sunfish
2 green sunfish
3 bluegill
Fish held at (Temperature): 21°C
Total Juveniles produced: 199
Days to transformation: 14 - 33
Culture System: Juvenile Recirculating System
Results: At 3 ½ months (4 April 2006), there were 11 survivors (5.5%), ranging in length from 0.6-1.3 mm. This was the last assessment of this cohort.

Discussion – Villosa vaughaniana

Overall, *V. vaughaniana* did not fair as well in our culture attempts as *V. delumbis*; however, our first trial had relatively good survival after 5 months indoors. Survival during the first year at the hatchery was likely affected by the inconsistent temperature and flow regime with the water supply. While *V. delumbis* experienced growth and high survival over-wintering indoors at 1 year old, *V. vaughaniana* experienced significant mortality in the lab under identical conditions. While not growing quite as much (only 2-3 mm smaller), the group that over-wintered at TRH had much better survival than those that were moved indoors. While very small juveniles (< 5 mm) clearly do not fair well in winter conditions, we recommend keeping larger juveniles of this species at the hatchery over winter until more appropriate conditions can be created in the laboratory. Those placed at MLH experienced significant mortality in a short period of time; however, the 1 individual that did survive the first 2 summer months also survived the winter and was alive through the last assessment in July of the following year. As with *V. delumbis*, conditions at MLH appear to be marginal, at best, for culture of this species.

Release of Propagated Juveniles

We originally hoped to be able to release juveniles in the spring of 2006, but it was clear that the juveniles were not large enough to release at that time. They needed an additional year of growth in the hatchery. At the time of this writing, we have successfully cultured 3 species from 2 separate river basins to a size which we would feel gives them a good chance at survival. From the West Fork Little River in the Yadkin Pee-Dee basin, we have *Villosa delumbis* and *Villosa vaughaniana* over 20 mm at 2 years old. From the Eno River in the Neuse River basin, we have cultured *Lampsilis radiata* 1 year to sizes over 20 mm. We are currently working with the NCWRC to establish proper stocking densities and stocking sites for this species within the Eno River State Park.

Conclusions

1. **Many of mussels of the subfamily Anodontinae (e.g. *Alasmidonta, Pyganodon*, and *Strophitus*) naturally release glochidia in the second half of winter up into early spring in North Carolina. Infested fish should be held at chilled temperatures that would reflect water temperatures during this time of year.** Newly transformed juveniles should also be cultured initially at these lower temperatures, and care should be taken during counting and sorting of juveniles not to raise their ambient water temperature. Breakthroughs in maintaining survival of these species in the lab may apply to other related rare and endangered animals in the state (e.g. *Alasmidonta heterodon*, *Alasmidonta raveneliana,* and *Lasmigona decorata*)
2. **Caging gravid mussels in close proximity with their host fish in a hatchery setting may be an effective way to allow the females to release glochidia when they are fully mature.** Rather than manually extracting glochidia of unknown maturity with a syringe, allowing the mussel to dictate development and larval release in response to the hatchery's natural temperature regime is likely beneficial. More experimentation is needed
3. **The Table Rock Hatchery near Morganton, NC has shown to be a valuable resource for culturing mussels.** Once the water supply was constant, growth and survival of older juveniles was high. Having two water supplies allowed us to avoid extreme summer temperatures that were shown to cause high mortality.
4. **The hatchery at McKinney Lake is marginal, at best, for culturing mussels.** High temperatures and low pH and hardness are less than ideal for the species we tried there. This facility could prove valuable for holding mussels for a short period of time. It did facilitate the successful culture of *Alasmidonta viridis* by caging the gravid mussels with their host fish. Survival of adult mussels was 100% during that time period, and infestation was achieved.
5. **Survival of newly transformed juveniles was poor at both hatcheries.** We recommend placing juveniles in the hatchery raceway after they reach approximately 2-3 mm in size.
6. **Our static culture tanks have proven to be just as effective - if not more effective - at growing mussels as those with recirculating flow.** The aeration in the static tanks created a more vertical, circular current in the tank than the more laminar flow of the juvenile recirculating system. This may have created better water exchange in the substrate where the mussels live.
7. **Some species of mussel may benefit from being moved indoors to over-winter after their first growing season.** With other species, the increased mortality in the lab may not be worth only a few millimeters in growth that warmer lab temperatures allow.
8. **While glochidial maturity may play an important role with some species, we were able to propagate some Lampsiline species early in their brooding period (November) with good survival.** This allowed us to get a head start on the growing season. Then, once waters at the hatchery warm to temperatures that support growth, the juveniles were already large enough to be placed there (2-3 mm).

Literature Cited

Bigham, SE. 2002. Host specificity in freshwater mussels: a critical factor in conservation. Master's thesis. Southwest Missouri State University. 48 pp.

Watters, GT, SH O'Dee, and S Chordas. 1998. New potential hosts for: *Strophitus undulatus*- Ohio River drainage; *Strophitus undulatus* - Susquehanna River drainage; *Alasimidonta undulata* - Susquehanna River drainage; *Actinonaias ligamentina* - Ohio River drainage; and *Lasmigona costata* - Ohio River drainage. Triannual Unionid Report. 15.

Chapter 4: Mussel Culture Experiments

Introduction

The science of freshwater mussel propagation and culture is still in its infancy. Many questions are unanswered and have to be addressed one at a time. Outside of host fish determination, we sought to address three questions related to mussel culture.

How much variation is there in brood health and propagation success between females at a given site and time?

Will the addition of detritus and/or commercial algae improve growth or survival over a simple diet of one species of cultured algae?

Do early nutritional deficits contribute to mortality that occurs around 1-2 months of age?

Methods

Variation between broods

We tested variation in production between different broods of 2 mussel species (*Villosa constricta* and *Lampsilis radiata*) collected from the same site at the same time. Each brood was extracted from the female mussel by flushing the marsupia with a water-filled syringe. Broods and the fish the brood was used to infest were kept separate to allow monitoring of propagation success of individual broods.

Villosa constricta – On 1 April 2005, we collected 8 gravid female *V. constricta* from the North Fork Little River (Neuse River Basin) in Orange County, NC. Gravid females were held at 6°C and fed cultured algae until 7 of the broods were extracted for use on 18 May 2005. Before infestation, viability of each brood was checked by exposing a subsample to a concentrated NaCl solution and checking for a valve closure response (Zale and Neves 1982). Fantail darters (*Etheostoma flabellare*) were collected from Upper Barton Creek (Neuse River Basin) in Wake County, NC and transported live to the laboratory. They were anesthetized using 100 mg/L tricaine methanesulfonate (MS-222) and were infested by hand with glochidia from 1 of the 7 broods. Fish were grouped in tanks by the brood with which they were infested. Because some of the gravid mussels were more fecund than others, the number of fish infested varied between broods. When over 20 fish were infested with a single brood, fish were divided equally between 2 tanks. Tanks were chilled to 18°C and siphoned routinely to check for sloughed (dead) glochidia as well as transformed juveniles. We calculated the percent of glochidia originally attached to the fish that transformed into juveniles.

Lampsilis radiata – On 5 May 2005, we collected 5 gravid female *Lampsilis radiata* from the Flat River (Neuse River Basin) in Durham County, NC. Gravid females were held at 6°C and fed cultured algae until the broods were extracted for use on 15 May 2005. Largemouth bass (*Micropterus salmoides*) were purchased from an aquaculture supplier

or collected from a farm pond maintained by NC State University. Fish were anesthetized using MS-222 and were infested by hand with glochidia from 1 of the 5 broods. With each of 4 of the broods, we infested 4 separate bass, and 5 bass were infested with the 5th brood. Individual fish were given separate tanks. Unfortunately, 4 bass died during the encystment period, and each of those bass were infested with glochidia from separate mussels. Tanks were maintained at 21°C and siphoned routinely to check for sloughed (dead) glochidia as well as transformed juveniles. We calculated the percent of glochidia originally attached to the fish than transformed into juveniles.

Feeding Trials

We conducted 2 feeding trials to assess the potential of multiple food sources for use in the culture of freshwater mussels.

Trial 1 – In our initial trial, we used 2-month-old *Villosa delumbis* propagated from the West Fork Little River (Yadkin-Pee Dee River Basin). Mussels were counted into 6 groups of 50 individuals each and randomly assigned to 1 of 6 treatments (Table 4.1).

Table 4.1. Treatments used in Feeding Trial 1.

Treatment Number	Treatment Type	Amount Fed
1	Cultured Algae	1 L/day
2	Cultured Algae + Detritus	1 L/day cultured algae 10mL/day of detritus mixture
3	Cultured Algae + Detritus + Commercial Algae	1 L/day cultured algae 10mL/day of detritus mixture 2 mL/day of each of 3 commercial algal diets
4	Commercial Algae *(low concentration)*	1 mL/day of each of 3 commercial algal diets
5	Commercial Algae *(medium concentration)*	2 mL/day of each of 3 commercial algal diets
6	Commercial Algae *(high concentration)*	4 mL/day of each of 3 commercial algal diets

Mussels were then cultured in 6 identical troughs in the Juvenile Mussel Feeding Trial Unit (See Chapter 2) with separate 38-L reservoirs for each trough. Each reservoir was fed daily according to its assigned treatment. The commercial algal mixes were Chromaplex™, Phytoplex™, and Micro-Vert™ available from Kent Marine. Detritus was prepared by collecting fallen leaves from Southern Sugar Maple Trees (*Acer barbatum*) at the College of Veterinary Medicine at NC State University. Leaves were placed in a blender with conditioned water and blended into a slurry. The slurry was maintained in conditioned water in a 19-L bucket and aerated for 8 months prior to use. To feed the slurry to the mussels, the bucket was stirred so that the slurry was well mixed and 10-mL were taken from the suspension. After 2 months, we ended the test by counting and measuring length of all surviving mussels.

Trial 2 – To follow up on the results of Trial 1, we conducted a second trial eliminating the treatments that used only the commercial foods and using 2 replicates for each of the 3 remaining treatments. We propagated *Villosa delumbis* from the Deep River (Cape Fear River Basin), and randomly assigned 30 groups of 51 newly metamorphosed juveniles to 1 of the 6 troughs for a total of 255 individuals per replicate. Feeding was carried out as in the first trial, and we assessed growth and survival at 1, 2, and 4 months. At each assessment, we sieved the sediment and counted all live juveniles in each trough. We then measured length on the first 30 individuals encountered using a stage micrometer. The trial ended after 4 months.

Time to First Feeding

Using a batch infestation (described in Chapter 1), we infested 32 largemouth bass (*Micropterus salmoides*) with glochidia of *Lampsilis radiata* from the Eno River (Neuse River Basin). After a 30 minute exposure in the glochidial bath, infestation was determined to be sufficient. We then placed two fish in each of 16 aquaria (38-L each). A mesh tank divider was used to keep the bass separate. Each tank was then randomly assigned to one of four treatments, so that there were 4 tanks per treatment (Table 4.2).

Table 4.2. Treatments assessing time to first feeding

Treatment Number	Treatment description
1	Tank siphoned every 3 days
2	Tank siphoned daily
3	Tank siphoned daily + algae added to tank
4	Tank siphoned daily + algae added to tank + sediment on bottom of tank

Each treatment represented the time to first feeding after dropping off of the host fish (excystment). Once excystment began, all tanks were siphoned to remove juveniles, and treatments began. Sediment (<200 µm) was added to tanks in treatment 4, and 1 liter of cultured algae was added to all tanks in both treatments 3 and 4. Treatments 2, 3, and 4 were then siphoned daily, Treatment 1 tanks were siphoned every 3 days. Sediment from treatment 4 was sieved with a 200-µm sieve to separate juveniles. We removed approximately half of the water volume in a tank each time it was siphoned. We then replaced the water with fresh water, and tanks in treatments 3 and 4 were fed after the water change.

Juveniles from each tank were counted and put into separate 250-µm mesh cages (see Chapter 2) in the secondary growout system. Each cage had a 4-8 mm layer of fine sediment (<300 µm) on the bottom. We stopped putting juveniles into each cage once that cage reached 200 individuals. We were unable to obtain 200 individuals in 4 of the 16 cages. Juveniles were maintained in these culture cages, and growth and survival was assessed at 40 and 100 days after initial excystment. We also monitored juvenile activity in a subsample of tanks each day during excystment. When a tank was siphoned, juveniles were counted using a dissecting microscope. At that time, we counted the number of juveniles in a tank that were actively moving their feet. An individual was regarded as active if it moved its foot within a 10-second observation.

Results and Discussion

Variation between broods

<u>*Villosa constricta*</u> - There was substantial variation in transformation rates between broods in *Villosa constricta* in the North Fork Little River (Table 4.3). While 5 of the broods yielded somewhat similar results, 1 brood yielded no transformed juveniles and another yielded only 1 juvenile out of two tanks of host fish. The poor health of the

brood that produced no juveniles was reflected in the glochidial viability test. Only 10 of the 41 glochidia tested responded to the salt test. Glochidia in this brood did not seem to be in early development, but they seemed to be in a state of decay or death. Individual glochidia tended to clump while glochidia from healthy broods lie completely separate. Contrary to what was expected, the brood that produced only one juvenile appeared healthy initially and was 100% viable according to the salt test commonly used to test glochidial viability. Because similar results were seen in both replicates, we believe this represents lack of glochidial viability that cannot be detected by exposure to a saturated salt solution. More research is needed to understand the health and maturity of glochidia prior to infestation.

Table 4.3. Total juveniles produced and percentage of attached glochidia that transformed across broods from 7 different *Villosa constricta* females collected from the same site.

Brood Number	(Number of Viable Glochidia/Number of Glochidia Tested)	Total Number of Fish Infested	Tank Replicate	Live Juveniles Produced	Dead Glochidia	Transformation %
1	72/72	27	A	28	129	17.8
			B	25	198	11.2
2	75/75	25	A	30	111	21.3
			B	47	110	29.9
3	21/21	18	A	24	72	25.0
4	10/41	5	A	0	28	0.0
5	99/99	26	A	1	23	4.2
			B	0	47	0.0
6	52/52	15	A	22	74	22.9
7	31/31	9	A	14	137	9.3

Lampsilis radiata – Transformation rates of *L. radiata* varied substantially between individual host fish, but there were no significant differences in transformation rates between broods (p = 0.332, Kruskall-Wallis Test) (Table 4.4). The reason for this variation in production between fish is unknown.

Table 4.4. Total juveniles produced and percentage of attached glochidia that transformed across broods from 5 different *Lampsilis radiata* females collected from the same location in the North Fork Little River (Orange Co., NC).

Brood Number	Viable glochidia in Brood Test	Tank Replicate	Live Juveniles Produced	Dead Glochidia	Transformation %	Mean Transformation % (±SD)
1	77/77	A	167	336	33.2	56.1 ± 31.5
		B	433	570	43.2	
		C	92	8	92.0	
2	187/187	A	338	479	41.4	42.8 ± 1.9
		B	442	609	42.1	
		C	36	44	45.0	
3	239/239	A	131	523	20.0	46.2 ± 39.1
		B	138	480	22.3	
		C	74	591	11.1	
		D	124	20	86.1	
		E	146	14	91.3	
4	99/99	A	212	491	30.2	54.8 ± 28.5
		B	867	929	48.3	
		C	147	24	86.0	
5	83/83	A	25	357	6.5	17.4 ± 19.7
		B	469	702	40.1	
		C	13	224	5.5	

Feeding Trials

Trial 1 – Juveniles fed only the commercial diets faired poorly, experiencing relatively low survival and little growth. Of the mussels fed cultured algae, there was a trend toward increased growth with more additives to the diet (Table 4.5). Additionally, the treatment with all three food types had the highest survival (100%).

Table 4.5. Survival and final length of *Villosa delumbis* after being fed one of six diets in Feeding Trial 1.

Treatment Type	Survival (%)	Mean Length (mm) ± SD
Cultured Algae	86	2.24 ± 0.38
Cultured Algae + Detritus	72	2.44 ± 0.51
Cultured Algae + Detritus + Commercial Algae	100	2.88 ± 0.54
Commercial Algae - low	86	1.43 ± 0.25
Commercial Algae - medium	50	1.34 ± 0.22
Commercial Algae - high	64	1.40 ± 0.24

Trial 2 – The results of Trial 2 did not confirm the results of Trial 1 that indicated additions of detritus and commercial algal mixes to the diet would benefit growth and survival. In fact, the treatment containing all three food types experienced the lowest survival (Table 4.6, Fig. 4.1). Growth varied as much within treatments as it did across treatments (Table 4.7). Based on this test, we abandoned the use of this particular commercial algae as well as the use of detritus.

One replicate of the treatment with all three food types experienced extremely low survival (2.7%), and very little growth occurred between 2 and 4 months. During that time, there was an outbreak of small snails (Physidae) in this trough that may have contributed to this high mortality and slow growth. For this reason, culture systems should be watched carefully for snail outbreaks, and all snails should be removed and destroyed.

Table 4.6. Survival of *Villosa delumbis* juveniles fed three separate diets over a 4-month period in Feeding Trial 2.

Treatment	Replicate	Survival		
		1 month	2 months	4 months
Cultured Algae	A	57.3%	29.4%	26.7%
	B	56.5%	43.5%	38.0%
Cultured Algae + Detritus	A	51.8%	31.4%	27.1%
	B	62.4%	37.3%	32.2%
Cultured Algae + Detritus + Commercial Algae	A	55.7%	22.4%	14.9%
	B	72.5%	22.0%	2.7%

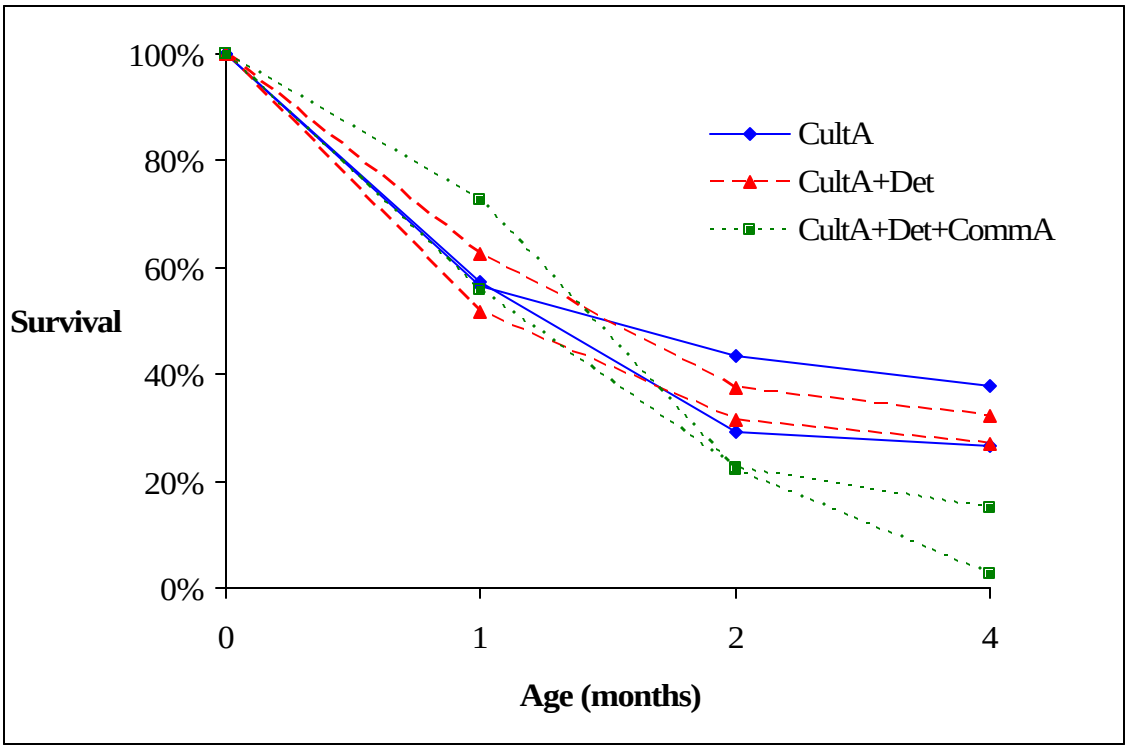

Figure 4.1. Survival of mussels fed either cultured algae (CultA), cultured algae and detritus (CultA+Det), or cultured algae, detritus and a commercial algal mix (Cult A+Det+CommA)

Table 4.7. Length of *Villosa delumbis* juveniles fed three separate diets over a 4-month period in Feeding Trial 2.

Treatment	Replicate	Mean Length (mm) ± SD		
		1 month	2 months	4 months
Cultured Algae	A	0.793 ± 0.115	1.248 ± 0.222	1.896 ± 0.423
	B	0.713 ± 0.117	1.189 ± 0.258	1.896 ± 0.484
Cultured Algae + Detritus	A	0.728 ± 0.133	1.252 ± 0.167	1.818 ± 0.329
	B	0.808 ± 0.122	1.455 ± 0.156	2.039 ± 0.404
Cultured Algae + Detritus + Commercial Algae	A	0.671 ± 0.093	1.157 ± 0.215	2.217 ± 0.321
	B	0.774 ± 0.108	1.046 ± 0.171	1.237 ± 0.414

Time to First Feeding

Mussels from aquaria that were siphoned and fed daily faired slightly better in growth and survival than those that were not fed (Tables 4.8 - 4.9). Mean survival was highest at 40 and 100 days in Treatment 3 (Fed daily - no sediment), but there were no statistical differences in survival ($p = 0.3881$, repeated measures ANOVA) or growth ($p = 0.1031$, repeated-measures ANOVA) between treatments. These statistical tests, however, may not mean that there is no value to having food available in the aquaria as soon as juveniles excyst from the fish. Even though not statistically different, we recommend supplying algae to aquaria during excystment until more definitive resolution is obtained.

Table 4.8. Mean Survival of *Lampsilis radiata* from 4 different treatments.

	Mean Survival (%) ± SD	
Treatment	**40 days**	**100 days**
Siphoned every 3 days	32.5 ±19.2%	18.5 ± 14.5%
Siphoned Daily	33.3 ± 4.4%	19.7 ± 8.6%
Siphoned and Fed Daily	46.1 ± 15.6%	33.5 ± 19.2%
Siphoned and Fed Daily (Sediment)	30.5 ± 9.2%	22.6 ± 8.2%

Table 4.9. Mean Length of *Lampsilis radiata* from 4 different treatments.

	Mean Length (mm) ± SD	
Treatment	**40 days**	**100 days**
Siphoned every 3 days	0.664 ± 0.136	1.812 ± 0.634
Siphoned Daily	0.694 ± 0.155	1.794 ± 0.668
Siphoned and Fed Daily	0.783 ± 0.158	1.871 ± 0.717
Siphoned and Fed Daily (Sediment)	0.779 ± 0.174	1.922 ± 0.683

Overall, 67.5% (SD = 16.5%) of juveniles were active under the dissecting scope immediately after they were collected from the fish tanks. When juveniles are active on the bottom of the aquaria, they are expending energy. If food is not available, this could result in an energy deficit early in life. Having food readily available may help prevent such metabolic deficits. Juvenile activity was significantly higher ($p = 0.003$, GLM) in the sediment treatment than in the other three treatments which were statistically similar (Table 4.10). The higher activity observed in the sediment treatment could have been related to the extra handling to find the juveniles, and we don't think this represents a biologically significant difference.

Table 4.10. Percent of juveniles in each treatment active immediately after excystment.

Treatment	Percent of Juveniles Active	SD (%)	n
1	57.8	17.2	8
2	62.0	9.8	5
3	67.6	8.6	5
4	88.4*	5.8	5

* = significantly different (α=0.05)

Conclusions

1. **The health and propagation success of broods can vary between individual adult females at a given site.** For this reason, broods cannot be mixed in mass propagation with the assumption of equal juvenile production and survival. When mussels are propagated for release into the wild, an accurate understanding of genetic contribution of the stock is crucial to population management. If multiple broods are mixed prior to assessment of long-term survival as juveniles in captivity, the genetic contribution of individual broods is unknown.
2. **Using a saturated salt solution to test glochidial viability may not accurately portray the true viability of a given brood.** It appears there can be variation in glochidial viability that is not detected by a simple response to salt. More research is needed to understand development of mussels in this lifestage.
3. **Results of these feeding trials indicate no significant advantages to feeding with these algal mixes or with detritus as used here.** Still, growth and survival in the lab is not optimal, and more research is needed to determine the role that different food resources might play in captive culture.
4. **Although no statistically significant differences were seen, supplying algae in fish tanks during juvenile excystment may increase growth and survival of juveniles.**

Chapter 5: Microsatellite Development

Introduction

A key component of this work was to be able to release captive-reared freshwater mussels into natural populations to augment and/or reintroduce threatened species in a manner similar to what fisheries scientists have done for decades. To avoid repeating the well-documented failures of some of these stocking programs (Waples 1991), genetic markers are needed to assess the contribution of released captive-reared stock to the genetic diversity of wild populations. These markers will facilitate the refinement of brood stock selection and management, determination of appropriate stocking densities, and the development of protocols for monitoring the long-term contribution of stocked individuals to augmented mussel populations. While other labs have been developing markers targeting North American species (Eackles and King 2002; Jones *et al.* 2004; Shaw *et al.* 2006; Zanatta and Murphy 2006), little effort has focused on the imperiled Southeastern fauna. Our effort represents the fourth Lampsiline to be examined and the first on the SE Atlantic slope.

Methods and Preliminary Results

Villosa vaughaniana (Lea 1838), the Carolina Creekshell, was selected as the target species for developing markers for population genetic studies. This species was targeted, in part, because of our previous success in culturing the species in our facility. Additionally, there is a need to determine genetic viability for existing populations of this state endangered species, many of which are in decline due to a variety of environmental impacts. Since *V. vaughaniana* has been extirpated from its type locality (Sawney's Creek, near Camden, SC) we chose a museum vouchered and georeferenced individual (NCSM 29619.1, NC: Cabarrus Co., Rocky River) as the individual from which to develop markers. We began our efforts by extracting DNA from this individual. Extracted DNA was sent to the University of Georgia, Savannah River Ecology Laboratory (SREL) to initiate the first steps of isolating markers (steps I-IV, Glenn and Schable 2005). The SREL laboratory provided us with a microsatellite enriched "library" of DNA fragments from which to proceed through their protocol.

Potential microsatellite containing fragments were PCR amplified, cloned into a plasmid vector and transformed into *E. coli* for screening to isolate unique sequences. Positive clones were subcultured overnight then PCR amplified using the M13 primers present in the plasmid used in transformation. These microsatellite containing amplicons (amplified DNAs) were then cleaned and sequenced in both directions again using the M13 primers. Sequences were cleaned and loaded onto an ABI 3130XL automated DNA sequencer for visualization. These steps follow the protocol outlined in Glenn and Shable (2005). This has yielded approximately 2,300 potentially unique sequences from which primer design has been initiated, but not completed. To date, we have designed approximately 15 primer pairs and have begun to test these for functionality against a collection of tissues obtained throughout the current range of *V. vaughaniana* and other related taxa. Tables 5.1 and 5.2 document the primers we have designed and/or tested to date.

Table 5.1. Primers designed and ready to be employed in genetic study.

Name	Primer Sequence	Repeat Unit	Preliminary Size Estimate
Vvau001	For: TAT CAA CCG CAC ATC TGC AT Rev: TCA CAA ACT CAC CCC TCC TC	$(CATA)_{24}$	171 bp
Vvau002	For: AAG CAG CGC CAT TAT CAT TT Rev: ACC AGA AGA GGC ATG GAA TG	$(TGNG)_5$	165 bp
Vvau003	For: GCA CAT GCA ATT GGA GAG AA Rev: AAA TTG GGG ATG TGC GTT AC	$(GA)_{36}$	118 bp

If our results should follow other's successes, we would expect to complete primer design and have approximately 20 useful primer pairs after testing 100 to 150 primer pairs designed in the course of this project. These microsatellite primers will be used to test genetic diversity and measure population genetic parameters throughout the range of *V. vaughaniana*. Though costly (both in terms of money and laboratory time), these techniques and the markers developed are invaluable in aiding researchers to define natural genetic conditions in sparse and dense populations. These markers will be available for use in tracking long-term viability of stocked individuals by tracking their genetic contributions to subsequent generations in the wild.

Table 5.2. Untested primers designed in the study.

Primer Sequence	Repeat Unit	Preliminary Size Estimate
For1: AGT GGC TAG AGA CAT GTG AT For2: GCA TAG TAT GGT GAA AGG TC Rev: GAG ACG AGT CTC CTT TAT GA	$(GT)_{25}$	For1: 283 bp For2: 235 bp
For: AGT ATT GGT GCT GGA CAC TC Rev: GCA TCT GTG AAT CAA ACA AA	$(TATG)_4(TA)$ $(TATG)(TG)_8(TATG)_5$	271 bp
For: GAG AGC CAA AAG CAA ATA GA Rev: TCA AGA TAC TCA CGC ATC TG	$(GT)_{20}$	184 bp
For: GGG CTG AAT TAG GAT TCT CT Rev: GTT CCG GTG AGA AAC TAT GA	$(TG)_{17}(TTT)$ $(GT)_7(GA)_{13}$	225 bp
For: AGC GTT CAA AGT GGA ACC AA Rev: TTC ATA GGA GCA AAG GAC ATC A	$(TCAA)_{16}$	213 bp
For: CAA TGC CTA CAG GAC AAT TT Rev: GGG ACA GAC AAA ATA AAG CA	$(CT)_{32}$	296 bp
For: CTG TGT AAT TCT TGG TTA GG Rev: AAG TGT TAC GTG GTG TTA TC	$(AC)_{11}$	259 bp
For: GCA TGC TCT TAT GCG ATC AA Rev: TTC ACC TTG CCC TTC AGA AT	$(CA)_6(CT)$ $(CA)_3T(CA)_2$	231 bp
For: CAC CCT CAA ACG TTC TCT CC Rev: GCT CAC TCC CTC ACA CAC AC	$(CT)_{25}$	180 bp
For: CAT GCT GCA TTA GTG GGA AA Rev: GAA TGA TCG TTC ACC ACA TCA	$(ACAT)_{20}$	226 bp
For: TGC CAA CTA AGA ATA ATA AGA Rev: CGG AAA TCT GAT ACA TAC AT	$(TATC)_9 \ldots (ATGT)_{24}$	171 bp
For: GAC AGT CAG GAG CAG CAG AA Rev: CAT GAA AGC CAC ACA GGC TA	$(GTAT)_{30}$	226 bp

Discussion and Future Research Directions

This grant has allowed us to generate an inordinate amount of preliminary genetic data from which to develop microsatellite primers. The 2,300 sequences we have generated are potentially unique but must be examined by hand until software (in development in several labs) becomes available to allow for rapid screening for microsatellite containing regions. As such, our raw genetic database is being compiled and sorted by hand to find useful regions in the genome. We will continue to sort through these sequences and design primers for testing in the lab over the course of the next 12-18 months.

During primer development, we have solicited tissues from existing collections of *V. vaughaniana* from biologists throughout North and South Carolina. We have chosen to exploit only existing collections so as not to further impact this imperiled species. If we can gather enough tissue from fellow biologists, we will be able to measure the population genetic parameters initially proposed (genetic bottlenecks, existing genetic diversity, numbers of individuals existing in a wild population, etc.). However, if we cannot gather statistically significant numbers of tissues from throughout its range, we will need to explore alternative DNA sources (mantle clips, hemolymph, or swab-based sampling being developed elsewhere) to gather the preliminary information needed to direct future population augmentation initiatives.

Once adequate baseline genetic data are acquired from native populations we will be able to make recommendations about augmenting with laboratory-reared stock. In addition we anticipate being able to genetically track these stocked animals and measure their contributions to subsequent generations. This is a long-term goal, but one which must be examined to measure the success of our augmentation efforts.

Smaller and more basic research questions that might be addressed could be generated from examining our captive populations. Many of these are question that have been impossible to answer previously. Microsatellites should facilitate parentage analysis, or how many males contribute to a female's brood. Given that mussels release sperm into the water column, it is unknown how many males are responsible for any given female's brood. Additionally, is there an effect of distance on fertilization success in a stream; in other words, is there an optimal distance between individuals for successful fertilization? Related to these questions are the following:

1) Is there an optimal density of males (or females) in a healthy population?
2) Is there an optimal ratio of males to females in a healthy population?
3) Do females generate multiple broods per reproductive season?

Once markers are developed, these questions might be addressed for the first time. As such, the expense and time devoted to microsatellite development will aid biologists in making informed decisions concerning wild populations of freshwater mussels.

Literature Cited

Eackles, M. S. and T. L. King (2002). "Isolation and characterization of microsatellite loci in Lampsilis abrupta (Bivalvia: Unionidae) and cross-species amplification within the genus." Molecular Ecology Notes **2**(4): 559-562.

Glenn, T. C. and N. A. Schable (2005). "Isolating microsatellite DNA loci." Methods Enzymol **395**: 202-22.

Jones, J. W., M. Culver, et al. (2004). "Development and characterization of microsatellite loci in the endangered oyster mussel Epioblasma capsaeformis (Bivalvia: Unionidae)." Molecular Ecology Notes **4**(4): 649-652.

Lea, I. (1838). "Description of new freshwater and land shells." Transactions of the American Philosophical Society **6**: 1-154.

Shaw, K. M., T. L. King, et al. (2006). "Isolation and characterization of microsatellite loci in Alasmidonta heterodon (Bivalvia: Unionidae)." Molecular Ecology Notes **6**(2): 365-367.

Waples, R. S. (1991). "Genetic interactions between hatchery and wild salmonids—lessons from the Pacific Northwest." Canadian Journal of Fisheries and Aquatic Sciences **47**: 968-976.

Zanatta, D. T. and R. W. Murphy (2006). "Development and characterization of microsatellite markers for the endangered northern riffleshell mussel Epioblasma torulosa rangiana (Bivalvia: Unionidae)." Molecular Ecology Notes **6**(3): 850-852.