

**2008 Cheoah Fund Report
Contract No. 1930
North Carolina Wildlife Resources Commission**

Captive Culture of Priority Fishes and Mussels to Restore Cheoah River Populations

Grant period- July 2008-June 2009

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Introduction

Minimum flows were recently established in the Cheoah River and other habitat improvements are underway as part of the FERC relicense agreement. Restoration of multiple species, including Appalachian elktoe, *Alasmodonta raveneliana* (Fed. & NC Endangered); Spottfin chub, *Erimonax monachus* (Fed. & NC Threatened); Wavy-rayed lampmussel, *Lampsilis fasciola* (NC Species of Concern); and Rainbow mussel, *Villosa iris* (NC Species of Concern), are part of the cooperative restoration plan for the Cheoah River.

In 2008, the Cheoah Restoration Fund Board granted the North Carolina Wildlife Resources Commission \$20,000 to support reintroduction of the species listed above to the Cheoah River (full proposal text attached in Appendix). The primary goal for use of these funds was to expand present NCWRC capacity to propagate and grow conservation priority aquatic species to releasable size to meet objectives for species restoration and conservation in the Cheoah River and elsewhere in North Carolina. Specific objectives were:

1. Construct propagation and grow-out facility in an existing structure at the NCWRC Marion State Fish Hatchery.
2. Grow-out Spottfin Chub fry propagated by Conservation Fisheries, Inc. (CFI) to releasable size at the Marion Hatchery and release in the Cheoah River (~9 months).
3. Begin propagation and grow-out of Wavy-rayed lampmussel and Rainbow mussels to releasable size at the Marion Hatchery for release in the Cheoah River (~2 yrs).
4. Begin experimental propagation of Appalachian elktoe and develop techniques for production of releasable size animals.

Schedule and Deliverables

Milestones

- July 2008 Begin construction of culture facility
- Sept. 2008 Complete construction and begin mussel grow-out
- Oct. 2008 Begin Spottfin Chub grow-out (dependent on availability from CFI)
- June 2009 Release Spottfin Chubs

Reimbursement Schedule and Deliverables

- October 2008 \$10k System operational and Spottfin chub and mussels on-site
- May 2009 \$10k Demonstrate survival and growth of fish and mussels and on schedule for release of fish in June

Results

Construction of culture facility In April 2008, NCWRC Western Aquatic Wildlife Diversity and Fish Production staff and NCSU cooperators completed final planning and funding allocations for the expansion of mussel and rare fish culture at the Marion Fish Hatchery. Construction began in July 2008 to convert an existing structure at the Marion Hatchery into approx. 1200 sq. ft. of space for controlled propagation and grow-out of freshwater mussels and non-game fishes. The facility includes 80 seven-gallon mussel grow-out containers, six 50-gallon fish and mussel holding tanks, two recirculating quarantine units with four nine-gallon tanks each, six 50-gallon host fish holding tanks configured to continuously collect juvenile mussel, and eight 300-gallon fish rearing tanks (see Figures 1-7). Water is supplied from the warm water supply pond at the hatchery. Water flows through the system and is filtered and UV sterilized to prevent escape of organisms before it's returned to the pond. Lighting is controlled by an astronomical timer to mimic annual seasonal photoperiod.

Mortalities among Spottfin chubs and mussels that occurred in late June and July were apparently related to rapidly rising water temperatures in the hatchery. A new water line was run to the upper, cooler water supply pond at Marion and infrastructure installed to allow mixing of water from each source to attenuate temperatures in the hatchery. Thermostat-controlled ventilation fans and vents were also installed in the building to aid in temperature control. The immediate problems were solved and we now have a relative range of water temperatures available in different parts of the hatchery.

Mussel culture In August, juvenile and adult mussels of several species were transferred to the new Conservation Aquaculture Center (CAC) from the Table Rock Fish Hatchery and a temporary shed at Marion (Figure 8). By early March, approximately 500 individuals of six mussel species were being held at the CAC, including the Federal and State Endangered Tar River spiny mussel (*Elliptio steinstansana*) and Carolina heelsplitter (*Lasmigona decorata*). Mussels included adults that were rescued from drying streams during 2007 and 2008 drought and juveniles that

were propagated in captivity at NCSU College of Veterinary Medicine. Several thousand early juvenile Wavy-rayed lampmussels were produced in 2008 at NCSU from Little Tennessee River stock and approximately 1000 of those survived into early 2009. Plans were to transfer these to the CAC in summer 2009 for grow-out and release to the Cheoah in late 2010. Unfortunately, water quality problems and an outbreak of predatory flatworms at NCSU's Mussel Barn resulted in a total loss of the cohort.

While not directly related to the Cheoah Restoration project, construction of the CAC allowed us to pursue important culture efforts with other high priority species. Three endangered Tar River spiny mussels held for nearly two years at Table Rock, again became gravid (brooding larvae) in captivity at the CAC in late March. This critically endangered species had first been propagated in captivity during host fish trials at NCSU in 2007. Lessons learned from the first attempt should improve chances for successful propagation this year and further advance our knowledge of its early life history and habitat requirements. Since being moved to the CAC, no problems have been observed among these sensitive species; in fact, they have apparently thrived showing good growth rates and normal reproductive activity.

Spotfin chub culture and reintroduction In September, approximately 1000 Spotfin chubs were transferred to the facility from the CFI facility in Knoxville, TN (Figure 9). No problems were observed as animals acclimated to the new facility. However, an outbreak of the protozoan parasite *Chilodonella* in February caused significant mortality among Spotfin chubs (~100) before it was brought under control. Nonetheless, growth rates exceeded expectations and no further significant mortalities were experienced through spring 2009. A heat wave in June resulted in a rapid increase in water temperatures in the water supply pond and in the CAC. This stressed Spotfin chubs and contributed to an outbreak of hemorrhagic septicemia that caused significant mortality before it was brought under control. Ultimately, ~600 chubs were successfully reared and released at mile 7.4 in the upper Cheoah River on June 25 and July 1.

Mussel propagation In March, gravid brood females of the Wavy-rayed lampmussel and Rainbow mussel were collected from the Little Tennessee River for captive propagation at the CAC. Female Wavy-rayed lampmussels used in this effort were selected to include a variety of mantle-flap lure morphs in numbers approximating the proportion of lure morphs seen in the Little Tennessee River population. Mantle-flap lure diversity is an indicator of genetic diversity (Zanatta et al. 2007); thus, a representative proportion of the genetic diversity in the source population should be included in our sample. Under the guidance of cooperating NCSU researcher Chris Eads, gills of appropriate host fishes (largemouth bass, rock bass, mottled sculpins) were infested with larvae (glochidia) extracted from the female mussels (see Figures 10-11). Infested host fishes were held in tanks configured to continuously collect the transformed juvenile mussels (see Figures 12-13) until they all drop from the fishes (~14-20 days). Brood mussels of both species were collected and host fishes were infested in late March, mid-May, and late June. Production was hampered by health problems with host fishes; however, ~18,000 Wavy-rayed lampmussels and ~2000 Rainbow early juveniles were recovered.

Prior to our efforts in 2009, limited experiments to determine host fishes were the only attempts to culture Appalachian elktoe in captivity. In April 2009, gravid females were collected from the Little Tennessee River and an array of known host fish species were collected for further experimental work at NCSU. These experiments are intended

primarily to help develop production techniques (e.g. identify most productive hosts, fine tune early juvenile life support systems), but any surviving juveniles will be reared and used for augmentation in the Cheoah River. Several thousand juveniles were successfully transformed in June. Adults were returned unharmed to the Little Tennessee River after glochidia were extracted.

Conclusions

All milestones and deliverables identified during the grant period were met. The primary achievement this year was the successful construction and initial operation of the Conservation Aquaculture Center. While there were challenges, they were met and the ability of the facility and staff to keep sensitive mussel and fish species alive and growing was proven. The first cohort of Spotfin chubs was successfully reared at Marion and released in the Cheoah River. The first steps to propagate Wavy-rayed lampmussel and Rainbow mussels on-site were successful. The survival and growth of other rare and closely related species is favorable for culture of Appalachian elktoe at the CAC in the future. Propagation of Appalachian elktoe was successful at NCSU and we are optimistic for their survival and growth through their first year.

Literature Cited

Zanatta, D.T., S.J. Fraley, and R.W. Murphy. 2007. Population structure and mantle display polymorphisms in the wavy-rayed lampmussel, *Lampsilis fasciola* (Bivalvia: Unionidae). Canadian Journal of Zoology. 85: 1169-1181.

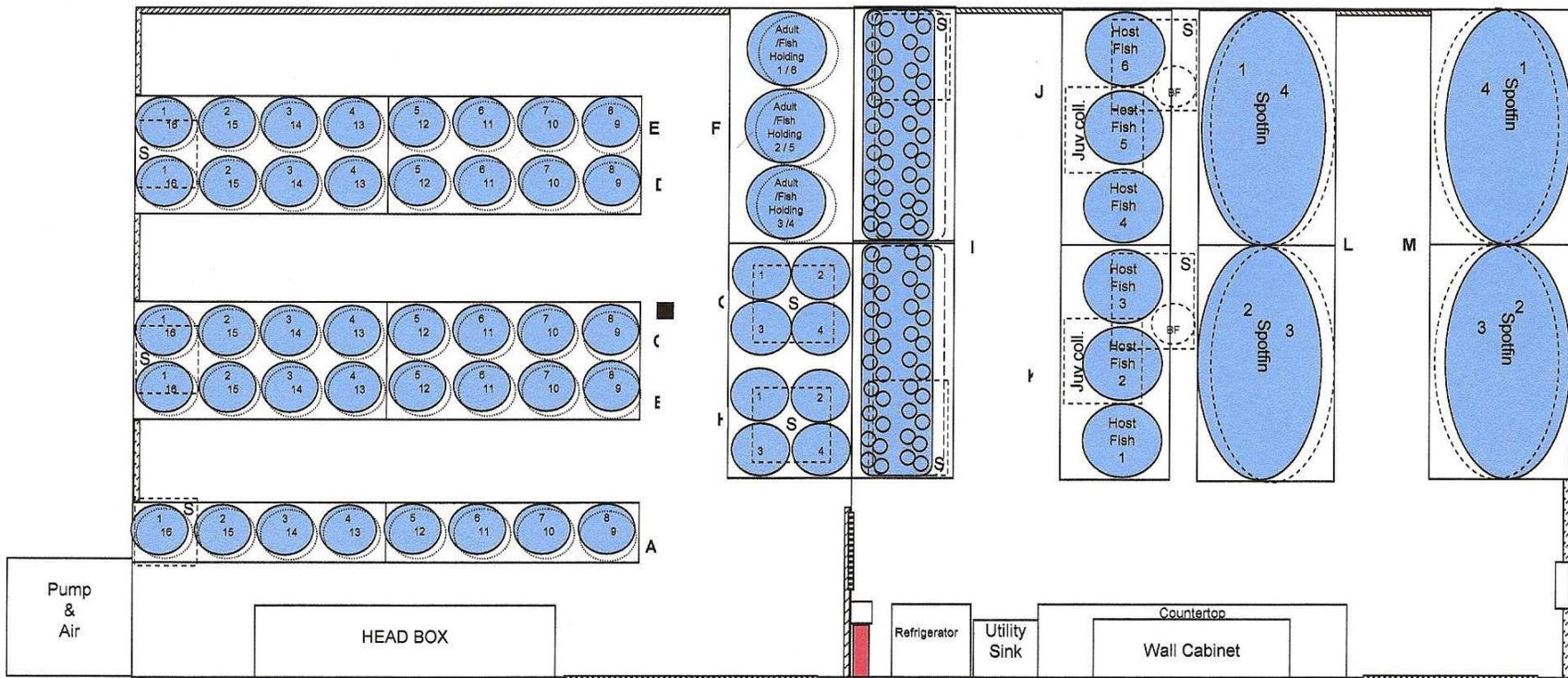


Figure 1. Floor plan, Conservation Aquaculture Center at Marion State Fish Hatchery.



Figure 2. Conservation Aquaculture Center at Marion, hatchery building in foreground, with water supply pond behind.



Figure 3. NCWRC Fish Production and AWD staff during construction.



Figure 4. Three-hundred gallon fish holding tanks used for Spottfin chub rearing.



Figure 5. Seven gallon mussel grow-out containers.



Figure 6. Primary system water and air pumps.



Figure 7. Filter and ultraviolet sterilization array on out-going water line.



Figure 8. Age 1yr.+ juvenile Wavy-rayed lampmussels from Pigeon River stock moved from Table Rock Hatchery (note: mussels attached by byssal threads, present only in juveniles).



Figure 9. First cohort of Spotfin chub fry arrive at Marion September 11, 2008.



Figure 10. Glochidia extracted from Rainbow mussels during first on-site propagation effort March 24, 2009.



Figure 11. Batch infestation of host fishes with Rainbow mussel glochidia.



Figure 12. Holding tanks for infested host fishes with double stand pipe to continuously draw transformed juvenile mussels out with overflow.



Figure 13. Overflow from host fish tank with filter to collect juvenile mussels.

Appendix

2008 NCWRC Cheoah Fund Proposal

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Grantee

North Carolina Wildlife Resources Commission

Statement of Problem

Minimum flows were recently established in the Cheoah River and other habitat improvements are underway as part of the FERC relicense agreement. Restoration of multiple species, including Appalachian Elktoe, *Alasmidonta raveneliana* (Fed. & NC Endangered); Spotfin Chub, *Erimonax monachus* (Fed. & NC Threatened); Wavy-rayed Lampmussel, *Lampsilis fasciola* (NC Species of Concern); and Rainbow mussel, *Villosa iris* (NC Species of Concern), are part of the cooperative restoration plan for the Cheoah River.

Reintroduction of Spotfin Chub via captive propagation is presently underway elsewhere in Tennessee and Alabama. Conservation Fisheries, Inc. (Knoxville, TN) produces fish for these efforts using brood stock from the Little Tennessee River in NC, but their space for grow-out is presently at capacity and cannot supply fish for the Cheoah effort until other efforts are completed. This is likely to be several years, if not decades, away.

A relict population of Appalachian Elktoe is presently extant in the Cheoah River and augmentation to improve demographics and genetic diversity and assist expansion into revitalized habitats is part of the restoration plan; however, beyond experiments to determine host fishes, controlled propagation of Appalachian Elktoe has not been attempted. Recent identification of additional host fishes and more extensive and successful propagation experiments with related species (*A. varicosa*, *A. viridis*, and *Lasmigona decorata*) supports optimism for successful propagation of Appalachian Elktoe in the near future. We are presently cooperating with Dr. Morgan Raley (NC Museum of Natural Sciences [NCMNS], Raleigh) to describe the genetic structure of all extant Appalachian Elktoe populations (also supported by the Cheoah Fund), making management of genetic diversity in reintroduction and augmentation efforts possible.

Wavy-rayed Lampmussel and Rainbow are known to be relatively easy to culture and brood stock is also available from the Little Tennessee River in NC. In October 2007, 35 three year-old Wavy-rayed Lampmussels were released in the Cheoah River. These were the products of controlled propagation experiments at NC State University (NCSU) and NC Wildlife Resources Commission (NCWRC) Table Rock Hatchery with Little Tennessee River stock. However,

present capacity for mussel culture is limited to experimental scales. Expansion of our culture capacity is necessary to provide the numbers of each priority species required to meet Cheoah River restoration objectives.

Proposed Action

The primary objective is to **expand present NCWRC capacity to propagate and grow conservation priority aquatic species to releasable size to meet objectives for species restoration and conservation in the Cheoah River** and elsewhere in North Carolina. Sub-objectives are:

- 5. Construct propagation and grow-out facility in an existing structure at the NCWRC Marion Fish Hatchery.**
- 6. Grow-out Spotfin Chub fry propagated by Conservation Fisheries, Inc. (CFI) to releasable size at the Marion Hatchery and release in the Cheoah River (~9 months).**
- 7. Begin propagation and grow-out of Wavy-rayed Lampmussel and Rainbow mussels to releasable size at the Marion Hatchery for release in the Cheoah River (~2 yrs).**
- 8. Begin experimental propagation of Appalachian Elktoe and develop techniques for production of releasable size animals**

We are presently partnering with NCSU (also supported by NC Dept. of Transportation, Project No. HWY 2005-07) to investigate controlled propagation of native mussels (including those mentioned above), including evaluation of three NCWRC hatcheries for suitability for mussel grow-out. Trials at Table Rock and Marion hatcheries proved most successful with good survival and growth rates (Eads, et al. 2007). Initially, two raceways were installed in existing facilities at Table Rock in 2005, with two more added in 2007. A small temporary shed containing grow-out facilities was installed at Marion Hatchery in 2007. Survival and growth equaled or exceeded that seen at Table Rock. Due to these results, the availability of a suitable existing structure and personnel at Marion, and anticipated renovations and other limitations at Table Rock, we propose expanding our controlled propagation capacity at the Marion Hatchery.

An existing enclosed shed located at the Marion Hatchery will be renovated and infrastructure fabricated and installed. Planning and execution are in cooperation with Chris Eads, NCSU College of Veterinary Medicine, and WRC personnel: Steve Fraley and T.R. Russ, Western Region AWD staff; Kyle Briggs, Fish Production Coordinator; David Deaton, Fish Production Technology Technician; Gene Wilson, Table Rock Hatchery Superintendent; and Mallory Martin, Mountain Region Fisheries Supervisor. We will cooperate with CFI and University of Tennessee for equipment design and technical guidance for Spotfin Chub grow-out. Further technical expertise will be sought through WRC Division of Engineering Services or commercial sources as needed.

Mussel culture and training will be overseen by Chris Eads, NCSU cooperator. Western Region AWD staff (Fraley, Russ, and technician); David Deaton, Fish Production Technology Technician; and, Chris Wood, Eastern Region AWD Biologist will assist and train in all aspects of propagation and grow-out. Primary on-site responsibilities will be shared by Marion-based AWD staff (Russ and technician) and Deaton. Additional personnel needs will be evaluated during the first year of operations.

Initially, juvenile mussels grown-out at the Marion facility will be exclusively produced at the NCSU mussel propagation facility ("Mussel Barn"). Within the first year of operations, host fish

infestations and propagation of juvenile mussels should begin at Marion. After that, grow-out will likely continue to be a mix of mussels produced both on-site and at the Mussel Barn. Most mussel species will be cultured in captivity for one to three years before release. Experiments to date show growth rates are sufficient in that time to reach sizes with greater chances for survival in the wild, and in some cases, sexual maturity. Spotfin Chub juveniles approximately 0.5 inches (13mm) TL will be acquired from CFI (Knoxville, TN) and could be available by October 2008. Spotfin chubs acquired in October could be ready for release (~1.5 in. [~40mm]) the following late spring/early summer (May-July).

Budget

This covers construction and part of first year operations. Continued funding to support culture of species for release in the Cheoah River will be sought annually until the need for cultured animals ceases.

Total cost of project	\$100,000
Cheoah Fund contribution	20,000
Balance sought from other sources (USFWS Section 6 Grant, State Wildlife Grant, and matching funds from a variety of sources)	80,000

Schedule and Deliverables

Milestones

- July 2008 Begin construction of culture facility
- Sept. 2008 Complete construction and begin mussel grow-out
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Reimbursement Schedule and Deliverables

October 2008	\$10k	System operational and Spotfin chub and mussels on-site
May 2009	\$10k	Demonstrate survival and growth of fish and mussels and on schedule for release of fish in June

We can present an interim report and/or final results to the Annual Cheoah Board Meeting.

Permits

The requestors are presently permitted by the NCWRC and USFWS to cover the activities proposed here.

Literature Cited

Eads, C.B., M.E. Raley, E.K. Schubert, A.E. Bogan, and J.F. Levine. 2007. Final Report: Propagation of freshwater mussels for release into North Carolina waters. North Carolina Dept. of Transportation Project Number: HWY-2005-07. NC State University, College of Veterinary Medicine and NC Museum of Natural Sciences, Research Laboratory, Raleigh, NC. 87p.