

**2010 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 2009-June 2010**

**Steve Fraley**

N.C. Wildlife Resources Commission  
50 Trillium Way  
Clyde, NC 28721  
Home/office: 828-627-8414  
Cell: 828-550-0064  
[stephen.fraley@ncwildlife.org](mailto:stephen.fraley@ncwildlife.org)

**Introduction**

In 2005, minimum flows were established in the Cheoah River and other habitat improvements are currently underway as part of the Federal Energy Regulatory Commission (FERC) relicense agreement for Santeetlah Dam. Restoration of multiple species, including Appalachian elktoe, *Alasmidonta 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.

We received support from the Cheoah Fund in 2008 to improve the NCWRC's infrastructure to enable propagation and culture of Wavy-rayed lampmussels, Rainbow mussels, and Appalachian elktoes, and to begin grow-out of Spottfin chubs for release in the Cheoah River. Those funds were used successfully to leverage further funding and an appropriate facility, the Conservation Aquaculture Center (CAC), was constructed at the Marion State Fish Hatchery, Marion, NC, and all deliverables were completed (see NCWRC Cheoah Fund Report 2009). Support from the Cheoah Fund was also provided in 2009 to continue to propagate and grow the target species to releasable size to meet objectives for species restoration and conservation in the Cheoah River. Specific objectives were:

1. Collect brood stock for third cohort (2010), receive and rear second Spottfin chub cohort (2009) propagated by CFI and release in the Cheoah River.
2. Collect brood stock and conduct production-oriented propagation and culture of Wavy-rayed lampmussel and Rainbow mussel at the Marion Hatchery for release in the Cheoah River.
3. Continue experimental propagation of Appalachian Elktoe and develop propagation and culture techniques for production of releasable size animals for release in the Cheoah River.

## Schedule and Deliverables

### Milestones

- July 2009 Complete mussel brood stock collection and host fish infestations.
- Oct. 2009 Obtain 2009 Spotfin chub cohort for grow-out (dependent on availability from CFI) and complete stage 1 and 2 early juvenile rearing of Wavy-rayed lampmussel and Rainbow and continue Appalachian elktoe propagation and culture experiments.
- June 2010 Release second captively propagated Spotfin chub cohort (2009) and collect both chub and mussel brood stock from Little Tennessee River.

### Reimbursement Schedule and Deliverables

- |           |      |   |
|-----------|------|---|
| Oct. 2009 | \$5k | Obtain 2009 Spotfin chub cohort for grow-out (dependent on availability from CFI) and complete stage 1 and 2 early juvenile rearing of Wavy-rayed lampmussel and Rainbow and continue Appalachian elktoe propagation experiments. |
| May 2010  | \$5k | Demonstrate survival and growth of Spotfin chubs, Wavy-rayed lampmussel, and Rainbow.   |

## Results

### Mussel propagation and culture

**2009 cohort:** The first cohort of Wavy-rayed lampmussel and Rainbow mussels transformed in spring and summer 2009 remained in the early juvenile culture unit until mid-March 2010. Samples taken periodically showed relatively good growth but poor survival rates were indicated. Measures taken to prevent flatworm infestation in the juvenile unit were apparently ineffective. High numbers of flatworms, as well as various cladocerans and many other small invertebrates, were seen in samples throughout the growing season. Predation and competition apparently contributed to survival of only 71 (0.4%) Wavy-rayed lampmussels and 48 (2.4%) Rainbows by 8 March, 2010, when both species were transferred to containers in the main grow-out unit. While survival was relatively poor, growth had been fair. Mean lengths were 5.2mm (range: 2.6-11.1) among Wavy-rayed lampmussels and 4.4mm (range: 3.1-6.2mm) for Rainbows when they were transferred in March (see Figure 1). Routine early season inventory on 4 May, 2010 showed good growth in their first two months in the flow-through grow-out containers (mean growth: Wavy-rayed lampmussels=1.7mm; Rainbow=1mm). Upgrades to the filtration system and other measures aimed to improve juvenile survival were put in place before propagation of the 2010 cohorts began (see *Infrastructure Improvements*, below).

**2010 cohort:** Gravid female brood mussels were again collected from the Little Tennessee River in March 2010. Host fishes for Wavy-rayed lampmussels (largemouth bass, *Micropterus salmoides*) were purchased from a commercial supplier, while hosts for Rainbows (rock bass, *Ambloplites rupestris*) were collected from local streams without native mussel populations to ensure naïve hosts. All fish were quarantined for one week and treated with a regime of formalin solutions to remove parasites and other unwanted organisms. Host fishes were infested on 26 March and held in tanks designed to continuously collect sloughed juvenile mussels. Transformed juveniles were collected from 13 April through 4 May and placed in the

early juvenile culture unit. Total number of transformed juveniles recovered by species: Wavy-rayed lampmussel~31,500, Rainbow~8,700 (totals are estimates extrapolated from samples).



**Figure 1. Rainbow mussels from 2009 cohort, May 2010.**

On 14 June, growth and survival of approximately half of the first batch of Wavy-rayed lampmussels were assessed. Substrata from holding baskets were sieved to 500 microns and 6304 juveniles were recovered between 500 microns and 1mm in length (~39% survival). We were pleased with this rate of growth and survival. Growth was sufficient in two months to exceed the size of major vulnerability to flatworm predation, and these juveniles were moved to the main grow-out unit. While a few flatworms were seen in the early juvenile unit during our assessment and transfer, densities were much lower than were seen last year. The remaining half of juveniles from the first batch were transferred to the second stage early juvenile unit for further growth in the partially recirculating system with supplemental feeding.

Gravid female Wavy-rayed lampmussels and Rainbow mussels were again collected from the Little Tennessee River on 16 June for production of a second batch of juveniles. Largemouth bass purchased from a commercial supplier were used as hosts for both mussel species. Wild rock bass have been difficult to maintain in the large holding tanks for infested hosts, apparently due to agonistic interactions between individuals. This is the first time we've tried this approach with Rainbows and the outcome was uncertain. After quarantine and disinfection, hosts were infested with glochidia on 22 June and transformation was completed within two weeks. Transformation and recovery rates of Rainbows with largemouth bass as host proved to be

more successful than previous attempts with rockbass or sculpin. Total transformed juveniles recovered and transferred to the early juvenile unit by species: Wavy-rayed lampmussel- 24,578, Rainbow- 21,652.

All Appalachian elktoe juveniles transformed in June 2009 were lost to flatworm predation and water quality problems at the NCSU facility. In March 2010, gravid female Appalachian elktoes were collected from the Tuckasegee River and host fishes (Central stoneroller, *Campostoma anomalum*) were collected from Cullowhee Creek, Jackson Co., and both were taken to the NCSU propagation facility. New measures and protocols to prevent flatworm invasion and improve survival of early juveniles, similar to those at the CAC, were put in place at NCSU. A total of 619 transformed juveniles were recovered from infested host fishes. A preliminary assessment in late June indicated survival of early juveniles of ~60%.

### **Spotfin chub culture and reintroduction**

In late August 2009, 750 Spotfin chub fry were obtained from Conservation Fisheries, Inc. (CFI). Low spawning rates early in the breeding season and lower numbers of brood fish limited the number of captively-propagated fry that were available from CFI this year. However, more successful spawning later in the season resulted in additional fry reared at CFI that were available later for release in the Cheoah R. (see below). Transport stress and a subsequent outbreak of disease resulted in substantial mortalities soon after arrival at the CAC. With the assistance of the USFWS Fish Health Lab, Warm Spr., GA, problems were successfully diagnosed and treated, but total mortality over the first month at Marion was 263. No further health problems were observed and growth was excellent.

By late June 2010, lengths were 50-70mm and all surviving 385 Spotfin chub fry were released in the Cheoah River on 28 June. An additional 459 fry that were reared at CFI were released into the Cheoah at the same time (844 total released in 2010). Staff also assisted CFI to collect fresh Spotfin chub and Wounded darter (*Etheostoma vulneratum*) brood stock from the Little Tennessee River for continued propagation for the Cheoah restoration effort.

### **Infrastructure and operations improvements**

It became obvious by the end of the growing season in 2009 that measures taken to prevent flatworm invasion in the host fish and early juvenile systems had not been effective and survival of early juveniles was unsatisfactory. After surveying other culture facilities (VA, AL, USFWS) for their experiences with similar problems, a system of improved filtration, recirculation, supplemental feeding, and improved host fish handling protocols was designed. Construction began in late winter 2010 to be completed before propagation efforts began in the spring.

Filtration to five microns of all incoming water to the early juvenile and host fish and juvenile recovery units was required to prevent flatworm invasion. A progressive series of water filters (600-5 microns) was installed in water lines feeding these systems (Figure 2). Plumbing and pumps were also installed to enable adjustments from full recirculation to various degrees of flow-through in each juvenile unit independently and isolation and sterilization of filters and all units in each system downstream.



**Figure 2. New filtration system, CAC, Marion State Fish Hatchery.**

Such fine filtration also removes a substantial proportion of the suspended food items for mussels. Supplemental feeding of juveniles with commercial mariculture diets (Reed Mariculture© Shellfish Diet and Nanochloropsis mix) was now necessary. A system of reservoirs and drip lines for delivering supplemental food was installed in the early juvenile units. Recirculation and flow-through ratios and drip rates were tested and adjusted to maintain water quality and provide a target feeding rate of 30k cells/mL of water.

New tanks for host fish quarantine were also installed (Figure 3). This improved our ability to hold and disinfect newly arrived host fishes more efficiently and increase our capacity to hold more fish longer. Protocols for quarantine and disinfection were also strengthened and improved to decrease the likelihood of host fishes as a vector for contamination of early juvenile systems. All host fishes are now quarantined for at least one week and are treated with formalin solutions in a two-stage regime.



**Figure 3. New host fish quarantine tanks, CAC, Marion State Fish Hatchery.**

## **Conclusions**

After two years of experience and challenges, there appear to be no significant obstacles to meeting our goals for production of the targeted numbers of captively-propagated Wavy-rayed lampmussels, Rainbows, and Spotfin chubs for release in the Cheoah River. Problems experienced during the first attempts to propagate and culture early juvenile mussels at the CAC have been identified and addressed effectively. As a result, propagation efforts in 2010 were much more successful. Early juvenile survival rates were substantial improvements over 2009 results. To date, approximately 11,900 Wavy-rayed lampmussel and 7400 Rainbow juveniles from the 2010 cohort have survived the most critical stage for mortalities. Spotfin chub rearing has also been successful with release of a total of 1440 yearling chubs to the Cheoah River in 2009 and 2010. Mortalities from transport stress and disease continue to be a challenge for improvement. While a setback in progress toward successful propagation and culture of Appalachian elktoe was experienced with the loss of the 2009 cohort, major problems have been addressed and limited success to date supports optimism for more successful results from 2010 propagation efforts.