FINAL PERFORMANCE REPORT

Federal Aid Grant No. F15AP00923 (E-75-R-2)

Propagation and Augmentation of the Ouachita Rock Pocketbook

Oklahoma Department of Wildlife Conservation

October 1, 2015 through September 30, 2018

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State: Oklahoma **Grant Number:** F15AP00923 (E-75-R-2)

Grant Program: Endangered Species Act Traditional Section 6

Grant Title: Propagation and Augmentation of the Ouachita Rock Pocketbook

Grant Period: October 1, 2015 – September 30, 2018

Reporting Period: October 1, 2015 - September 30, 2018

Principal Investigator: Chris Barnhart, Missouri State University

A. ABSTRACT

This project was initiated to propagate larval Ouachita rock pocketbook (*Arkansia wheeleri*) for population restoration and augmentation in the Little River of Oklahoma and Arkansas. The project continued a previous project in 2013-15. During three field seasons a single female *Arkansia wheeleri* was obtained and metamorphosed using golden shiner (*Notemigonus crysoleucas*) as host fish, and also using the *in vitro* approach of placing glochidia into a nutritive liquid medium in lieu of the fish host (Barnhart 2015). Glochidia were obtained in January of 2018. *In vitro* metamorphosis was unsuccessful. Although 242 juveniles were recovered from fish hosts, these failed to thrive and survived no longer than 1 month. Failure was attributed to immaturity of the larvae. Although glochidia were removed from the female near the same date as in previous years, persistent egg membranes were present which are associated with immaturity.

The main obstacle to captive propagation of *A. wheeleri* has been obtaining brooding females. Two females have been obtained in the past 4 field seasons (including the previous project). Other factors include low and inconsistent metamorphosis success on known host fish, and grow-out of early juveniles. Both of these latter factors may depend on timing of collecting and holding of females to ensure fully mature glochidia. Further efforts with this species may hinge on sequestering a population in the field either by caging or by pit-tagging for relocation of free-ranging females. Sequestering would facilitate monitoring, collection of brood, and timely return of females to the field.

B. BACKGROUND

The Ouachita Rock Pocketbook (*Arkansia wheeleri*) is known from only three river systems in southeastern Oklahoma and southwestern Arkansas - the Little River (AR and OK), the Kiamichi River (OK) and the Ouachita River (AR). The population in the Ouachita River is very small and only one live *A. wheeleri* has been collected in recent surveys. The Kiamichi River population is larger, but with an estimated population size of less than 1,800 individuals. There is, however, no evidence for recent reproduction or recruitment into the Kiamichi River population. In the Little River, three small and apparently disjunct populations occur. One population occurs in

Oklahoma between the US 70 bridge and the confluence of the Mountain Fork River. The other two populations occur in Arkansas. One occurs within a 4.5 mile reach of the river immediately east of the Oklahoma/Arkansas state line, and the other population occurs in the lower Little River below Millwood Reservoir but upstream from the river's confluence with the Red River. The only population for which gravid females and juveniles have been found in recent years is the population that occurs below Millwood Reservoir.

Several factors appear to have played a role in the decline of the Ouachita Rock Pocketbook, including the modification of river flows and in-stream habitat as a result of reservoir construction and management, and the loss of suitable in-stream habitat as a result of siltation and channel modification. Additionally, reservoir construction has nearly eliminated the potential for genetic exchange between the remaining populations, and the small population size and scattered dispersion of individuals within these populations has likely reduced their breeding success and reproduction. The remnant populations currently face additional threats including a proposed electric power plant and a proposed inter-state sale and transfer of water out of the region and into north-central Texas. Given the species' small population size, declining population trends and the uncertainty surrounding potential new threats, it is important to examine means to enhance or augment these populations.

C. **OBJECTIVE:**

To augment current populations of the Ouachita Rock Pocketbook in the Little River. The activities of this grant will assist in implementing the recovery goals of the U.S. Fish and Wildlife Service for this species by increasing the stability of wild populations.

D. PROCEDURES:

Upon receipt of gravid female *A. wheeleri* originating from the Little River, Missouri State University (MSU) staff made preparations to extract the glochidia in the mussel propagation lab on the MSU campus. Larval mussels would then be propagated on fish hosts and/or *in vitro* until metamorphosis. Outside of the grant, resulting juveniles were planned to then be reared to a size suitable for caging on site in the Little River. When transformed juvenile mussels reached suitable size, they were to be placed into in-stream cages in the Little River on the Little River National Wildlife Refuge, where existing mussel populations occur. At this location, the mussel cages would have been monitored by U.S. Fish and Wildlife Service (USFWS) refuge staff to ensure successful establishment of the young mussels.

Propagation activities were slightly modified throughout the life of the grant to comply with the propagation and augmentation/reintroduction plan currently being drafted by the USFWS for the Ouachita Rock Pocketbook. The propagation of closely related mussel species may provide further insight into the successful rearing of *A. wheeleri*; therefore, plans were made to use grant funds for the propagation of similar species that will act as surrogates for the Ouachita Rock Pocketbook. A closely related species, the Rock Pocketbook (*Arcidens confragosus*), is biologically similar to *A. wheeleri* and is likely a suitable surrogate for propagation trials.

E. RESULTS AND DISCUSSION

Successful propagation of *Arkansia wheeleri* is contingent upon collection of viable glochidia from mature female mussels collected by ODWC and U.S. Fish and Wildlife Service (USFWS) biologists outside of the grant. Throughout the reporting period, obtaining brooding female *A. wheeleri* downstream of Millwood Dam in Arkansas was logistically challenging. A minimum number of federally-permitted, SCUBA-certified divers had to be available during the narrow brooding season period (December – January) to search for mussels. Unfortunately, increased river flows from heavy precipitation events narrowed the survey windows during the winters of 2015 - 2016 and 2016 - 2017 and no brooding females were located in those years. A single brooding female was obtained in 2017 - 2018. Despite planned efforts to do so, surrogate mussel species (i.e. *A. confragosus*) were unable to be obtained for the project and thus were not propagated during the project period.

Year 1 (2015 – 2016)

During this period, flow conditions in the Little River prevented USFWS and ODWC biologists from collecting gravid female Ouachita Rock Pocketbooks for captive rearing. Therefore, no propagation or culture of *Arkansia* took place in early 2016. The project period was extended for 1 year (to September 30, 2017) in anticipation of resumed collecting efforts for *Arkansia* in the winter of 2016 - 2017. During October-March 2016, experiments with culture methods continued with *A. wheeleri* juveniles derived from 2015. However, these activities were terminated after March 2016 in order to conserve funds for *Arkansia* work in 2017.

Year 2 (2016 - 2017)

As experienced in 2015 – 2016, flow conditions in the Little River in the winter of 2016-2017 prevented USFWS and ODWC biologists from collecting gravid female Ouachita Rock Pocketbooks for captive rearing. The project period was extended for an additional year (to September 30, 2018) in anticipation of resumed collecting efforts for *Arkansia* in the winter of 2017 - 2018.

Year 3 (2017 - 2018)

A single brooding female Ouachita Rock Pocketbook was collected on November 30, 2017 from the Little River below Millwood Reservoir by USFWS biologists David Martinez and Chris Davidson and transferred by car to Missouri State University. The mussel was marked by engraving as 18-1. Dimensions were L80.3, W60.5, H80.3 mm and mass 315.5 grams. The mussel was held in a recirculating aquarium system at 10C for one month anticipating further development of the brood. After glochidia removal the mussel was shipped to Kendall Moles from the Arkansas Game and Fish Commission on January 3, 2018, and was returned to the site of collection by AGFC within 24 hours of receipt.

Glochidia (126,000) were recovered on January 2, 2017. It was noted that egg membranes were intact on a large proportion of the glochidia (Figure 1). The glochidia were agitated by gentle shaking in water to disrupt these membranes (a routine procedure). Viability of a subsample by

salt test was 85%. Glochidia were propagated on Golden shiner (*Notemigonus crysoleucas*) and *in vitro*. Fish (n=78) were inoculated with 5700 viable glochidia per liter. From these 242 metamorphosed juveniles were recovered (average 3 juveniles per fish) which is a lower metamorphosis success than was observed previously with this species pair (10.4%, Barnhart 2015). A subset of the glochidia were propagated in cell culture medium using methods described previously (Barnhart 2015). Metamorphosis was achieved but the juveniles failed to survive after transfer to water. No juveniles from either fish or *in vitro* metamorphosis survived beyond February 10.

The failure of the glochidia to complete metamorphosis or thrive in culture may be attributable to incomplete larval development. The relatively resistant egg membranes seem to be a sign of this, but one that it not evident until after glochidia have been removed from the female. In our previous experience, mature glochidia were obtained from Little River *A. wheeleri* as early as December 1 (Barnhart 2010). However, the timing of spawning and development may vary from year to year, depending on temperature or other factors. *A. wheeleri* is a winter brooder and it is still unclear when spawning and glochidia release occur, or how many degree-days are required for complete development. It would be very useful to monitor field temperatures and attempt to improve predictions for reproductive timing of this and other species. In any future effort I recommend that we collect brooding mussels as late as possible and hold them until spontaneous glochidia release is observed, rather than extracting glochidia.

We must consider that the numbers of females (2) that we have obtained over the past 4 years (including a female found in 2014 before the grant began) is insufficient to support a restoration effort. From a practical point of view, it is very difficult for cooperators using contract funds to budget for, and commit staff and students to, projects that have a high probability of not proceeding. From a biological point of view, a small number of females may not give good genetic representation even if we are able produce a large number of juveniles. All juveniles derived from a female will be at least half-siblings, and probably most will be full siblings (Ferguson et al. 2013). Although effects of inbreeding have not been investigated in Unionids, heterosis (loosely, outbreeding vigor) is strongly expressed in other bivalves (Hedgecock 2007). An effective effort to restore *A. wheeleri* or other unionid species should ideally characterize the genetic diversity in the remaining populations and set a goal of maintaining that diversity in the propagated population (Jones et al 2006). Of course, there may be no other practically accessible populations to utilize.

In conclusion, we suggest that further propagation work with *Arkansia* should be preceded by an effort to sequester a group of 12 or more mussels in a protected but accessible area so that access to brooding females is eased. These mussels should be monitored to determine the timing of reproduction with respect to water temperature, which appears to be the key variable affecting timing (Loyd 2017). Further propagation efforts should utilize glochidia released spontaneously from females to ensure full development. Ideally, local cyprinids should be collected and utilized as hosts to identify species supporting higher metamorphosis success. *In vitro* metamorphosis requires relatively few glochidia and should continue to be used as a back-up or primary method.

Photos depicting propagation activities for *Arkansia wheeleri* can be seen in Appendix 1 of this report.

F. SIGNIFICANT DEVIATIONS:

The original grant was scheduled for one year (October 1, 2015 – September 30, 2016) but was extended for two additional years to attempt to collect additional brooding female Ouachita Rock Pocketbooks through the winter of 2017-2018.

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Approved by:

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APPENDIX 1

Figure 1. Ventral view of a glochidium of *Arkansia wheeleri*, showing the intact egg membrane (arrows). (*C. Barnhart*).

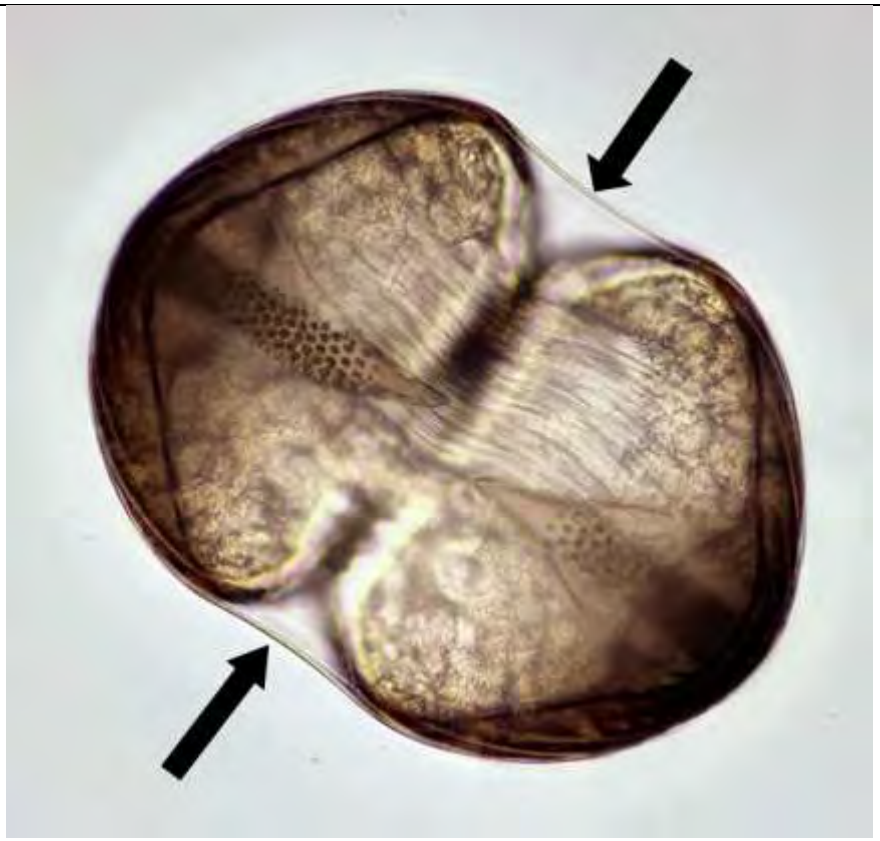

Figure 2. Extraction of glochidia from female *Arkansia wheeleri* on the campus of Missouri State University *(C. Barnhart).*

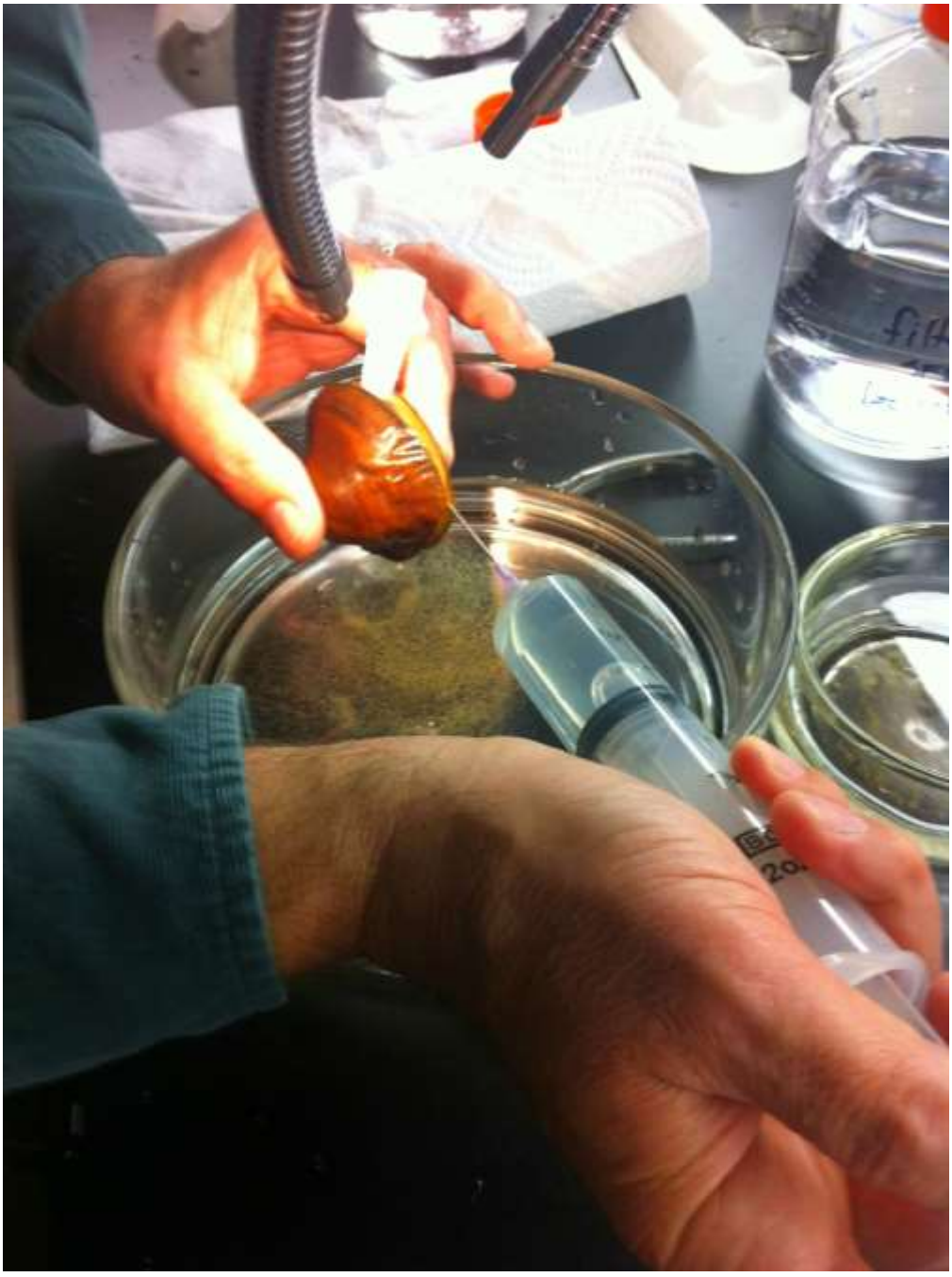

Figure 3. *In vitro* solution for mussel glochidia. Each contains 3.5 mL solution and ~300 glochidia *(C. Barnhart).*

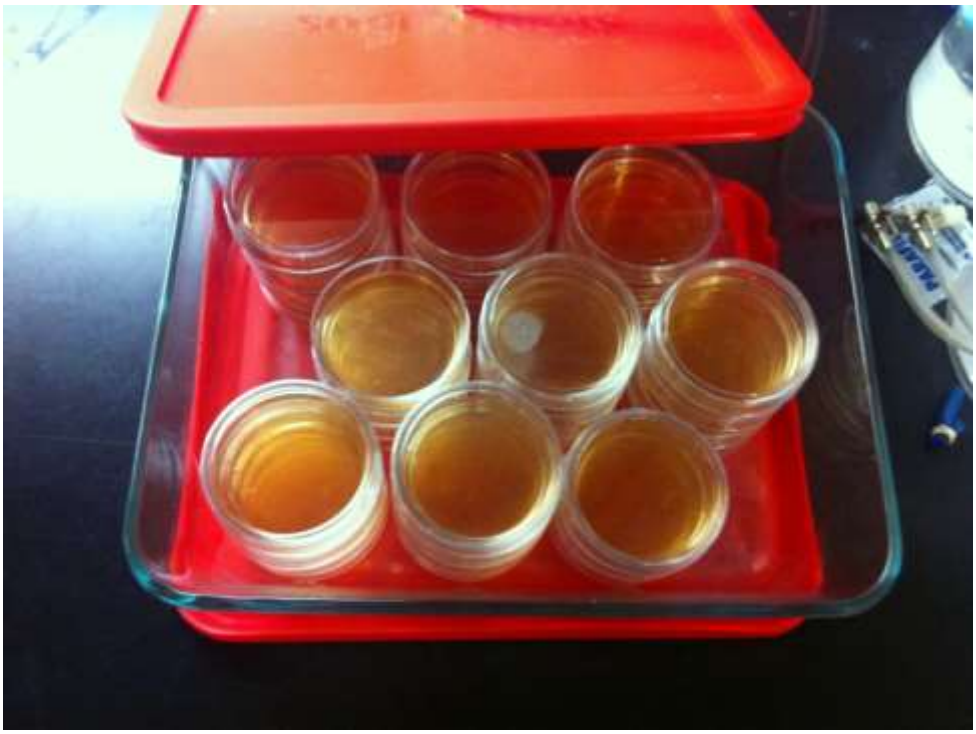

Figure 4. Larval mussel (glochidium) attached to gill filament of host fish *(C. Barnhart).*