Growing Mussels in the Desert: *ex situ* propagation of California Floater mussels (*Anodonta californiensis*

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Summary

The Phoenix Zoo's Arthur L. and Elaine V. Johnson Foundation Conservation Center (Johnson Center) is the epicenter of our local species conservation involvement. We work closely Arizona Game and Fish Department (AGFD) and U.S. Fish and Wildlife Service (USFWS) to assist with species conservation. Our conservation mission is to support field conservation by developing husbandry techniques and propagation protocols for species to be released back into their natural habitat and to assist with species surveys and habitat improvement. We are entrusted by AGFD and USFWS to work with threatened and endangered species such as the desert pupfish (*Cyprinodon macularius*), Gila topminnow (*Poeciliopsis occidentalis*), Chiricahua leopard frog (*Lithobates chiricahuensisi*), Mt. Graham red squirrel (*Tamiascirus hudsonicus grahamensis*), black-footed ferret (*Mustela nigripes*,) Three Forks springsnail, (*Pyrgulopsis trivialis*) and the recently listed narrow-headed gartersnake (*Thamnophis rufipunctatus*). At times we work with local species that are not federally listed, but are in serious decline due to pressures such as habitat deterioration and competition from introduced species. These species may require research to determine if they can be sustained outside of their natural habitat and capable of reproducing, in the event that future population augmentation is necessary. AGFD biologists have been tracking the declining populations of the California floater (*Anodonta californiensis*), a freshwater mussel indigenous to Arizona. The Johnson Center is working with them to develop an *ex situ* husbandry program that allows the mussels to reproduce as they would naturally. The California floater is the only freshwater mussel species indigenous to Arizona (Myers 2009). It has declined in range and population in the wild, but is not yet considered a candidate for federal listing as threatened or endangered as are nearly 70 percent of the United States' 300 freshwater mussel species (Williams et al. 1992). It is however, considered a "species of greatest conservation need" (AZGFD 2001) as populations have all but disappeared in Arizona (Myers 2009).

Introduction

According to archeological history and recent AGFD-supported surveys, the former range of the California floater (*Anodonta californiensis*) within Arizona included the Colorado River Basin and Rio Yaqui Basin, which comprise nine rivers (Myers 2009). Now the California floater is severely restricted in the Upper Black River drainage of east-central Arizona (Myers 2009). This population may also represent the last remaining California floater population in Arizona (Terry Myers personal communication).

The California floater prefers the soft sediments of undercut banks in slow-moving backwaters, cienegas and sheltered ponds of the rivers where the phytoplankton they filter and the fish they need to complete their life cycle might flourish (AGFD 2001). The mussels range in length from 5 - 7.5 cm and width from 3 - 4.5 cm as adults, with no visual dimorphism between the genders. Its color varies from olive to reddish brown or black, with clearly visible growth lines on its outer shell. The nacre is usually white, but may be tinted flesh-colored or purplish (Nedau, et al. 2005). Their preferred habitat consists of shallow areas, less than 2 m. deep in clean lakes, rivers and perennial streams at an altitude of 1,220 - 2,644 m Adult California floaters typically live in soft mud or sand and juveniles in loose sand (AZGFD 2001). The longevity for California floaters is not well documented, but estimates suggest a life span of 10 – 15 years (AGFD 2001). In the life cycle of the California floater, females brood their eggs and new larvae, or glochidia, in one of their gill pouches. The female will release the mature glochidia into the water where they opportunistically attach themselves to a fish's gills or fins (AGFD 2001). It appears that the fish host aids in the dispersal and is required for metamorphosis of the larvae into juvenile mussels. They may receive some nutrients from the fish, but that is not clear. Juvenile California floater will drop off the fish in as little as one week and then use its foot to settle into the substrate of the new habitat. If the juvenile California floaters are dispersed to suitable habitat, they will reach sexual maturity within five years (Myers 2009).

Nearly three years ago, the Johnson Center began working to develop an *ex situ* husbandry protocol for this species in hopes of producing a breeding group that would produce California floaters to augment declining wild populations. Our goal for this project was to first determine if the California floaters could survive long term in a desert aquatic environment and naturally reproduce by completing their two-stage life cycle which requires a host fish that the California floaters' larvae (glochidia) attaches to. We developed a novel floating habitat that submerged the California floaters within one of the Zoo's lakes allowing them the ability to feed and produce larvae on their own. Un this paper we present successes, lessons learned and information gained from this endeavor and provides the status of the population located at the Zoo.

Desert Management of *Anodonta californiensis*

In April 2012, the Johnson Center received a group of four California floaters from AGFD. These mussels had been collected from the Black River and housed temporarily at a hatchery until we were prepared to receive them. Our goal with this first group was to establish whether we could maintain them in one of the lakes on Zoo grounds throughout the year, especially during the summer temperatures. We developed a floating enclosure to hold them securely . This specially designed float, dubbed "the floater float" suspends the mussels within a tub filled with a finely sifted substrate of silt. The tub consists of a 36 x 30 x 8 inch deep hard plastic tub. The tub is suspended in the lake by a 36 x 30 x 48 inch frame submerged nearly five feet below the lake's surface where water temperatures are cooler.Throughout the summer months, the temperature within the float averaged 29 C. The float frame is covered with a fine mesh, and the tub coverings have lattice 2 x 2 inch openings, providing refuge for smaller fish, enticing them to enter the area but preventing potential predators from entering the enclosure. Creating a refuge for small fish to move in and out of the float also provides opportunities for California floater larvae to attach to their gills and complete their life cycle, possibly distributing juveniles within the lake. The fish species located within the Zoo's lake are largemouth bass (*Micropterus*

salmoides), green sunfish (*Lepomis cyanellus*) and mosquito fish (*Gambusia affinis*). It has been reported that *A. californiensis* larvae have used green sunfish and mosquito fish as hosts (Xerces Society).

We also monitored ph. μ=8.2, and dissolved oxygen μ=7.2 ppm of the lake, and we measured the length and width of the California floaters monthly. They did well over the year, increased in size and were able to thrive even when the summer water temperatures were above 30 C. We have since added 11 California floaters, but have also suffered four mortalities for unknown causes. Two mortalities occurred when the entire group was located within the lake. It is possible that the substrate became anoxic and they succumbed due to poor water quality; however, none of the remaining mussels suffered the same fate. Two mortalities occurred once the group was transferred to the pond. We believe that these mortalities may have occurred due to a reduction of available algae to consume. Although there was plenty of algae present within the pond that they were transferred to, within a week the water was crystal clear. We supplemented the pond algae by feeding a commercially available algae (*Nannochloropsis*) (~68 billion/ml concentration).

We now have a population of 11 California floaters at our facility and have achieved a milestone of maintaining them within our lake over two summers and hope to detect evidence of successful reproduction by discovering juveniles established on the lakebed. While the natural lake provided plenty of algae for food and a healthy environment for the California floaters, in June 2013, a scheduled lake shoreline restoration project required us to move them to a pond located at the Johnson Center out of concern that the lake's water quality would be compromised temporarily as a result of the project. The smaller size of the temporary enclosure requires us to supplement feeding with commercial live algae, as opposed to the abundant naturally occurring lake algae. One advantage of the smaller enclosure is that we are able to see the California floaters more easily. The increased visibility provides us the opportunity to monitor their health without disturbing them and to observe changes in their activities, including evidence of reproduction. The pond setting also allows us to house a population of native fish, including longfin dace (*Agosia chryogaster chryogaster*) and possibly Gila topminnow (*Poeciliopsis occidentalis*) in with the California floaters. These native fish are known host preferences for the larvae. In December 2013, Johnson Center technicians collected a substrate sample from the California floaters' enclosure within the pond. After we examined the sample under magnification, we discovered a living ~0.5mm larva. This discovery was the first evidence that the California floater population is at least producing larvae; however, we have not observed any glochidium attached to fish gills or fins. We now plan to leave the entire group of California floaters in the temporary pond until after the breeding season to better observe evidence of recruitment. We intend to place a portion of the population back into the float within the lake once we are comfortable that the water quality has returned to normal. With luck, we may see juvenile California floaters living in the pond and lake in the near future. Johnson Center technicians will continue to monitor the lake and the pond for juvenile California floaters using an underwater camera on loan from the Bureau of Reclamation.

Specific Information Gained and Lessons Learned

With this project, we have determined that the California floater can be maintained in the desert lake located at the Zoo. They have thrived while suspended with a submerged float withstanding temperatures as high as 32 C, with an average Do2 level of 7.2 ppm. The advantages of housing them within the lake system are that there is a plentiful supply of presumably appropriate algae (based upon the appearance of the mussels) and there are plenty of fish available as host to the glochidium. Some disadvantages discovered with the use of the floater float are that it is difficult to observe the mussels, because they are submerged five feet below the surface of a rather turbid lake. It is difficult to determine whether juveniles are being produced or may be living within the lake, because of the depth and turbidity of the lake in general. The substrate within the tub has a tendency to become anoxic unless we change it periodically. We now change the substrate every five months. We also have added an aerator and bubbler to the system to increase substrate aeration and Do2 levels.

The advantages of housing the California floaters in the Johnson Center pond are that we are able to observe and monitor their health and activity daily. We are also able to monitor fish to determine if they are infested with glochidia. Additionally, we are more likely to observe juvenile California floaters that have been produced.

Overall, we have gained a great deal of information about how to maintain this species *ex situ*. With reproductive success, we hope to harvest adult California floaters for release into rivers in their existing and viable historical ranges. Special thanks to Bureau of Reclamation biologist, Rob Clarkson, for his equipment support and Dr. Terry Myers and AGFD Biologists Jeff Sorensen and Ross Timmons, who have all supported the effort to collect and establish California floaters at the Zoo. They provide excellent guidance and motivation as we continue to learn more about this species.

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Appendix

The “Floater Float” is designed to suspend a California mussel groups beneath the surface of the lake where the water temperature is cooler.

The tub consists of a 36” x 30” x 8” inch deep hard plastic. It is filled to a 5-inch depth with finely sifted silt.