Chronicles of Ex Situ Springsnail Management at Phoenix Zoo's Conservation Center

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Summary

The Phoenix Zoo's Arthur L. and Elaine V. Johnson Foundation Conservation Center (Johnson Center) is the hub 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 for release protocols for species to be released back into their natural habitat. We are by AGFD and USFWS to work with them on the conservation of threatened and endangered species such as the desert pupfish, Gila topminnow, Chiricahua leopard frog, Mt. Graham red squirrel, black-footed ferret and the recently listed narrow-headed gartersnake.

In 2004, a sudden dramatic reduction in the range of the Three Forks springsnail started discussions about creating a refugium as a hedge against extinction. In 2008, representatives from AGFD and USFWS asked the Zoo to collaborate on this project due to our historical involvement with native species conservation. Very little was known about the conditions necessary for sustained *ex situ* management and reproduction of hydrobiids. It was not clear if they could be maintained long-term nor whether they would be able to reproduce, and there was no record of this species ever reproducing outside of its natural habitat.

In this paper, we chronicle the milestones, lessons learned and successes achieved while developing a consistent and reliable method of maintaining springsnail species *ex situ*. We also report recently discovered information gained about the reproductive characteristics and seasonality of Page springsnails and on mysteries remaining about Three Forks springsnails that thwart our attempts to maintain the species *ex situ* long-term.

Hydrobiid Characteristics

Hydrobiids are aquatic gill breathing mollusks that possess a conical shell ranging in size from 2 mm. – 4 mm as adults. Pyrgulopsis are known to be egg laying, reproducing seasonally. It's estimated that they live from one to one and a half years (Hershler and Landye 1988). Worldwide, 423 hydrobiid species are listed as either vulnerable or endangered (IUCN 2014). Arizona has 12 identified springsnail species of the genera *Pyrgulopsis* (Family Hydrobiidae) (Hershler and Landye 1988). All *Pyrgulopsis* species are considered "species of greatest conservation need" by the AGFD (AGFD 2006). Several are candidates for federal listing as threatened or endangered under the Endangered Species Act (USFWS 2010) and two species, Three Forks springsnail (*P. trivialis*) and San Bernardino springsnail (*P. bernardina*), are now listed as endangered under the Endangered Species Act (USFWS 2012). Factors implemented for these species' decline are habitat loss, predation from introduced species, such as crayfish, and effects of fire. In general, hydrobiid mollusks are grazers, feeding on periphyton and detritus found within their natural habitat. They are sensitive to changes in water chemistry (Wells et al.

2012) and very minor changes in water quality can be detrimental to their survival (Wells et al. 2012). Their presence in a habitat may serve to regulate the type and density of algal growth as seen in other hydrobiid snails (Korpinen, et al., 2007). When hydrobiids occupy a significant portion of a spring system, it is an indication that the system is highly oxygenated and relatively unpolluted (Mehlhop and Vaughn 1993); however, loss of springsnail populations can indicate deteriorating local ecological conditions and perhaps predict significant ecological system declines.

Species Held at the Johnson Center

The Three Forks springsnail (*P. trivialis*) is a medium-sized hydrobiid species with adults reaching up to 4 mm in size. Adult males tend to be smaller than adult females. This species occurs at an unusually high elevation within habitats located at 8400 ft. (Taylor 1987). They are found in a spring-rich meadow with springs and creeks of various sizes that flow into a 200 ft. pond at temperatures of 15 C (springs) to 17 C (pond). They are limited to this pond and a spring rich meadow which is perched above a steep canyon of the North Fork of the East Fork of the Black River (Cordeiro, J. 2012). A firm substrate of cobble, gravel, woody debris and aquatic vegetation is essential for egg laying and feeding (Cordeiro, J. 2012). In 2012, USFWS issued a final rule listing Three Forks springsnail as endangered under the Endangered Species Act. (USFWS 2012). Reasons stated for listing included severely limited species population and distribution declines due to habitat instability, water quality and habitat disturbance.

The Page springsnail (*P. morrisoni*) is a medium-sized hydrobiid species with an adult shell height reaching 1.8 – 2.9 mm (Hershler and Landye 1988). Newly emerged juveniles range from in size from .03 mm – .08 mm within the first week of hatching (Wells et.al, 2010). Page springsnails are locally endemic to Arizona's Verde Valley (Hershler and Landye 1988). All extant populations are known from a complex of permanent springs along the east and west sides of Oak Creek near the community of Page Springs, Yavapai County, Arizona (AGFD 2009). The spring maintains an average temperature of 20 C year round (Martinez et.al.). It is considered a "species of greatest conservation need" by AGFD who along with USFWS has developed and implemented a "Candidate Conservation Agreement with Assurances" (AGFD 2009), which provided the opportunity to improve habitat and increase wild populations in avoidance of federal listing of the species. This conservation agreement has been very effective and in recent years, the population has had excellent recruitment throughout its range (Mike Martinez, personal communication).

Developing Consistent Husbandry Protocols

Since 2008, the Johnson Center has been working in collaboration with AGFD and USFWS to develop a husbandry protocol for springsnail species of Arizona. We initially established refugia for the Three Forks springsnail (*Pyrgulopsis trivialis*, Taylor 1987) and later for the Page springsnail (*P. morrisoni*, Hershler and Landye 1988) at the Johnson Center.

In 2008, when we acquired a sample population of 30 Three Forks springsnails, we designed holding tanks to resemble their natural habitat. We used substrate and plants found within their habitat at Boneyard Bog and provided rocks that they could use for refuge. We found that the natural set-up, although aesthetically pleasing and similar to their natural habitat, was difficult to manage. Although we could count the snails, it was not certain that our counts represented an accurate representation of the snail population. An additional consequence of the natural set-up was that some organisms that were present in their natural habitat would hitchhike along with the vegetation and stream water we collected and proliferate in the *ex situ* habitat, since there were no controls for these hitchhikers. It was also difficult to note changes in activity indicative of changing health, observe feeding or to locate mortalities. To remedy this, we set up two 30 gal ABS insulated tanks with white granite pebble-sized substrate covering 50 percent of the bottom of the tank and we used fewer rocks in the enclosure. The tanks were filled with five gallons of water to a depth of 6 inches. We set up two additional tanks using our Aquatic Habitat (AHAB) system. These tanks were smaller versions of the 30 gal tanks. Our hopes were if we could maintain springsnails in smaller containers, we could more easily observe and harvest springsnails that may hatch. Maintaining springsnails in the AHAB system proved to be problematic and we ultimately abandoned the use of this system for springsnail propagation. The ABS tanks however, have worked out well as habitat for springsnails. This set-up appears spartan, but it does contain the necessary elements that exist in the natural habitat, such as refuge and consistent environmental parameters, while at the same time allowing us to monitor the springsnail numbers and their activity more effectively.

One concern with this arrangement was whether there was an adequate supply of food for the springsnails to consume, since their natural habitat was a lotic or open system, which is presumably recharged by nutrients from upstream, The ex situ setting is a lentic or closed systemwhich needs supplemental nutrients. We did not supplement the first Three Forks population with diet because we anticipated they would get enough nutrients from the plants, natural spring water and substrate that we had provided; however, the current holding tanks were a completely nutrient free environment at the start since no algae was present at the time that the Three Forks springsnails were introduced. To address this issue, we provided diet in the form of commercially available algae wafers. We developed a feeding station that we could place a measured amount of diet into and that allowed us to remove leftover diet. We found that initially that both springsnail species were very actively feeding on the algae wafers, but over time as the algae growth began to develop in the tanks there fewer snails observed feeding on the wafers.

Water Quality is Critical

Maintaining consistent water quality and temperature were chief concerns. Previously, we maintained water quality by using spring water retrieved from the habitat. This was not practical for long-term management and led to the loss of two small groups of springsnails due to an unknown contamination within one of the transport containers used to collect the water. As a result, we researched alternatives to replace spring water while attempting to develop methods of achieving the same chemical makeup. One alternative was to use aged tap water, which would remove the threat of chlorine while maintaining the trace minerals believed to be essential to the springsnails, such as calcium and magnesium. However, when we analyzed Phoenix area tap water we found that it contained high levels of chlorides and sodium, along with high concentrations of dissolved solids and trace metals that could be detrimental to the springsnails. Although the snails seemed to persist using this water, they appeared to move around less frequently and ultimately did not reproduce. We decided to use reverse osmosis water (RO) instead and to implement aggressive water changes, 50 percent changes every 30 days. We also implemented water chemistry tests every month to ensure that water chemistry remained consistent. We used a chiller system in order to regulate and maintain water temperature at a value consistent with the Page springsnails habitat, 17 - 21 C. We filtered the water using 160 gph filters and maintained the water level in each tank at six inches in depth and used bar bubblers to maintain adequate dissolved oxygen levels of ~80 ppm. What we found is that using RO water along with a pebbled granite substrate, water changes and bubblers, resulted in water chemistry values nearly identical to spring water retrieved from the both Page and Three Forks springsnail habitats. This summer we added a current generating apparatus to the Page springsnail tank in hopes of simulating faster stream flow, which is where springsnails are found in higher abundance in their natural habitat (Rogowski and Martinez 2011).

Although we held Three Forks springsnails at the Zoo from 2008 through 2010, we had not observed successful reproduction and recruitment of these mollusks. In late 2009, after the sudden loss of an *ex situ* Three Forks population due to contaminated spring water, we decided to work with Page springsnails as a surrogate, to attempt to refine our husbandry techniques using this more abundant species. In July 2010, we observed the first ever newly emerged juvenile Page springsnail (*P. morrisoni*). Evidence of reproduction (newly emerged juveniles) continued from mid-July – October 2010, with peak reproduction occurring in August. Evidence of reproductive activity occurred within the population the following year beginning again in mid-July and exhibiting the same reproductive pattern and duration as the previous year, demonstrating seasonality. This population remains housed at the Johnson Center springsnail laboratory and continues exhibiting the same seasonality. The reproduction, which began in 2010, resulted from husbandry strategies developed at the Johnson Center specifically for the propagation of springsnails (Wells, et al., 2012) (Pearson et al. in Press). To our knowledge, this is the only population of Page springsnails that has reproduced consecutively outside of its natural habitat. Laboratory observations and research allowed us to collect information about the reproductive seasonality, ovipositional interval and developmental characteristics of this species as well. These observations and recorded data would have been difficult if not impossible to collect in the field.

We planned to apply the insights gained from previously held Page springsnail populations to holding subsequent groups of Three Forks of springsnails in hopes of developing a sustaining *ex situ* population of the species as a hedge against declining wild populations. We had another opportunity to work with Three Forks springsnails in June 2011, when we participated in an emergency action by holding approximately 800 Three Forks springsnails for USFWS and AGFD in our springsnail lab due to concerns that erosion and monsoonal wash activity would adversely affect their habitat and consequently the population. During the period time that we held this group, we observed evidence of reproduction within a month of their arrival, matching the reproductive season observed in the Page springsnail population. However, we believe that the reproduction was incidental to the timing of the acquisition, which was just two weeks prior to the reproductive season that we observed in our labs (Wells et al. 2012). We were able to maintain the population for their recorded lifespan of one year and presumably did not have enough recruitment to sustain an F2 population. We recorded an interesting drastic population decline in the Three Forks population that we did not observe in Page springsnails. Beginning in early September, we noted that our counts of observed snails had reduced by 50 percent from just two weeks prior. In examining our data from the 2008 – 2010 Three Forks population counts, we discovered that this population decline had occurred during the same period in each of the *ex situ* groups. Incidentally, AGFD and USFWS biologists conducted a field survey of

Three Forks springsnail habitat during this same September period and discovered an alarmingly small number of Three Forks springsnails present throughout its range. This suggests that their may be a seasonal die off occurring within the *P. trivialis* species, regardless of *ex situ* or *in situ* status. Reduced counts in the field could result due to the generally small size of juvenile springsnails, ~.03 mm, making them difficult to detect. The *ex situ* population declines were not offset by subsequent presence of more mature offspring, suggesting that the conditions were not suitable for the survival of juveniles; however, a subsequent field survey within five months revealed a robust population present within the same habitats surveyed previously. Although we have maintained a population of Page springsnails at the Johnson Center since 2010, which has exhibited predictable reproductive seasonality, recruitment is below expected based upon information gained through research and observation, which suggests a single female could produce up to four eggs per month (Wells et. al 2012).

Conditions That May Affect Recruitment

After examining our management closely, we have narrowed down three possible factors that may affect recruitment. One possibility is that we do not have enough appropriate food for them. We have observed algal growth, and we have observed the springsnails grazing on it, but we do not see the same type of algae growing in our lab as we do in their habitat. Another possibility is that although the water chemistry is very close to the spring water chemistry, there may be a shortage of some key trace mineral responsible for shell development or critical for juvenile development. In the future we would like to development methods for testing these two possibilities, by identifying the dominant algae species growing within their natural habitat, and by more closely examining the differences in water chemistry. The third possibility is proximity to other snails. We conducted an informal exploration of this by isolating a group of snails within a small container and placing the container in the main tank. We did this to see if recruitment would be higher in the smaller container than in the main tank. It was, but there are a number of variables that could have been at play, gender ratio not being the least.

Goals of This Work

We set out to first to determine if it was even possible to keep Three Forks springsnails alive outside of their natural habitat. We were able to demonstrate that it was possible with the acquisition of Three Forks springsnail populations in 2008 and 2009; however, this work helped us to identify areas that needed further understanding and improvement in order to provide environmental conditions for sustaining a reproducing population of Three Forks springsnail. We implemented a system for monitoring water quality and developed supplemental feeding protocol to compensate for the closed system that we had to set up *ex situ*. Providing stable environmental conditions and adequate resources should provide an ideal setting for achieving reproductive success.

In May 2010, we implemented a new approach to springsnail management with the collection of 186 Page springsnails from Page Springs. We found that the current system has allowed us to more closely monitor snail activity, determine feeding requirements and maintain consistent water chemistry and temperature. Additionally, within a couple of months after we collected the Page springsnails we began observing the presence of juvenile Page springsnails. This allowed us to monitor and record photographically the growth and development of Page springsnails. We were also able to closely monitor the presence of new juveniles, helping us develop an estimate for their ovipositional interval. This was further facilitated by the use of the sealed tubs, where we could easily monitor the presence juveniles, their development rates, the gestation period, and to definitively determine when juveniles were no longer being produced.

Field biologists can apply some of this information (i.e., development and reproductive period) towards defining when to conduct surveys to determine the most accurate population estimation. With the ongoing decline of these species, it is important to continue to learn more about their life histories and their environmental requirements necessary for survival. More study on reproduction, feeding preferences and longevity are needed to help us understand, and perhaps begin to reverse, the decline of this genus. We are pleased to have an opportunity to develop and implement a husbandry protocol that may serve as a tool for the conservation of many springsnail species and look forward to our continued partnership with USFWS and AZGD on these important conservation projects.

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Appendix

Chemical comparison of tank water to stream water

Element ppm	Bubbling Springs	Tank 2 RO	Tank 2 Tap	AHAB
Sodium	14.5	17.1	569.6	146
Chlorides	26	21	754	203
Calcium	56	24.7	49.5	31.7
Potassium	4.2	11.5	21.9	6.4
Iron	<.01	.02	<.01	<.01
Zinc	<.01	.01	<.01	.04
Copper	<.01	<01	.01	.03

Descriptions of juvenile page springsnails observed in the lab at different weeks during development.

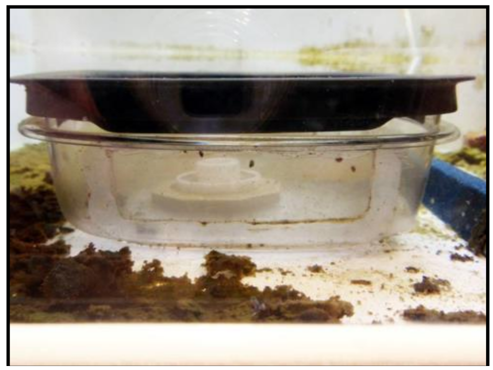
Age	Description
< 1 week	• ≤ .8 mm length and width • Only a single flat circular whorl visible. • No shell coloration. • Dark brown matter (ingested or digested material) visible through translucent shell
1-2 weeks	• 0.8 - 1.6 mm length • The beginning of a second whorl is present • No shell coloration • Dark brown matter more clearly visible in gut location. • Dark brown material evident on body whorl/inner lip (could be diet being consumed.) but inner lip is clear
2-3 weeks	• 1.6 - 2.2 mm x 1 mm. • Two whole whorls have developed. • The body whorl/inner lip of the shell is still the clear to white coloration while the whorls have a dark brown color. • Dark brown material still clearly visible in gut location
3-5 weeks	• 2.2 – 2.6 mm body shape becoming longer than width • Three complete whorls present • Shell whorls elongate to torpedo shape • The body whorl/inner lip coloration remains at the white-clear color. • Dark material still visible through shell but less distinct.
≥ 6 weeks	• ≥ 2.6 mm x 1-1.5 mm wide. Body length distinctly longer than width • Three complete whorls present but appear slightly thicker • Inner lip of shell is still clear • Dark brown material not as easily observed due to algal growth on shell, growth or mantle pigmentation appears to add coloration to the shell.

Current Springsnail habitat set-up

Original Springsnail habitat set-up

Feeding station

Specially designed closed tub



Aquatic Habitat (AHAB) set=up

AHAB System