EX-SITU HUSBANDRY AND ENVIRONMENTAL PARAMETERS RESULTING IN REPRODUCTION OF THE PAGE SPRINGSNAIL, *PYRGULOPSIS MORRISONI*: IMPLICATIONS FOR CONSERVATION

STUART WELLS, Phoenix Zoo Conservation and Science Department, 455 N Galvin Parkway, Phoenix, AZ 85008;
DREW PEARSON, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501 (current address: 16047 S. 7th Dr, Phoenix, AZ 85045);
TARA SPRANKLE, Phoenix Zoo Conservation and Science Department, 455 N Galvin Parkway, Phoenix, AZ 85008;
JEFF SORENSEN, Arizona Game and Fish Department, 5000 W Carefree Highway, WMNG, Phoenix, AZ 85086;
and MIKE MARTINEZ, U.S. Fish and Wildlife Service, 2321 W Royal Palm Road, Ste 103, Phoenix, AZ 85021

ABSTRACT

Detailed taxonomic information on Arizona’s springsnail species (*Pyrgulopsis*) exists, but few studies have looked at their life history and the environmental conditions required for reproduction. These biological parameters are difficult to observe and quantify in the field due to the small size and aquatic nature of the genus. Biologists at the Phoenix Zoo’s Arthur L. and Elaine V. Johnson Conservation Center used Page springsnails (*Pyrgulopsis morrisoni*) to develop and refine husbandry and management protocols that allowed us to monitor the snails’ numbers, changes in water temperature, dissolved oxygen levels, and water chemistry. Additionally, we were able to track their reproductive cycle, and record changes in their appearance and size during development. These biological parameters and developmental changes would be very difficult, if not impossible to observe in the field. Information gained from this work has direct applications towards both *ex situ* and in situ conservation of rare hydrobiid snails. Most notably this approach allowed us to maintain Page springsnails *ex situ*, and to observe the first reproduction of this species outside of its natural habitat.

INTRODUCTION


Because hydrobiids are gill breathing, they rely on wet, highly oxygenated conditions to thrive. Reproduction occurs annually or more often depending on water temperature (Hershler 1984, Mladenka 1992, Taylor 1987) and survivorship is estimated to be approximately one year (Pennak 1989).

Arizona has 12 identified springsnail species of the genera *Pyrgulopsis* (Family Hydrobiidae). All are considered “Species of Greatest Conservation Need” by the Arizona Game and Fish Department (2006). Several are candidates for federal listing as threatened or endangered under the Endangered Species Act, and two are listed (U.S. Fish and Wildlife Service 2010). Very little information exists regarding population demographics and/or reproduction of springsnails in the field. Due to the imperiled nature of several of these species, the Arizona Game and Fish Department and the U.S. Fish and Wildlife Service (2009) have identified the need to develop husbandry techniques and establish refuge populations to aid in the conservation of springsnails in hopes of furthering our understanding of these species’ needs. Since 2008, the Phoenix Zoo’s Arthur L. and Elaine V. Johnson Conservation Center (Conservation Center) has been working in collaboration with the Arizona Game and Fish Department and the U.S. Fish and Wildlife Service 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 Conservation Center. Although we successfully held Three Forks springsnails at the Conservation Center from 2008 to 2009, we did not observe reproduction in the population during that time and, ultimately, the population expired due to...
natural aging. In 2010, we decided to further develop and refine our husbandry management protocols using Page springsnails as a surrogate species, since their wild populations appeared to be more stable and robust. Our goal was to develop methods for creating an ex situ habitat that was similar to the Page springsnails’ in situ biological parameters and that allowed reproduction to occur. We hoped that lessons learned during the husbandry refinement process could be applied to threatened or endangered springsnail species.

The Page springsnail is a medium-sized hydrobiid species with a shell height reaching 1.8 to 2.9 mm (Hershler and Landye 1988). As with other hydrobiids, they are sexually dimorphic, in that females are larger than males as adults (Hershler and Landye 1988). 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 (Arizona Game and Fish Department 2009). Their population was in serious decline, but appears to have stabilized as a result of several habitat improvement projects conducted by the Arizona Game and Fish Department (Martinez, pers. comm.). Although P. morrisoni is a candidate for protection under the Endangered Species Act, a candidate conservation agreement was finalized in 2009, which outlines specific conservation measures and projects, and obviates the need for further conservation measures in the event that they become federally listed. (Arizona Game and Fish Department and U.S. Fish and Wildlife Service 2009).

In general, hydrobiid mollusks are grazers, feeding on periphyton and detritus in their natural habitat. Their presence in a habitat may serve to regulate the type and density of algal growth as seen in other hydrobids snails (Korpinnen 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, declines or disappearances in springsnail populations can be an early indicator of detrimental changes to local ecological conditions and perhaps a harbinger of a more significant ecological system decline.

In 2001, Martinez and Thome (2006) conducted a study of the habitat use of the Page springsnail in the wild. They reported that P. morrisoni occurrence and abundance were associated with gravel and pebble substrates, lower levels of dissolved oxygen and conductivity, and shallow water depths (Martinez and Thome 2006). Furthermore, they speculated that P. morrisoni may prefer substrates larger than sand because they provide more stable and reliable surfaces for egg deposition in flowing water, as well as the large substrates help facilitate snail mobility and provide more suitable surface for periphyton growth – the preferred food source for these mollusks (Martinez and Thome 2006). Mladenka (1992) also reported that the Bruneau Hot Springs springsnail (P. bruneaenensis, Hershler 1990) preferred gravel to sand substrates for egg deposition. Female Pyrgulopsis are believed to deposit single egg capsules on hard substrate surfaces (Hershler 1998).

**METHODS AND MATERIALS**

In March of 2010, we began a system of managing springsnails in hopes of developing ex situ husbandry methods for producing reproductively viable springsnails suitable for release to the wild. On May 17, 2010, we collected 187 Page springsnails of unknown gender from Bubbling Springs Pond. These snails were transported in small plastic Gladware™ containers to the Phoenix Zoo’s Conservation Center where they were floated inside aerated Gladware™ containers until they had acclimated to the water temperature in their new tanks (17.1ºC). Four tanks had been prepared in advance to receive these snails. Two, 91.44×60.96×60.96 cm ABS™ insulated aquatic tanks (referred to as ABS Tank 1 and ABS Tank 2) were filled with 19 liters (L) of reverse osmosis (RO) filtered water. ABS Tank 1 was left without substrate while ABS Tank 2 had a pebble-sized granite substrate, 1-inch deep covering one-half of the bottom of the tank. Each tank had a single Zoo-Med™ 511 Canister Filter system and a Current USA™ 1/15 HP Prime Chiller to maintain temperature.

The two additional tanks were set up using Aquatic Habitats™ (AHAB) system tanks (referred to as AHAB 1 and AHAB 2) that held 11.4 L of RO filtered water cycled from the 114-L capacity system reservoir. Each of the four tanks had a single full-spectrum Sun-Glo® T-8 lamp as a lighting source for 12 hours each day. The AHAB tanks were miniature versions of the larger tanks, i.e., one tank (AHAB 2) contained a granite substrate, while the other (AHAB1) did not. Each AHAB tank was separate and smaller than the ABS Tanks, allowing for the same level of monitoring, as did the isolation containers in ABS Tank 1 and 2. The AHAB reservoir was outfitted with an Aqua Logic™ Cyclone drop-in coil chiller to maintain a consistent water temperature.

Seventy-five snails were distributed into ABS Tank 1 and ABS Tank 2, respectively. Sixty snails in each tank were placed in an open plastic 12.7×10.16×7.62-cm Gladware™ container (referred to as “container”), provided with supplemental food, and allowed to disperse from the container into the ABS Tanks on their own. Each day we recorded the number of snails remaining in the container until all snails had dispersed. The 30 remaining snails were
distributed into 12.7×12.7×5.08-cm Rubbermaid™ containers. Each container housed 15 snails and one container was placed in each ABS tank. These isolation containers were modified with fine nylon mesh (100 μ) on two opposite sides so that water could flow through easily, but the snails could not get out. The lid was made of soft rubber with a 7.62×7.62-cm clear window in the center. The two containers with 15 isolated snails each, allowed for easier visualization of feeding, health and reproduction. Eighteen snails were placed into “AHAB 1” and 19 snails were placed into “AHAB 2.”

Temperature data collected, but unreported, by Martinez and Thome (2006) indicates that the mean temperature at Bubbling Springs Pond in 2001 was 20.44±0.16°C (n=50) (unpubl. data). Using a chiller, we attempted to maintain all four tanks at a temperature of 18°C. Each tank was monitored daily for water temperature, pH, and dissolved oxygen content using a HANNA pHep4® Waterproof pH Tester for temperature and pH and an YSI Ecosense® DO200 for dissolved oxygen. Nitrate levels were monitored weekly using Lifegard® All-Purpose test strips. Water samples were analyzed monthly for copper, chloride, zinc, iron, and total dissolved solids by Dairy One Forage Analysis Lab. Fifty percent water changes were conducted every 30 days.

While algae grew naturally in each tank, we also provided a commercial algae wafer (Hikari Tropical Algae Wafer™), to the snails as a supplemental food source. An algae wafer weighing approximately 0.10 grams was divided into four equal portions. One algae portion was placed in a centralized feeding location in each tank. The centralized feeding location consisted of a feeding platform and a cupped area for containing and monitoring feeding activity and food quality. Leftover food was removed at minimum every 4 days or more frequently if necessary, based upon appearance due to decomposition.

The snail populations were surveyed once a day, as part of the Conservation Center servicing routine. During a 5-minute scanning period, we recorded the number of snails visible, and noted whether they were active. Additional observational data were collected: Snails observed feeding on algae wafers, coupled snails, and any juvenile snails present. We used a handheld manual counter, a handheld light source and a magnifying glass to conduct the surveys. Time of day for conducting survey was not held as a constant due to differences in staff schedules.

### RESULTS

Monthly water changes resulted in consistent water chemistry levels in the Zoo tanks (see Table 1), with two exceptions noted below. While chloride did not appear to contribute to high mortality, chloride levels in the water appeared to affect the overall activity of snails negatively. Also increases in copper levels, from ≤0.01 to 0.05 ppm, appeared to be detrimental to springsnail activity. Two occurrences of elevated copper values in AHAB Tank 1 and AHAB Tank 2 (0.05 ppm in May and 0.03 ppm in June, compared to the typical ≤0.01 ppm observed) corresponded to a marked drop in snail activity.

| Table 1. Monthly water chemistry values for chloride (Cl), iron (Fe), zinc (Zn), and copper (Cu) from May through October. Results are presented in μg/L. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | ABS Tank 1 | | | | | | | | | | | | | | | |
| | Cl | Fe | Zn | Cu | Cl | Fe | Zn | Cu | Cl | Fe | Zn | Cu | Cl | Fe | Zn | Cu |
| May | 162 | 0.01 | 0.01 | 0.02 | 229 | 0.01 | 0.01 | 0.01 | 230 | <0.01 | 0.02 | *0.05 |
| Jun | 96 | 0.01 | 0.01 | 0.01 | 91 | <0.01 | 0.02 | <0.01 | 203 | <0.01 | 0.04 | *0.03 |
| Jul | 115 | <0.01 | 0.01 | 0.01 | 104 | <0.01 | <0.01 | 0.01 | 179 | <0.01 | 0.01 | 0.01 |
| Aug | 97 | <0.01 | <0.01 | 0.01 | 89 | <0.01 | <0.01 | <0.01 | 158 | <0.01 | 0.03 | 0.02 |
| Sep | 74 | <0.01 | <0.01 | 0.01 | 57 | <0.01 | <0.01 | <0.01 | 59 | <0.01 | 0.03 | 0.01 |
| Oct | 35 | <0.01 | 0.01 | 0.01 | 31 | <0.01 | 0.01 | 0.01 | 68 | <0.01 | 0.03 | 0.02 |

**Water Chemistry from Field Data**

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<tr>
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<th>ABS Tank 1</th>
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<tr>
<td>Date</td>
<td>Cl</td>
<td>Fe</td>
<td>Zn</td>
<td>Cu</td>
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<td>May</td>
<td>25</td>
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</table>

*Two records of copper level exceeding 0.02 were followed by death of snails in AHAB tanks.
†Bubbling Springs Pond.
(all snails consistently remained in nearly the same place for multiple days) and, presumably, the eventual death of all the snails in the AHAB tanks. Mortality in these tanks was 100% within 60 days of exposure to the elevated copper levels. Water chemistry analysis results from AHAB tank samples taken in May and June found an elevation in copper levels. The source, or cause, of this increase in copper level was thought to be a brass shut-off valve that had been installed onto the AHAB unit. Subsequently the valve was replaced with a plastic valve and there were no further recorded increases in copper levels.

Table 2 presents the average values for pH, DO₂, and water temperatures recorded during this study. Although we set temperatures for each tank at 18°C, there were temperature fluctuations due to outside air temperature changes affecting room temperature, as well as variations in the effectiveness of the chiller.

Snails were observed feeding on the commercial algae wafer placed in the feeding platform. The number of snails feeding was not recorded, only whether snails were observed feeding. Snails were observed feeding on the supplemental algae tablets 27 times in ABS Tank 1 (16.07%, n=168), and 77 times in ABS Tank 2 (45.8%, n=168). The time of day when observations were done was correlated with servicing times, which varied depending on the day. We observed that as algae grew within the tanks, fewer snails were observed on or near the feeding platforms. Typically, after 4 days, any remaining commercial algae wafer appeared to begin decomposing, breaking down from its original shape and developing a thin, gelatinous coating. Upon removal it emanated a putrid smell. The thin gel-like material appears to be produced by the snails as they feed on the algae pellet.

Table 3 shows that the combined average number of snails observed daily for Zoo Tanks 1 and 2 was 16.4±9.53 with Zoo ABS Tank 2 having the higher number observed and 9.54±5.33 for the AHAB tanks with AHAB 2 having the higher number observed.

Maintaining a known quantity of snails in isolation container within ABS Tanks 1 and 2 has allowed us to observe mortality and longevity. After the 6-month observation period, 11 of the original 15 snails in the ABS Tank 1 isolation container were still living—a 73.3% survival rate. However, on August 10, 2010, after a routine cleaning, the ABS Tank 2 isolation container population suffered complete mortality. Nothing obvious occurred during the routine cleaning to cause the mortality, however it is possible that an unknown toxin may have been introduced during the cleaning.

Hershler (1998) reported that the genus *Pyrgulopsis* lays eggs in a capsule, but no egg capsules were observed in our population. Rogowski (pers. comm.) noted Three Forks springsnails lay a single egg. In our setting, one single egg was discovered adhering to the cover of the isolation container in ABS Tank 1.

From May 17 until July 19, 2010, no juvenile springsnails were observed in either ABS Tank 1 or ABS Tank 2. A single juvenile snail, approximately 0.03 mm in length, was observed in ABS Tank 2 on July 19. The number of juvenile snails observed increased steadily for the first 10 weeks after the first juvenile was observed with a total 60 new juveniles observed (≤0.04 mm) during that time period (“new juvenile” refers to animals <1 week old). After this initial reproductive increase, the average number of juveniles observed dropped and remained constant at an average of 9.67±2.34 for the remaining six weeks of observations (see Fig. 1). Newly hatched snails (~0.03mm) were observed on average every 6 to 10 days throughout a 5-month period. We saw no further juveniles present in either tank after November 2010.

### Table 2. Daily average of temperature, pH, and dissolved oxygen levels during the May-October observation. For ABS Tank 1 and 2, n=168. For AHAB 1 and AHAB 2, n=86.

<table>
<thead>
<tr>
<th>Tank ID</th>
<th>Temperature °C</th>
<th>pH</th>
<th>DO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS Tank 1</td>
<td>18.51±0.793</td>
<td>8.3±0.526</td>
<td>8.9±0.319</td>
</tr>
<tr>
<td>ABS Tank 2</td>
<td>17.98±0.484</td>
<td>8.4±0.417</td>
<td>8.7±0.548</td>
</tr>
<tr>
<td>AHAB 1</td>
<td>19.98±2.181</td>
<td>8.3±0.302</td>
<td>8.6±0.292</td>
</tr>
<tr>
<td>AHAB 2</td>
<td>20.27±2.188</td>
<td>8.3±0.209</td>
<td>8.5±0.221</td>
</tr>
</tbody>
</table>

### Table 3. The daily average number of snails observed for each of the four tanks.

<table>
<thead>
<tr>
<th>Tank ID</th>
<th>Average snails observed over 168 and 86 days</th>
<th>~ Snails after first 3 days</th>
<th>~ After 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS Tank 1*</td>
<td>11.06±6.35</td>
<td>10.73±8.85</td>
<td>8.91±4.12</td>
</tr>
<tr>
<td>ABS Tank 2*</td>
<td>21.74±7.92</td>
<td>21.46±7.70</td>
<td>20.46±7.73</td>
</tr>
<tr>
<td>AHAB 1**</td>
<td>5.19±2.97</td>
<td>4.77±2.00</td>
<td>4.05±1.54</td>
</tr>
<tr>
<td>AHAB 2**</td>
<td>13.90±3.14</td>
<td>13.76±3.11</td>
<td>13.36±2.95</td>
</tr>
</tbody>
</table>

* n=186 for ABS Tanks 1 and 2  ** n=86 for AHAB Tanks
There was a difference in the snail reproduction rates of ABS Tanks 1 and 2, with ABS Tank 2 being more productive, with at least 60 juveniles observed, than ABS Tank 1 where only three juveniles were observed. However, we did see reproduction in the isolation container in ABS 1 but not until October. In October of 2010, we introduced a smaller, presumably male, snail into the isolation container in ABS Tank 1, which had contained only larger, presumably female snails. We did this because there had been no evidence of reproduction in the container for the previous three months and we presumed that the snails were all female. Within 10 days of this introduction, and every subsequent 6 to 10 days, we observed at least a single new ~0.03 mm springsnail in this container, suggesting that our presumption that the container had previously housed only females may have been correct, and that the ovipositional interval is 6 to 10 days in this species. In July 2011, we began to see juvenile springsnails in both tanks again, suggesting that July may be the start of their reproductive season.

It is unclear why there was a disparity in reproductive success between ABS Tanks 1 and ABS Tank 2 outside of the isolation containers. Successful reproduction in the isolation container in ABS Tank 1 argues against substrate presence as a primary regulatory factor for reproduction as there was no substrate present in the isolation container in that tank. One possible explanation is that in mid-June and early July, a large population of ramshorn snails \((\text{Planorbid rubrum})\) invaded ABS Tank 1. ABS Tank 2 had no such invasion. The appearance of ramshorn snails in ABS Tank 1 occurred at the same time that springsnail reproduction initially occurred in ABS Tank 2. It is possible that the presence of ramshorn snails inhibited reproduction in ABS Tank 1, perhaps due to competition for resources or predation. It has been shown that \(\text{Pyrgulopsis thompsoni}\) and \(\text{P. trivialis}\) compete and partition habitat with other pond snails (Tsai et al. 2007, Martinez and Rogowski 2011). Once the ramshorn snails were purged from ABS Tank 1, there was evidence of springsnail reproduction, but never at the level of reproduction observed in ABS Tank 2.

The smallest juvenile snails observed were approximately 0.3 mm in length, and developed to adult size of 2-3 mm within approximately 6 to 7 weeks. The newly hatched snails’ shell is translucent, which matches coloration found in Bruneau hot springsnail \((\text{P. bruneauensis})\) young as reported by Mladenka and Minshall (2001). They report that during growth the entire shell of the hot springsnail turned black by a length of 0.7 mm. However, in our study, during development, the Page springsnails’ shell appeared to remain translucent. The appearance of coloration changes during early snail development is perhaps due to digested food that is visible through the shell. The adult snails’ shells remain translucent, but either body color, inception of mantle pigmentation, algae growing on the shell, or stains from substrate appear to provide coloration (Table 4).

**DISCUSSION AND CONCLUSIONS**

Through our monitoring, we found that using RO water to supplement water levels in the tanks and conducting monthly water changes produced water chemistry levels nearly identical to field water
chemistry levels (Table 1). A minute increase in copper levels in the AHAB tanks had a deleterious effect on the springsnails, suggesting an extreme sensitivity to copper presence. We believe that the source of copper was due to the installation of a brass water valve onto the AHAB unit. We subsequently replaced the brass valve with a plastic valve and saw no further increases in copper. Copper sulfate is a known molluscicide, used to target snails and slugs that host liver flukes (O’Sullivan et al. 1989). Copper sulfate levels of <0.05ppm have been known to be lethal to Hydrobiidae. Anecdotal lab observations of activity in snails also suggested that the snails were more active when chloride levels were low. This could be explained by the inverse relationship of dissolved oxygen and salinity levels, which are tied to chloride levels (Best et al. 2007). With high chloride levels there is less oxygen available in the water, which snails may adapt to by decreasing activity. For example, field research results show that Page springsnails are found at higher densities where conductivity of the water is lower (Martinez and Thome 2006).

The fact that snails in the Zoo tanks were observed feeding on commercial algae wafers is an advantage for those seeking to maintain snails in an environment outside of their natural habitat. Snails may have been feeding on natural algae growth as well, but we were unable to determine this during this study. We were able to confirm that springsnails did feed on the commercial algae wafer diet even in the presence of natural algal growth. Much of the nutrients supplied in the snails’ natural habitat may rely on recharges that occur in a lotic system that are not available to animals in a closed system like a tank or aquarium, which is similar to a lentic system. However, since we observed fewer snails feeding at the platforms as algae grew in the tanks, supplemental feeding may be an essential requirement to establish springsnail populations initially, but not as critical after algae growth has developed.

It has been reported that the longevity of aquatic gastropods is approximately one year (Pennak 1978). The Jackson Lake (Pyrgulopsis robusta) springsnail is reported to have an average lifespan of 382 days (Lysne et al. 2007). In the development of this report we maintained a population of snails in isolation containers, thus allowing us to monitor longevity of a certain population of snails. After 6 months, 73% of the original population had survived in the isolation tub.

In December 2010, in order to measure species longevity more directly, we removed all adult snails from the ABS 1 isolation container and replaced them with a population of 27 juveniles that had been hatched in October 2010. In July of 2011, an inven-

<table>
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<th>Age</th>
<th>Description</th>
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| <1 week | • ≤0.4-0.8 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.  
• 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 wide.  
• Two whole whorls have developed.  
• The body whorl/inner lip of the shell is still clear to white in 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.                                                                 |

Table 4. Descriptions of juvenile Page springsnails observed in the lab at different weeks during development.
However, if the breeding cycle observed triggered the start of reproduction. No particular environmental change was until the end of October 2010, though reduced in was evidence of reproduction in the ABS Tank 2 ning in mid-July and not ending until late fall; there were reproductive activity observed shortly after reproduction. This differs from our snails reproduce in December and that adults die off an important role in the reproductive success of this species. Subsequent reproduction occurring with the use of these methods will provide more support for the effectiveness of the protocols.

Raisanen (1991) suggested that Page springsnails reproduce in December and that adults die off shortly after reproduction. This differs from our findings. Evidence of reproductive activity observed ex situ suggests a robust reproductive period beginning in mid-July and not ending until late fall; there was evidence of reproduction in the ABS Tank 2 until the end of October 2010, though reduced in level. No particular environmental change was observed that triggered the start of reproduction. However, if the breeding cycle observed ex situ remains consistent in the wild, it could serve to inform field conservation efforts for this species. For example, Martinez and Sorensen (2007) found that sampling of P. morrisoni from July to September appeared to have a significant negative impact on the population in the short term. The negative impact could also be due to the fact that July through August were peak reproductive periods in our ex situ population and that sampling snails during that time could interrupt reproduction. Additionally, our findings on reproduction would suggest that adults present during this period would be less evident due to life-history cycles, and that juveniles would be more likely to be present in higher numbers. Because newly hatched snails are ≤0.4 mm they will be less observable in the field, and since they do not all hatch at once the there will likely be smaller juveniles present throughout this period. To maximize reproductive potential and minimize population impact, collection would best be conducted in October since snails readily observable would be adults nearing the end of their reproductive life history.

This study generated useful information about the husbandry requirements, life-history parameters, and the ideal reproductive conditions required to maintain the species outside of its natural habitat. The management strategy resulted in the first ever reproduction of this species outside of its natural habitat. Many of the insights gained are applicable directly to field conservation efforts, in terms of understanding how time of year can affect population density assessments. Additional studies conducted in managed settings, focused on other Pyrgulopsis species may provide key insights into the life histories and reproductive strategies of this genus, and may also provide valuable knowledge on how to propagate a population outside of its natural habitat that will be suitable for reintroduction into the wild or maintained as a refuge population.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Tsai, Y. J., K. Maloney, and A. E. Arnold. 2007. Biotic and abiotic factors influencing the distribution of the Huachuca springsnail (Pyrgulopsis