Development of Propagation Techniques for Loach Minnow

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EXECUTIVE SUMMARY

Loach minnow, *Tiaroga cobitis*, were collected by seine from the west end of Aravaipa Canyon (WAC; N = 18), Arizona, in August, 2002, and from the east end of Aravaipa Canyon during spring 2002 (EAC1; N = 20) and September, 2002 (EAC2; N = 77). Fish were transported by hatchery truck to a wetlab located at Bubbling Ponds Hatchery near Cornville, Arizona. WAC fish were emaciated and infected with yellow grub at the time of collection, and were not used in spawning experiments. Photoperiod in the laboratory was set to April (12.5 h L : 11.5 h D), and water temperature was held constant by ambient room temperature. Holding tanks in the laboratory consisted of recirculating systems, employing three 1900 L circular tanks, five 3 m X 0.25 m linear raceways, and two rows of 37 L aquaria connected in series (six tanks per row). Substrates provided included pea gravel in linear raceways, bare fiberlass bottoms in circular raceways, and bare glass bottoms in 37 L aquaria. Three broken clay potsherds (5-10 cm dia.) were placed in each tank for cover, and spawning substrates consisted of broken clay potsherds and fist-sized (5-10 cm) cobbles.

Spawning activity began in October 2002, and continued through April 2004, with multiple spawning events occurring during every month that photoperiod was set to April. A single spawning event consisted of a male and female loach minnow aligning laterally, followed by a shuddering movement, during which eggs (1-20) were expelled and fertilized. Typically, multiple spawning events occurred over several hours, as clusters of 1-50 eggs were usually found upon inspection of spawning substrates. Occasionally, false spawning events occurred, whereby all spawning behaviors were exhibited, except egg release. Spawning in the laboratory usually took place on the downstream or lateral sides of cobbles, and male loach minnow retrieved displaced eggs in their mouths as they drifted downstream following spawning events, depositing them in one or two clusters underneath the spawning substrate. Both male and female loach minnow guarded eggs, but male loach minnow were both more aggressive and more often found guarding eggs in the laboratory. Video of spawning behavior was recorded during May 2003.

Loach minnow eggs are adhesive and demersal. Spawning substrates, with eggs

attached, were moved to either 37 L aquaria or to hatching jars for egg incubation. Eggs typically hatched after 5 d at 21°C. Hatching success was highest (69.7%) for eggs incubated in an inverted position (facing down, underneath spawning substrate to which they were attached) within hatching jars. A variety of starter foods were provided to larval loach minnow, but the highest larval survival (91.1%) was obtained by feeding a combination of ground Silvercup® trout starter, powdered egg yolk (chicken), and a 100 Fm larval diet (Aquatic Ecosystems, Inc.) at 2-h intervals between 0800 and 1700 hrs, and adding *Artemia* nauplii to the diet at one week post swim-up. Although most juvenile F1 progeny were sent to the University of Arizona for research, survival of juvenile and adult F1 loach minnow was estimated at over 95%.

Loach minnow are short-lived, stream obligate species. Senescence in captive adult loach minnow populations can be expected to account for 33-50% mortality per year, because the species only lives for 24-36 months. In addition, the species is highly susceptible to disease, particularly to chronic, systemic bacterial infections by *Aeromonus*. Bacterial infection rate can be reduced by changing environmental conditions slowly (especially current velocity) so as to reduce stress, and also by periodically removing adult loach minnow from environmental conditions that stimulate spawning (e.g. April photoperiod). In order to ameliorate annual losses from captive adult loach minnow populations, captive populations should be supplanted annually with adult fish originating from the source population.

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Introduction

Loach minnow (*Tiaroga cobitis*) is a small secretive member of the family Cyprinidae. The fish is endemic to the Gila River Basin in Arizona, New Mexico, and Sonora, Mexico. Minckley (1973, 1987) described historic distributions of the species as locally abundant, but these populations have declined drastically in recent years, now restricted to sparse tributary populations in Arizona and New Mexico (Marsh 1991; P. C. Marsh and D. L. Propst, pers. comm.). The species is listed as threatened by the U.S. Fish and Wildlife Service (1986) and is listed as a species of special concern in Arizona and New Mexico (AZGF 1996).

Marsh (1991) and Vives (1996) reviewed studies dealing with habitat use, reproductive biology, growth, foods utilized, and species co-occurrence and competition, for wild populations of loach minnow. There are few studies dealing with hatchery propagation and husbandry of loach minnow. Unpublished research investigations at Arizona State University in the early 1990s represent the first known efforts to rear and propagate loach minnow in an artificial environment (S. P. Vives, pers. comm.; E. Jahrke, pers. comm.). Both researchers captured adult loach minnow from Aravaipa Creek, Arizona, and both experienced fish mortality as a result of infestations of *Ichthyophthirius multifilis* ("Ich") soon after transport to the laboratory. This early work was largely unsuccessful, save for a few spawning events observed by Vives (1996). Vives (1996) followed up his earlier work on loach minnow at Georgia Southern University, but was unable to elicit natural spawning during that study.

David and Wirtanen (2001) improved techniques for captive propagation of loach minnow. In that study, adult loach minnow were collected from the East Fork of the White River, Arizona (B. David, pers. comm.). As in previous work with the species, adult fish were lost to infestations of Ich, but this disease was controlled using repeated treatments of formalin (David and Wirtanen 2001). Fish were held at the Alchesay Unit of the Alchesay Williams Creek National Fish Hatchery under a natural photoperiod, supplemented with artificial lighting. Chillers were used to mimic water temperatures occurring in the natural environment, and natural spawning was first observed in April, ten months after fish were collected from the wild, at a water temperature of 12.2 C. Adult loach minnow were fed an

alternating diet of frozen foods, including bloodworms, mosquito larvae, adult brine shrimp and krill. Food was provided at a rate of 0.40 to 0.66 g/fish/day. Initial larval food for loach minnow included single-celled microalgae and rotifers. Although considerable numbers of naturally spawned eggs were lost to fungal infection (David and Wirtanen 2001), survival rate of sac fry to the juvenile life stage was 92%.

Speckled dace are closely related to loach minnow and exhibit similarities in egg deposition during spawning (M. Childs, pers. obs.). Research on captive breeding of speckled dace (Childs 1998), indicates that spawning in artificial aquaria can be elicited by manipulation of water temperature and photoperiod to mimic conditions associated with natural spawning of wild fish. Because of this earlier research, it was speculated that loach minnow could be induced to spawn in the laboratory by similarly manipulating temperature and photoperiod. Loach minnow typically spawn February through May in Aravaipa Creek (Minckley 1973), in the same riffles occupied by non-breeding adults at other times of the year (Marsh 1991). Fall spawning has also been recorded in Aravaipa Creek (Vives and Minckley 1990). Water temperatures recorded at sites of known egg deposition range from 16 to 20 C (Propst and Bestgen 1991) to as high as 23 C (Vives and Minckley 1990). David and Wirtanen (2001), however, recorded egg deposition in the laboratory at temperatures as low as 12.2 C. In the wild, eggs are typically deposited underneath and on the downstream side of cobbles and boulders that are partially embedded (Britt 1982; Propst et. al 1988; Vives and Minckley 1990). Spawning usually occurs in riffles that have approximately three percent gradient, and suitable cobbles and boulders are typically found on the upstream edge and the sides in a horseshoe-shaped pattern around the riffle (D. L. Propst, pers. comm.). David and Wirtanen (2001) successfully used clay potsherds instead of embedded cobble as spawning substrate for loach minnow.

Development of captive propagation techniques for loach minnow has been proposed as a means to facilitate species recovery (Marsh 1991). The primary goal of the present study was to develop and refine techniques for hatchery maintenance and

propagation of this species. The specific goals were to:

- Assemble and review published and gray literature dealing with husbandry and reproduction of loach minnow
- Acquire and assemble all equipment and supplies necessary for holding and propagation investigations of loach minnow
- Capture approximately 100 adult loach minnow from Aravaipa Creek
- Induce reproduction by loach minnow in the laboratory through manipulation of environmental variables, and preserve samples of eggs for future research
- Determine preferred spawning substrate by loach minnow in the laboratory
- Determine typical environmental conditions at spawning sites, and record number of eggs per spawn, frequency of spawning events, production potential, and optimal sex ratios for spawning
- Inject carp pituitary extract if manipulation of environmental variables does not induce reproduction
- Videotape and describe reproductive behavior
- Determine growth rates of larval and juvenile loach minnow, and determine hatching success, larval survival, and recruitment
- Determine egg type produced by loach minnow
- Determine if mortality of adult loach minnow in the laboratory is due to senescence or some other cause
- Provide recommendations for adapting laboratory results to hatchery production.

Methods

***Laboratory Preparation*–**All reproductive work on loach minnow was carried out in a wetlab at Bubbling Ponds Hatchery (BPH) near Cornville, Arizona, that is isolated from surrounding bodies of water. Water for the lab is supplied by an artesian well (18.6 C, 110 ppm CaCO3, pH 7.6), and all tanks and aquaria are recirculating systems (not flow-through). Tanks were filled with water and allowed to recirculate for one week prior to

fish collection to help biofilters become functional. A complete lab schematic (Figure 1) shows all tanks and raceways in the wetlab.

Fish Collection

West end of Aravaipa Canyon (WAC)–Loach minnow were collected from the West end of Aravaipa Canyon (WAC) on August 22, 2002, on land owned by The Nature Conservancy. Eighteen fish were collected between 1145 and 1500 hrs. Fish were collected from Aravaipa Creek, Arizona, using a 3 m seine with 0.80 mm mesh. Swift riffles were kick-seined in 3-5 m stretches, catching fish as they were dislodged and swept downstream by the current. Slower riffles and runs were seined in a downstream direction. Most loach minnow were captured in shady areas of riffles where algal mats and cobble were present. Few loach minnow were captured over sand/gravel or silt substrates (N=1-2). Loach minnow were noticeably emaciated in appearance, the gills were frayed, presumably due to recent flooding events, and none exhibited breeding coloration. Water temperature at time of collection was approximately 26 C.

Loach minnow were transported in aerated water obtained from Page Springs Hatchery. Salt was added to the water to create a 0.6% solution, and Stress Coat® was added to create a 0.027% solution (10mL per 37L water). One WAC fish died during transport, likely due to stress, as the fish was not visibly damaged. The remaining 17 fish were split into two of six 37 L aquaria connected in series (Row 1 aquaria; Figure 1). Substrate was provided as a commercially available pea-gravel (2-8 mm). Three clay potsherds (roughly 10 x 10 cm diameter) provided cover in each tank. Because none of the fish exhibited breeding coloration, photoperiod was set to approximate an April photoperiod (12 h L : 12 h D), and water temperature was held at a constant 21 C by ambient room temperature. Fish were fed *ad libitum*, one to three times each day with freeze-dried bloodworms, an artificial plankton diet, and a dry flake food (Aquatox®). All foods were obtained through Aquatic Ecosystems, Apopka, FL.

Yellow grub (*Clinostomum marginatum*) was visible on 13 of the 17 fish placed into aquaria. Treatment was not attempted due to likelihood of subsequent necrosis in surrounding tissues of infected fish. Prophylactic treatments using Ich Guard® (Malachite green, nitromersal, acriflavine) were administered on August 23, 24, and 25 at one-half the

manufacturer's recommended dosage, to control possible outbreaks of Ich. Water in the tanks was replaced between treatments using artesian well water. The gills and opercula of collected fish (apparently frayed due to recent flooding) appeared to have healed by early September 2002. Due to generally poor health, however, WAC fish were never used for breeding experiments.

East end of Aravaipa Canyon (EAC1)–A. Widmer (University of Arizona) provided twenty adult loach minnow on September 3, 2002, for use in the propagation study. D. Ward transported the fish from Tucson to BPH. The fish had been collected previously from Aravaipa Creek, East of Aravaipa Canyon, (EAC1), approximately 16 km upstream from the WAC fish. EAC1 fish were split into two 37 L tanks that were set up identically to those described for WAC fish (Row 2 aquaria; Figure 1). Prophylactic treatments and feeding regimes were identical to those used with WAC fish. Three days after their arrival (at the completion of prophylactic treatments), all 20 EAC1 fish were moved to two 37 L aquaria that were connected in series to the two tanks holding WAC fish (Row 1 aquaria). This was done in order to make room for new loach minnow from Aravaipa Creek.

East end of Aravaipa Canyon (EAC2)–On September 8, 2002, 77 loach minnow were collected from the East end of Aravaipa Canyon (EAC2). Water temperature at time of collection (0830-1130 hrs) was approximately 20 C. Spikedace (*Meda fulgida*) and loach minnow were common, and loach minnow were most often found in shallow water in riffles over gravel/cobble substrates. Fish were collected using a fine-mesh seine, as for WAC fish. Sixty-five of the fish were tentatively identified as females, and 12 fish were easily identified as males, since they had red fins and red fin bases, indicative of male loach minnow in breeding coloration (Minckley 1965). Many of the fish thought to be females later developed breeding coloration typically displayed by males. Sex ratio of collected fish was likely 1:1 (Marsh 1991). EAC2 fish were transported and handled as for WAC fish, and were split into all six available 37 L aquaria (Row 2 aquaria; Figure 1). With the arrival of these fish, the photoperiod was changed slightly in order to more closely mimic an April photoperiod (12.5 h L : 11.5 h D); lights were turned on at 0615 and off at 1845 each day by means of an automatic timer. Feeding regimes were modified

on September 9, when all fish in the lab were fed *ad libitum* two to three times each day (0800, 1200, 1700) with a combination of Aquatox® flake food, freeze-dried blood worms, and artificial plankton (all foods available from Aquatic Ecosystems, Inc.).

Adult Mortality–All dead loach minnow found in laboratory aquaria during autumn 2002 and spring 2003 were preserved in 10% formalin. These fish were then sent to D. Rogers (AGFD, Phoenix) in March 2003, so that otoliths could be aged to determine if senescence was the cause of death. Full-spectrum lighting was installed during April 2003. In addition, a new well was installed in September 2003 to replace an old, sediment-laden well at the wetlab. Sediment load from the old well was resulting in poor water quality in recirculating tanks, and it was speculated that this could have contributed to adult mortality**.**

Egg Deposition and Incubation, and Larval Rearing–All EAC fish were moved to either linear raceways (LR1 or LR2, Figure 1) or to the first circular tank (CT1, Figure 1) on October 11, 2002. Circular tanks had bare fiberglass bottoms, and linear raceways bottoms were covered with a thin layer of pea gravel. Spawning substrates consisted of equal numbers of inverted clay potsherds (curved surface of potsherds facing down) and small (5-10 cm diameter) cobbles. Cover was provided in all tanks and raceways using clay potsherds that were not inverted, thus providing a hiding space underneath. Photoperiod was held at April (12.5 h L : 11.5 h D) throughout the entire study, with the exception of January 2003, when photoperiod was set to mimic the natural winter photoperiod (10 h L : 14 h D). Water temperatures were held constant throughout the study at 21 C by ambient air temperature. Again, the only exception to this was in January 2003, when temperature was reduced slightly to 18 C.

Spawning substrates in circular tanks and linear raceways were picked up individually, turned over and inspected for presence of eggs, at approximately 0800 and 1700 h each day. Eggs, when found, were moved to either single 37 L aquaria, or to one of eight available hatching jars (Figure 1), or were discarded. Eggs attached to spawning substrate were incubated in either an upright position (eggs on top of spawning substrate) or the spawning substrate was inverted and placed on top of a plastic, 5 cm diameter “Bio Barrel” (Figure 2; Aquatic Ecosystems, Inc.) to allow water flow around the eggs. A few

samples of eggs and newborn larvae were preserved in 10% formalin to provide a developmental series for D. Snyder (Larval Fish Lab, Colorado State University, Ft. Collins). Number of eggs spawned, spawning substrate, tank information (circular or linear), egg condition, days to hatching and number hatching all were recorded in a daily logbook. Initiation of larval feeding, foods provided, and larval survival, also were recorded daily. Growth rates of larvae, juveniles and adults were calculated from total length estimates made during the course of the study. Survival to the adult lifestage was also recorded.

Influence of spawning substrate (clay potsherd vs. cobble) on number of eggs per clutch was assessed using analysis of variance (ANOVA). Backward stepwise logistic regression was used to determine which factors were most important for egg-hatching success, for eggs spawned by EAC females. Percent survival of larvae to four weeks post-hatch was arcsin transformed, and the best diet combination was assessed using ANOVA and Duncan's multiple-range test. Linear regression was used to determine growth rate of loach minnow larvae/juveniles, females, and males in the laboratory. All statistical analyses were run using SPSS 11.5 (SPSS 2002).

Breeding experiments were conducted during September 2003 through January 2004 in order to assess optimal sex ratios. The first linear raceway was divided into five sections using 4-mm hardware cloth. One male was placed into each compartment of each raceway, and combinations of one to five female loach minnow were added with each male. The fish were left to spawn over the ensuing five days. This experiment was repeated four times. Another experiment consisted of placing a single female loach minnow into each compartment, and adding one to five male loach minnow with each female. This experiment was not repeated. Finally, male-female pairs were placed into each of the five compartments. This experiment was repeated three times (15 total pairings). In all experiments, EAC fish were selected from the circular holding tank (CT1) based on presence of secondary sexual characteristics, when available. Due to space limitations and low numbers of gravid females, only a small number of experiments were run, and thus no statistical analysis was attempted.

Egg deposition by loach minnow F1 progeny was also monitored during this study. However, most juveniles were delivered to the University of Arizona for research, and the smallest available F1's were usually selected for this purpose. As a result, sex ratios of the remaining F1's were strongly biased towards males, which grow larger, and faster (see Results). Thus, only a few female progeny reached reproductive maturity in the laboratory.

***Spawning Behavior*–**Loach minnow spawning behavior in the first circular tank (CT1; Figure 1) was documented with a digital video camera. Hour-long recordings were made during May 2003, until eggs were found underneath a cobble placed in the tank. Spawning behavior was described in detail, and sketches of typical spawning behaviors were made during review of the videotape.

Results

***Adult Mortality*–-**Fish began to show signs of poor health in early October 2002. WAC fish still appeared emaciated (as when collected) and yellow grub were growing larger and becoming more visible on these fish. The gills of all EAC fish were turning bright red in early October, a sign of nitrite poisoning, indicating that the biological filters in the recirculating systems were not functioning properly. High nitrite levels were verified with a commercially available test kit. On October 5, 2002, EAC2 fish were moved to a single 1900 L circular tank (CT1, Figure 1), connected in series to two other 1900 L tanks, and equipped with biological, chemical, ultraviolet and physical filtration. Water was pumped to and from the tanks using a 1 hp Jacuzzi® continuous-duty pump. The WAC fish and the EAC1 fish were not moved, but water was flushed from the system to eliminate nitrite buildup. Beginning on October 6, 2002, Aquatox® flake food and artificial plankton were removed from the feeding regime and replaced with Tetramin® flake food, and all fish were fed only two times each day to help reduce ammonia and nitrite buildup in the recirculating tanks.

The first loach minnow mortality (WAC fish) occurred on October 8, 2002, apparently due to a combination of yellow grub infection, secondary bacterial infection, and possibly latent effects of nitrite poisoning. A general prophylactic treatment using Ich

Guard® at half the manufacturer's recommended dosage was administered to WAC and EAC1 fish (Row 1 aquaria) on October 9, 2002 to treat for possible secondary bacterial or parasitic infection. In addition, Cycle® bacterial supplement was added to all tanks in the lab on October 11, 2002, to aid in biofilter function. Nitrites, however, remained high in the circular tanks on October 15, 2002, so all water was flushed and replaced. WAC fish were moved out of Row 1 aquaria on October 15, 2002 to a separate 74-L aquarium equipped with an under-gravel filter. These fish were also treated with 100 ppm formalin for one hour as a prophylactic measure against secondary parasitic infection. On October 24, 2002, all EAC2 fish were moved back into Row 2 aquaria for two days as a result of continuing nitrite buildup in the first circular tank (CT1). EAC2 fish were also given a prophylactic treatment of nitrofurizone, and returned to the first circular tank after flushing the system with fresh water. Thereafter, biofilters began to function normally.

Mortalities occurred sporadically over the ensuing months, in both WAC and EAC fish, as well as in some of the F1 progeny from EAC fish (Figure 3). Most WAC fish that died appeared emaciated and infected with yellow grub. Some EAC fish that succumbed appeared emaciated, while other EAC mortalities occurred in fish that appeared perfectly healthy and were free of external pathogens. Unfortunately, otoliths from loach minnow found dead in the laboratory were unreadable, a result of degradation caused by preservation in formalin. As a result, it is impossible to differentiate between senescence and other causes of loach minnow mortality during this study.

Monthly adult loach minnow mortality for EAC fish finally decreased during late spring 2003. The highest mortality rates observed during the study for EAC fish were in January and March 2003 (Figure 3), both points coinciding with rapid increases in current velocity from near zero to 10 cm/sec. These changes in current velocity occurred as a result of changing the direction of inflow into the first circular tank, thus causing changes in current velocity within the tank. After fish were able to acclimate to the current, mortality rates declined. No attempts were made to modify the diet of adult loach minnow during spring-summer 2003, primarily due to the fact that mortality rates in EAC fish declined after March 2003, and for the remainder of the study (Figure 3). Full-spectrum

lighting was added to the laboratory in April 2003, and may have played a role in improving fish health.

Another probable contributor to adult mortality was an apparent systemic bacterial infection by *Aeromonus hydrophila* (or "motile aeromonad septicemia"), detected by W. Cavender (AGFD) in April 2004. This bacterium is common in recirculating freshwater systems, but usually causes disease only if fish are stressed (e.g. during spawning activity). Diseased fish typically stop feeding, lose equilibrium, and eventually die. It is likely that all fish in the laboratory were affected to some degree by this disease, as no prophylactic treatments for systemic bacterial infections, such as medicated feed, were ever administered.

Two spikes in mortality also are evident for F1 loach minnow (Figure 3). The first spike occurred during August 2003, and was apparently associated with an external bacterial infection, which was successfully treated with an oxytetracycline bath (15 ppm for 24 h) on August 28, thus reducing mortalities in September 2003. However, on September 5, 2003, all F1 progeny in the laboratory were moved from 37-L aquaria (where flow velocity was near zero) to a 1900 L circular tank (CT2) with a current velocity of 10 cm/sec. Like the adult loach minnow, F1 progeny did not acclimate quickly to these new conditions, and became both fatigued due to higher current velocities and possibly malnourished due to rapid loss of food down the tank drain. Mortalities increased during October, and on October 22, 2003, all remaining F1 progeny were split into several 37-L aquaria. After moving fish to these low-velocity aquaria, mortalities declined (Figure 3).

Reproductive Development–As stated above, about 10 of the EAC2 fish exhibited breeding coloration when collected from Aravaipa Creek. By the end of September and into early October 2002, breeding coloration had continued to develop in most of the fish in the lab, particularly in male loach minnow. Fish were not counted, but approximately half the fish in the lab demonstrated male breeding coloration. Thus it appears that sex ratios at collection sites were approximately 1:1. Spawning events began in late October in the laboratory, and occurred during every month when the photoperiod was set to April (12.5 h L : 11.5 h D).

Egg Deposition–Environmental conditions (temperature, photoperiod, substrates, flow velocities) within individual tanks and raceways remained primarily static throughout the study. Spawning was documented only in circular tanks or linear raceways where water velocities were usually at or near 10 cm/sec, but varied from 5 to 10 cm/sec. Eggs were found underneath the potsherds and cobble, at the interface between spawning substrates and either bare fiberglass bottoms (circular tanks) or pea gravel (linear raceways). No spawning events were ever recorded in any of the first or second row of 37-L aquaria (although adult fish were not held in these tanks for long periods of time).

EAC loach minnow deposited a total of 7,183 eggs under clay potsherds and cobbles, between October 23, 2002 and April 1, 2004. Most of these eggs (N = 5,874, or 82%) were deposited by at most 22 breeding pairs of loach minnow (i.e. after April 2003; Figure 4) because 52 of the 97 collected EAC fish died prior to May 2003 (Figure 3). Thus, each EAC female deposited an average of 267 eggs between May 2003 and April 2004. Average clutch size during this study was 26.1 (1.6 SE) eggs/clutch, indicating that the average number of clutches produced by EAC females was a little over 10 per year, when exposed to an April photoperiod. Average number of eggs per clutch did not vary by spawning substrate (potsherds vs. cobble; ANOVA, F = 1.325, 1 df, P = 0.251). However, loach minnow clearly preferred cobble as spawning substrate to clay potsherds (Figure 4). A majority of eggs found during the study (4,032 of 7,183) were discarded due to space limitations. Eggs that were disturbed and replaced in spawning tanks and raceways were usually cannibalized.

Breeding experiments produced a large number of spawning events (Table 1). Male-female pairs spawned more than 50% of the time, within five d of being placed together in compartments within linear raceways. Although groups of three, four and five females placed with a single male spawned 75% of the time, only four such tests were run, so this percentage might be somewhat inflated.

Spawning by F1 progeny also was documented, with a total of 13 separate spawning events, and 148 eggs deposited between September 10, 2003 and March 31, 2004.

Hatching Success–Factors determining hatching success, in order of importance, were location of incubation (hatching jar or 37-L tank), position during incubation (inverted or upright), and raceway where eggs were deposited (circular or linear) (Table 2). The primary difference between the two raceway types was the fact that linear raceways contained pea gravel. Thus, in linear raceways, eggs often adhered directly to pea gravel, or to exposed fiberglass tank bottoms between pea gravel and spawning substrates. As a result, eggs were often more difficult to move, without damage, from linear raceways to incubation jars or tanks. Highest hatching success was for eggs deposited in circular raceways, incubated in an inverted position (underneath spawning substrates to which they were attached) with spawning substrates placed on top of "Bio Barrels", and placed in hatching jars. Five of 13 egg clutches produced by F1 progeny were incubated, spawning substrates were inverted in hatching jars, and hatching success for these eggs was 80%.

Larval Survival–Larval loach minnow proved difficult to culture. Biokyowa® B-250 larval fish food has been a reliable, highly effective early larval food for Colorado River Basin cyprinids and suckers (Childs 1998; Childs and Clarkson 1996; Clarkson and Childs 2000). Unfortunately, this larval diet is no longer available in the U.S. Therefore, the first food provided to larval loach minnow was an artificial plankton diet (Aquatic Ecosystems, Inc.). Numerous larvae died of apparent malnutrition between October 28, 2002 (date of first hatching), and November 5, 2002. On November 9, 2002, the artificial plankton diet was ground to a fine powder using a mortar and pestle, to provide a smaller size food particle. No larval fish were observed ingesting this modified plankton diet, so on November 12, 2002, a new larval diet was provided (100 Fm larval diet, Aquatic Ecosystems, Inc.), in addition to the powdered artificial plankton. Although larval fish did appear to feed on the new diet, the fish weakened and died over the next couple of weeks. On November 23, 2002, a single-celled, spray-dried microalgae (*Spirulina*; Aquatic Ecosystems, Inc.) was added to the larval diet. This diet is high in protein, and was effective at increasing larval survival. Fish eggs that were spawned on November 11 and hatched on November 16 represent the first group of larval fish to survive to the juvenile life stage in the laboratory. On November 30, 2002, Silvercup® trout chow starter was ground to a fine powder with the mortar and pestle, and added to the larval diet to increase

protein content of ingested food. Artificial plankton and Spirulina were excluded on from the larval diet on December 7, 2002, and brine shrimp (*Artemia*) was added to the larval diet on December 10, 2002. This diet item was readily ingested by young loach minnow, but *Artemia* can not be used as a starter food, as newly hatched loach minnow larvae are too small to eat even the smallest of brine shrimp (David and Wirtanen 2001). Loach minnow larvae were capable of feeding on brine shrimp at approximately 1 week after they began feeding (i.e. one week post-swim-up). Finally, on March 29, 2003, powdered egg yolk was added to ground trout chow and the 100 Fm larval diet. Survival increased dramatically with this new starter food. Overall, larval survival was highest for fish that were fed a combination of egg yolk, ground trout chow, and the l00 µm larval diet, and adding *Artemia* to the diet at one-week post-swim-up (approximately 12-14 d post-hatch) (Table 3). These foods were continued into the juvenile life stage, until the fish had grown to approximately 30 mm TL. Of the 1,330 eggs that hatched in the laboratory, 979 larvae survived to the juvenile life stage.

Larval loach minnow placed into 37 L aquaria with adult loach minnow were quickly cannibalized, so different life stages of this species must be separated.

Juvenile Survival–Although relatively few F1 progeny were allowed to survive to reproductive maturity, juvenile survival was high in the laboratory (estimated at more than 95%). Most F1 progeny were sent to the University of Arizona, where they showed a high survival rate as well, prior to use in experiments (A. Widmer, pers. comm.). These fish were fed a dry diet of ground trout chow, freeze-dried bloodworms, and two commercial flake foods (similar to Tetramin® flake), as well as occasional additions of powdered egg yolk and *Artemia*. Overall survival of fish sent to the University of Arizona was estimated at more than 99%. These fish were not exposed to the higher current velocities present in circular tanks and linear raceways at the BPH laboratory.

Growth Rates–Larval foods were ignored when assessing growth rate, but all F1 fish were fed a diet of Tetramin® flake and freeze-dried bloodworms when they reached approximately 30 mm total length. Female growth rate was lower than that for either male or juvenile loach minnow, based on 95 % confidence intervals for slopes of regression

lines (Figure 5). Lower growth rate of females reflects sexual dimorphism in loach minnow.

Spawning Behavior–Loach minnow are usually found in direct contact with substrates, and usually under any available cover (such as broken clay potsherds). Prior to, during, and after spawning events, a single, apparently dominant male typically guards the spawning substrate (usually cobble), chasing other males and females away. Male-male chases are more aggressive, and sometimes escalate to fin biting. When a dominant male is present at the spawning cobble, one to four females often approach simultaneously, position their snouts underneath the edge of the cobble, and exhibit a shuddering movement (also described by David and Wirtanen 2001), thus apparently attracting the dominant male to approach. Female loach minnow compete for a single dominant spawning male, and the male chooses one female for spawning events, usually chasing all other females away. Finally, with the chosen female and dominant male positioned side by side, both fish again display the shuddering movement. The female then rubs her snout back and forth across the lower jaw of the male. Less than one second from time of this behavior, the male arches his spine around the female, positioning his vent near that of the female, and the female contorts in similar fashion, turning sideways, and broadcasting 1-20 eggs in an upstream direction. The male fertilizes the eggs as they are expressed from the female. Spawning in the laboratory usually takes place on the down-current or lateral sides of cobbles (Figure 6). Male (and possibly female) loach minnow retrieve the demersal adhesive eggs in their mouths as they drift downstream following spawning events, depositing them in one or two clusters underneath the cobble. Typically, multiple spawning events occur over several hours, as clusters of 1-50 eggs are usually found upon inspection of spawning cobbles. False spawning events occur frequently, with all spawning behaviors exhibited, except gamete release. A DVD of spawning behavior will be submitted with this report.

Discussion

In general, the secretive nature of loach minnow makes hatchery propagation difficult. Unlike spikedace, which spend a majority of time suspended in the water

column, loach minnow are usually found at the bottom of culture tanks, under cover. An obligate stream-dwelling fish, loach minnow in the wild feed opportunistically among benthic substrates for a select few benthic insects (Marsh 1991; Schreiber and Minckley 1981). This feeding pattern makes culture of this species quite difficult, as food must be provided frequently, especially to younger life stages, and leftover food must be cleaned out of recirculating tanks to avoid ammonia and nitrite buildup. However, many of the difficulties encountered during the first year of study were solved or ameliorated over the subsequent 12 months. Adult mortality proved the most difficult problem to overcome.

Adult Mortality–Most successful captive breeding programs involve either long-lived species or short-lived species that are prolific (Hendrickson and Brooks 1991). Because loach minnow are neither long-lived nor prolific, senescence will always be a visible and problematic factor concerning captive propagation of this species. A fairly high and steady level of senescence (33-50% mortality/year) can be expected in any captive population of adult loach minnow, due to their short lifespan (24-36 months).

Increases in mortality rates were associated with rapid changes in current velocities within tanks. High flow velocities in circular tanks resulted in rapid loss of food items down tank drains, and food was therefore available to adult fish for only brief periods during the day. These two factors, lack of acclimation to current (i.e. moving adults from low velocity 37-L aquaria to circular tanks) and rapid loss of food items from tanks, both likely contributed to increased mortality rates, over and above natural senescence. These factors represent additional stress factors, in addition to spawning activity, which could have resulted in systemic bacterial infection by *Aeromonus hydrophila*. Motile aeromonad septicemia (MAS) might explain the emaciated condition of many loach minnow that died during this study, as a common side effect of this disease is cessation of feeding (Camus et al. 1998). Prophylactic treatments for systemic bacterial infections (e.g. medicated feeds) could be used to alleviate the onset of MAS, but a careful balance must be met between over-treating with antibiotics vs. allowing disease to impact captive populations.

Thus, in order to prevent high mortality rates in captivity, environmental conditions such as current velocity should be changed slowly, and perhaps low velocity conditions should be restored periodically to ensure fish health. Alternatively, low-velocity, off-

channel areas could be provided in linear raceways to provide fish with a choice of flow velocities. Starvation resulting from rapid loss of food down tank drains could be ameliorated by reducing inflow to tanks, and maintaining desired flow velocities for spawning (ca. 10 cm/sec) using submersible pumps. Finally, since mortality rates of adult loach minnow have remained relatively low in the laboratory since April 2003, it is reasonable to assume that the extended breeding period (April photoperiod) was not the ultimate cause of adult loach minnow mortality during this study.

Reproductive Development–Despite lack of reproductive coloration at time of collection, development of secondary sexual characteristics during this study confirmed that sex ratio of collected fish was close to 1:1. Thus sexing of wild fish is probably unnecessary when collections or translocations are made. Male loach minnow held at an April photoperiod developed and retained full breeding coloration throughout the study. Breeding coloration in males, however, was not an accurate indicator reproductive maturity. Female loach minnow reproductive coloration was far less obvious, but yellowing of fins (Minckley 1965) was noted in females engaged in spawning activity. Reproductively mature females were easily identified by their distended abdomens.

Egg Deposition and Hatching Success–Loach minnow spawned extensively when held at an April photoperiod, in water of 21 C and flowing at a velocity of approximately 10 cm/sec, and when also provided small cobbles as spawning substrate. Although all spawning documented in the laboratory occurred at these flow velocities, current is apparently not critical to induce spawning in loach minnow, as David and Wirtanen (2001) documented spawning in low-velocity tanks similar to the rows of 37-L aquaria used during this study.

Breeding experiments indicate that pairs of reproductively mature male and female loach minnow can be induced to spawn greater than 50% of the time in the laboratory. This finding is important insofar as out-crossing of spawning adults can be maximized in a hatchery setting with minimal effort, and without the use of hormones or manual spawning procedures.

Egg hatching success likely could have been improved, if flow-through artesian water had been used for egg incubation, rather than recirculating water. This option was

not available, however, during the present study. It is also possible that hatching success could have been improved if eggs had been treated with malachite green (8.4 mg/l for one hour, David and Wirtanen 2001), to prevent fungal infection upon recovery from raceways. A treatment of 11mg/l was attempted three different times during the present study, but was discontinued when it was found that 100% of eggs within untreated clutches often hatched successfully. The malachite green treatment thus appears to be relatively unimportant. Similar to David and Wirtanen (2001), egg clutches that appeared to be fertile and developing normally were usually unaffected by fungal infection. A majority of eggs that did not hatch appear to have been unfertilized.

Finally, placement of small sheets of plexiglass under spawning cobbles in both linear raceways and circular tanks would have allowed for efficient recovery of spawned eggs because cobbles and plexiglass could have easily been moved to hatching jars, along with any adhered eggs. Without plexiglass, spawned eggs often adhere to tank bottoms and/or to pea gravel substrates, and are easily damaged when removed (siphoned into an eye-dropper). Limited testing suggests that loach minnow spawning is uninhibited when plexiglass is placed under spawning cobbles. This procedure should be followed during future propagation work with loach minnow.

Larval and Juvenile Survival–Larval survival was vastly improved after swim-up fry were fed powdered egg yolk as a starter food. As with egg hatching success, larval survival probably would have been improved if the fry had been reared in flow-through, pathogen-free artesian well water. Early in the study, rearing tanks were cleaned on a daily basis in an attempt to prevent bacterial build-up on the bottom of tanks, but this proved to be harmful to larval loach minnow, as they, like adults, typically remain in contact with the substrate, and often were accidentally siphoned from rearing tanks during cleaning. Subsequently, larval tanks (37-L aquaria) were not cleaned until larvae were at least three weeks old. Because excess food settles to the bottom of tanks, a beneficial side effect of not cleaning the rearing tanks was that larval loach minnow were able to feed continuously throughout the day.

Juvenile loach minnow fed actively on ground trout chow, *Artemia*, and artificial larval diets, as well as on adult loach minnow diets (Tetramin® flake and freeze-dried

bloodworms), and survival was high in the laboratory. Some juveniles died as a result of external bacterial infection, and others likely died due to systemic bacterial infection by *Aeromonus hydrophila*. Like adult loach minnow, some juveniles were lost when current velocities were changed rapidly in tanks. This stress, combined with rapid loss of food down tank drains, and continuous exposure to an April photoperiod, likely triggered the systemic bacterial infection. Regardless of age, EAC loach minnow remained healthy when held in low-velocity tanks.

Spawning Behavior–Male loach minnow build nests, which they guard and defend against other males and females, until they have chosen a single female to spawn with. A single dominant male vigorously guards a nest (or, in this study, a spawning substrate), but it is impossible to tell if the guarding behavior is displayed in an effort to protect eggs, or if this behavior is displayed merely to protect spawning substrate from other males. The latter is more likely, as males were often observed guarding spawning substrate prior to egg deposition. In any case, adult loach minnow were observed preying on newly hatched larvae, so brood guarding is likely absent in this species.

Hatchery Production of Loach Minnow–An outline summary of successful results from this study can be found in the Appendix. This protocol should be followed for all future propagation work on loach minnow.

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Table 1. Summary of breeding experiments. "Successful tests" indicates the number of independent tests that resulted in spawning within 5 d; actual number of clutches was somewhat higher.

Pairing	Tests (N)	Successful tests (N)	Eggs (N)
1 male / 1 female	19	10	520
1 male / 2 female	4	1	37
1 male / 3 female	4	3	81
1 male / 4 female	4	3	193
1 male / 5 female	4	3	41
2 male / 1 female	1	0	0
3 male / 1 female	1	0	0
4 male / 1 female	1	1	2
5 male / 1 female	1	0	0

Table 2. Coefficients of logistic regression model equation for egg-hatching success ($P < 0.05$). The equation predicts percent hatching success of loach minnow eggs, depending upon egg treatment. Coding for each variable was dichotomous: incubate = 1 when eggs were placed in hatching jars, and incubate = 0 when eggs were placed in 37-L aquaria; position = 1 when spawning substrates were inverted so that eggs were attached but underneath the substrate, and position = 0 when eggs were incubated on top of attached spawning substrates; raceway = 1 when eggs were deposited in circular raceways, and raceway = 0 when eggs were deposited in linear raceways. The variable substrate was eliminated from the model ($P = 0.055$). N = number of clutches; n = number of eggs incubated. Hatching success for each possible incubation condition is summarized below the model coefficients.

		Model Coefficients					
N	N	Constant	Incubate	Position	Raceway		Percent Hatch* (%)
		-1.569	+1.232	+0.950	+0.222	= Z	
23	565		Hatching jar	Inverted	Circular	0.835	69.7
21	543		Hatching jar	Inverted	Linear	0.613	64.8
7	485		Hatching jar	Upright	Circular	-0.115	47.1
20	328		Hatching jar	Upright	Linear	-0.337	41.6
5	157		37-L aquarium	Inverted	Circular	-0.397	40.2
17	467		37-L aquarium	Inverted	Linear	-0.619	35.0
6	188		37-L aquarium	Upright	Circular	-1.347	20.6
10	185		37-L aquarium	Upright	Linear	-1.569	17.2

* Percent hatch = $1/(1 + e^{-Z})$

Table 3. Survival of larval loach minnow to four weeks post-hatch, by food type. Food types are as follows: P = artificial plankton; 100 = 100 μm larval diet; S = *Sprirulina***;** T = ground Silvercup® trout chow starter; E = powdered egg yolk; A = *Artemia*, fed one week post swim-up. N indicates number of fish raised on each food type. Homogeneous groups were determined using Duncan's multiple range test (SPSS 2002). Table-wide $\alpha = 0.05$.

Food type	N	Homogeneous subsets (percent survival ± standard error)				
P	14	0.0 + 0.00				
P100	18	0.0 + 0.00				
SP100	17		35.3 + 0.07			
TS100	22		54.5 + 0.08	54.5 + 0.08		
S100	4			75.0 + 0.00	75.0 + 0.00	
TSA100	64				71.9 + 0.05	
TEA	63				73.0 + 0.05	
TEA100	951					91.1 + 0.01
Significance		1.000	0.067	0.187	0.249	1.000

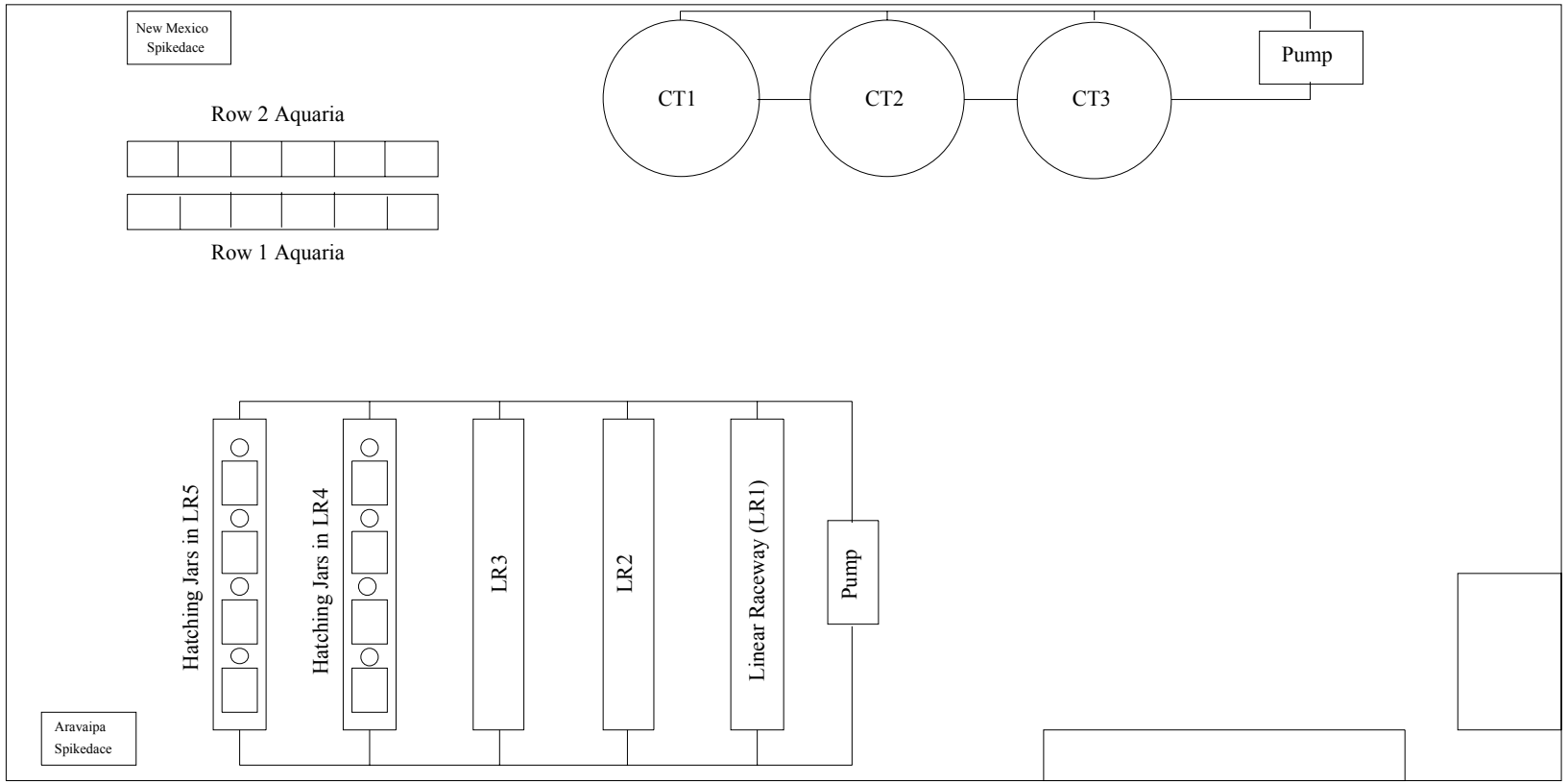

Figure 1. Diagram showing the layout for Bubbling Ponds wetlab. The two rows of 37 L aquaria (Row 1 and Row 2 aquaria) are connected in series within each row to a 1/3 hp continuous duty water pump. Three large circular tanks (1900 L; CT1-CT3) on the West wall are connected in series to a 1 hp continuous duty pump, as are the five raceways (25 X 300 cm) perpendicular to the East wall of the lab. All tanks and raceways are fitted with inline biological (bacterial), chemical (zeolite), physical (cloth) and ultraviolet filters. Hatching jars (N = 8) were placed into the bottom of raceways 4 and 5 (LR4 and LR5, respectively) for egg incubation. They each empty into separate 37 L aquaria, and are connected in series to the water supply for the other three raceways.

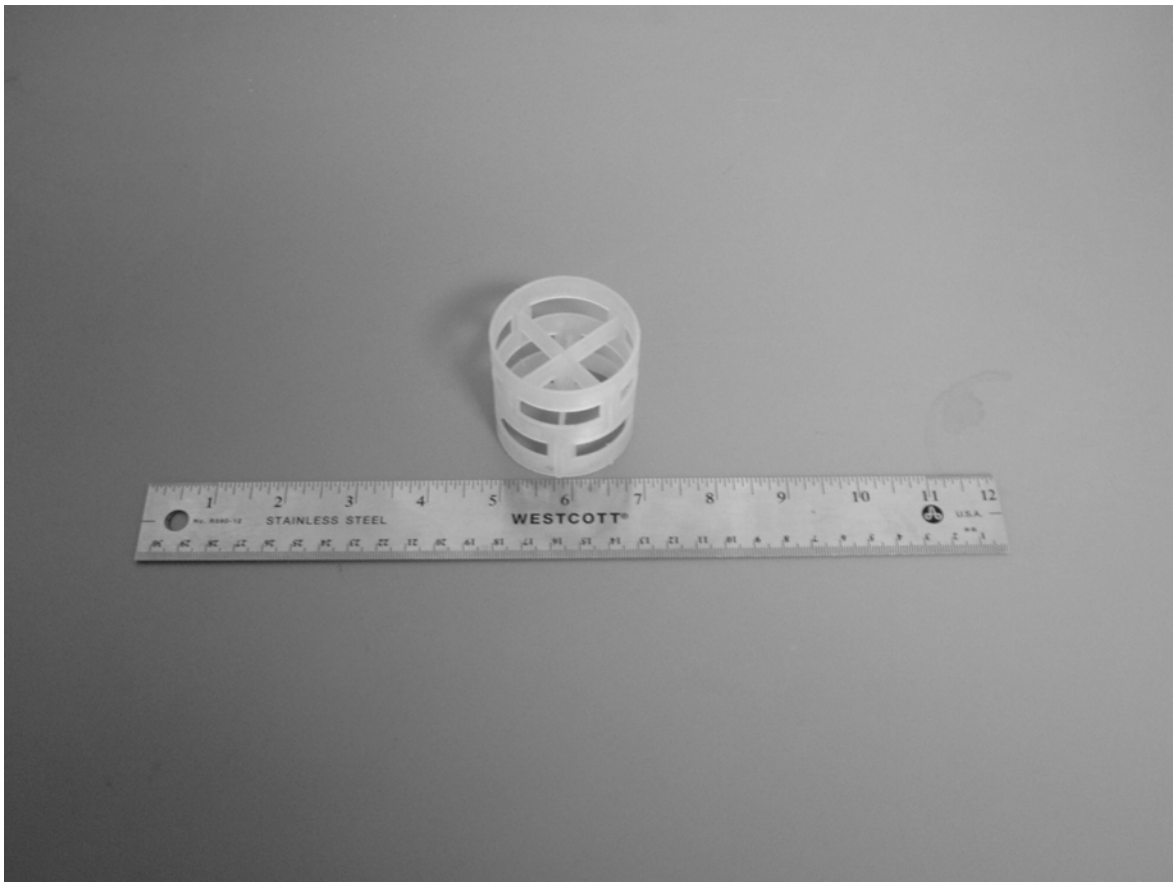

Figure 2. Image shows a "Bio Barrel" (Aquatic Ecosystems, Inc.) used for incubating loach minnow eggs attached to cobbles. Cobbles were placed on top of "Bio Barrels", and then placed into hatching jars for egg incubation.

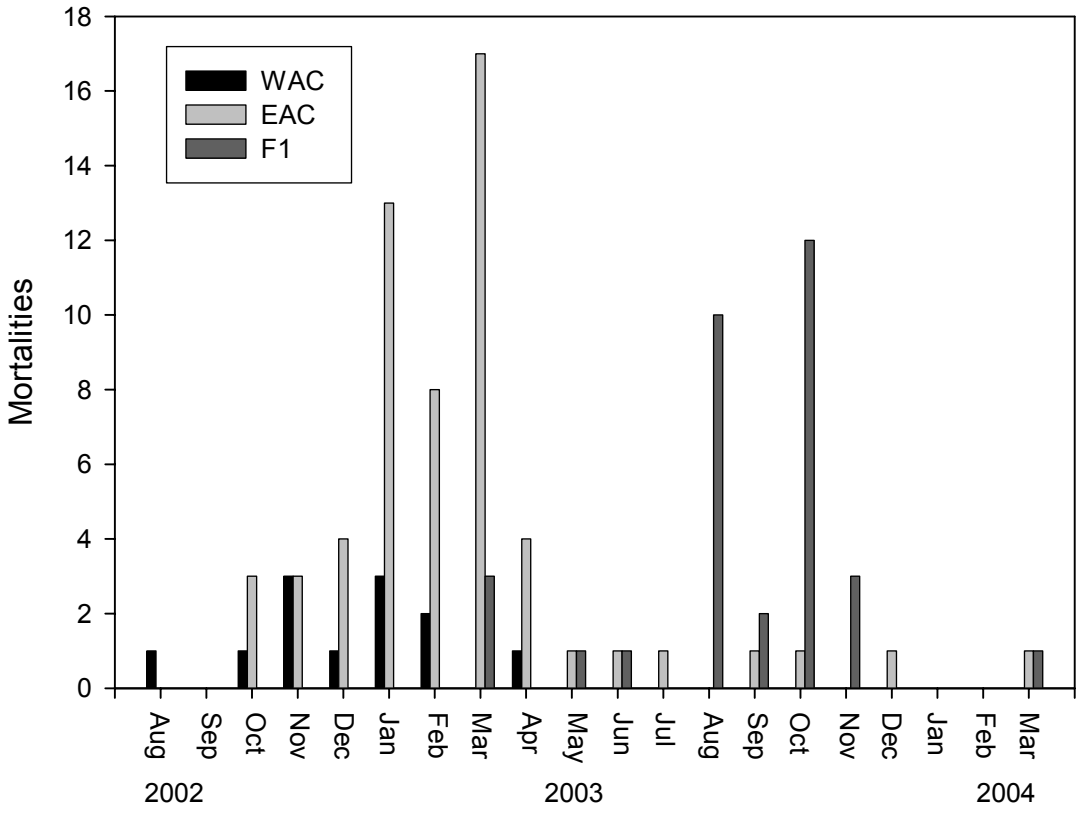

Figure 3. Mortality of adult loach minnow (WAC and EAC) and F1 loach minnow progeny, by month, during the loach minnow study at Bubbling Ponds Hatchery.

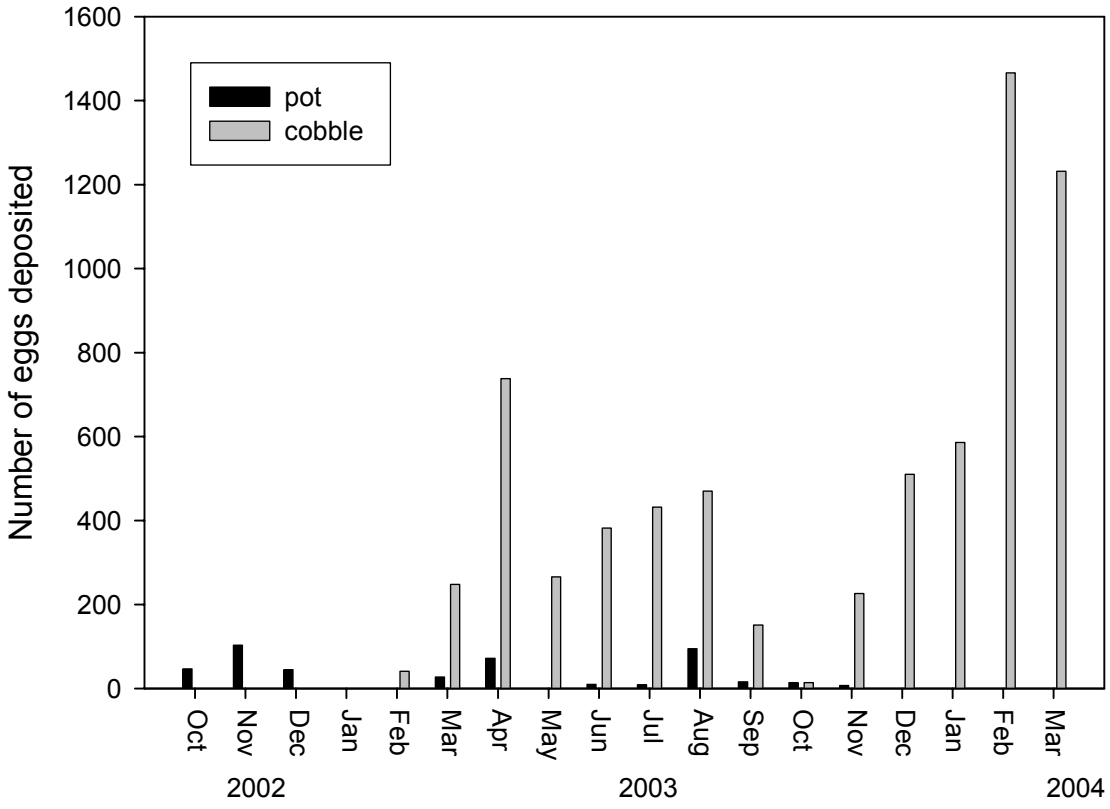

Figure 4. Egg deposition in the laboratory, between October 2002 and March 2003, by loach minnow collected from the East end of Aravaipa Canyon (EAC).

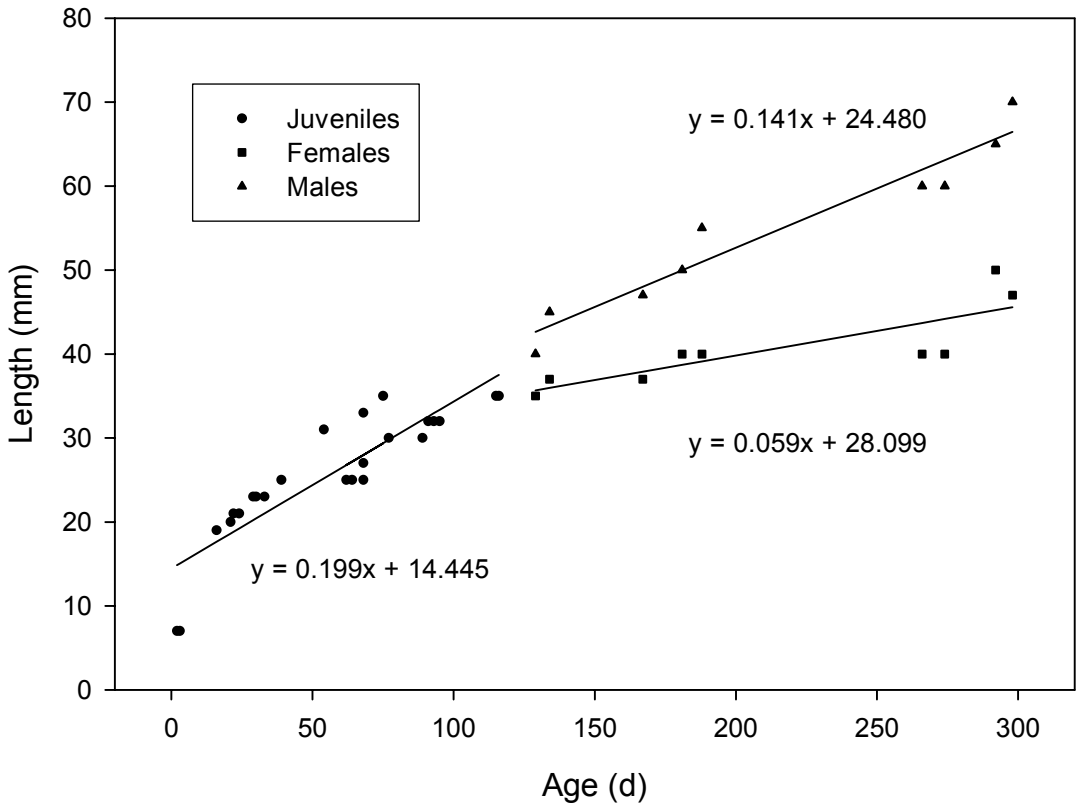

Figure 5. Linear regression of length at age for larval and juvenile (F = 79.2, $P < 0.000$, adjusted $r^2 = 0.773$), female (F = 14.7, $P = 0.006$, adjusted $r^2 = 0.631$), and male (F = 96.6, $P < 0.000$, adjusted $r^2 = 0.923$) loach minnow in the laboratory. Twenty-eight independent length estimates were taken for larval/juvenile loach minnow, and nine independent length estimates were taken for male and female loach minnow.

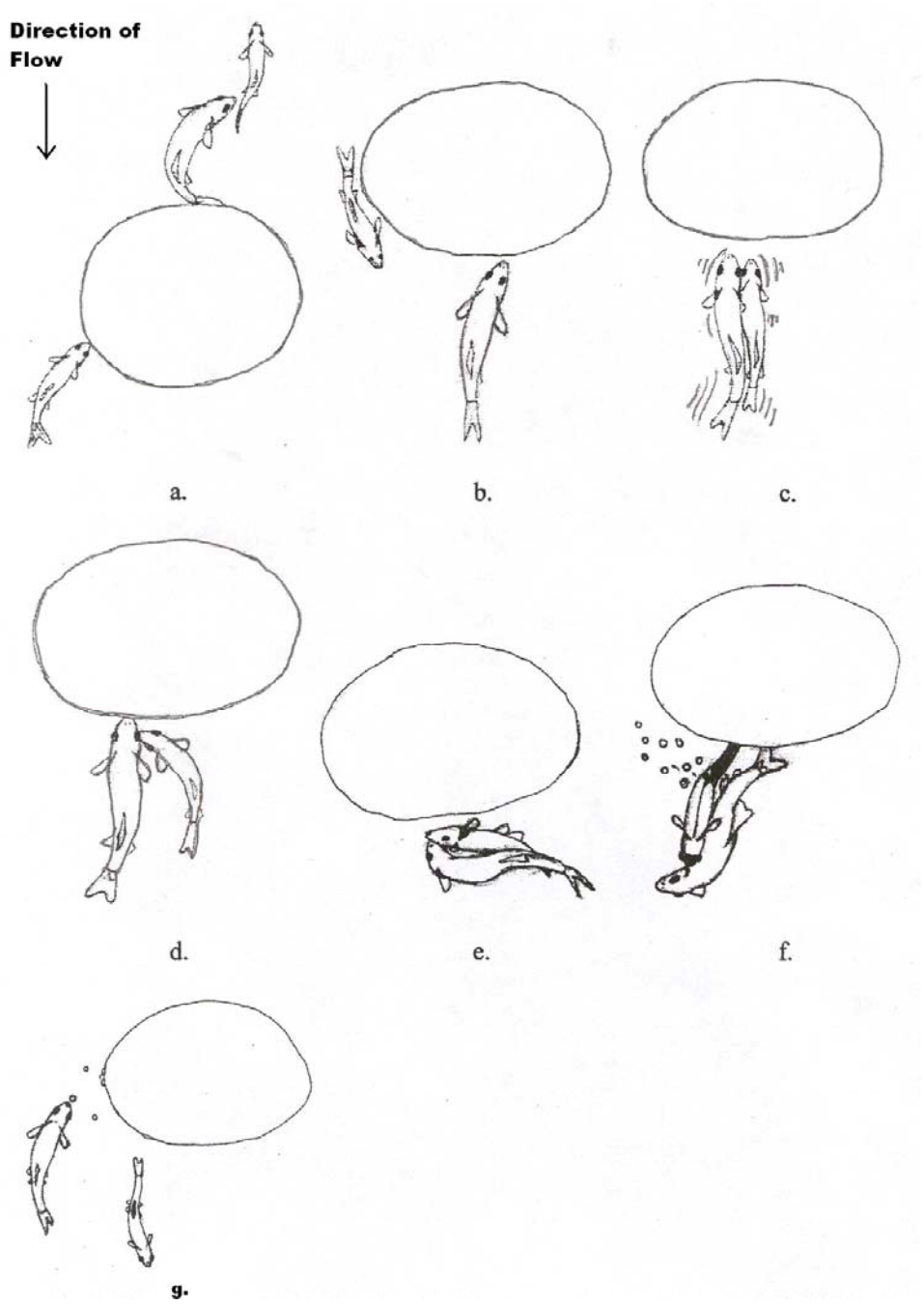

Figure 6. Spawning behavior by loach minnow in laboratory tanks. After one to several females approach the spawning cobble, the male (larger fish) typically chases off all but one female (a). The remaining female and male approach one another (b), align laterally and begin a shuddering movement (c). The female then rubs her snout back and forth across the lower jaw of the male (d), the two fish begin to arch their spines and align their vents (e), and eggs and sperm are expelled (f). The eggs are demersal and adhesive. Finally, the male retrieves eggs that do not settle under the edge of the cobble (g), and places them with the other eggs. Sketches provided by S. Halford, AGFD.

Appendix. Protocol for Collection, Maintenance and Propagation of Loach Minnow.

A. Facility preparation

1. Circular or linear raceways are adequate, but linear are preferred if attempting to control numbers of spawning adults (maximizing outcrossing in the laboratory)
2. Obtain hatching jars for egg incubation, fist-sized cobbles for spawning substrate, "Bio Barrels" for increasing water flow around incubated eggs, plexiglass plates (place under spawning substrates) for improving egg recovery, and 37-L aquaria for rearing larvae and juveniles
3. Flow-through is preferable for all life stages to optimize water quality
4. Submersible pumps are needed for current (max 10 cm/sec), where inflow volumes are low (and to reduce food loss down tank drains)
5. Physical, biological, chemical, and ultra-violet filtration for recirculating systems
6. Temperature control (15-20 C), by ambient air temperature, or water chillers/heaters
7. Photoperiod control (12.5 h L : 11.5 h D) for induced spawning activity
8. Full-spectrum lighting (e.g. Paralite® bulbs)
9. Fill tanks and run pumps for 2 wks prior to fish delivery, for recirc. systems
10. Foods: powdered egg yolk (The Egg Store, online), Silvercup® trout chow starter, LD100 (Aquatic Ecosystems, Inc.), *Artemia*, Tetramin® flake, and freeze-dried bloodworms. Keep foods refrigerated.

B. Collection

1. Kick-seine swift riffles in 3-5 m segments, catching fish in the seine as they are dislodged from underneath cobbles.
2. Seine in a downstream direction in slower riffles and runs.
3. Focus on shady areas where cobbles and algal mats are present.
4. Hold captured fish in 19 L buckets, flushing with fresh stream water every 5 min. Move captured fish to hatchery tank as soon as possible.

5. Portable aerators for capture buckets are advisable.

C. Transport

1. Hauling tank should be aerated, devoid of sharp objects and/or hardware cloth (where fish can gill themselves). Fish density should never exceed 1 fish/ 1.89 L (i.e. 2 fish/gal). This is due to the fact that loach minnow are almost exclusively benthic in nature.
2. Salt solution (0.6 % unionized NaCl)
3. Stress Coat® (manufacturer's recommended dosage)
4. Aeration, backup O_2
5. Temperature acclimation to hatchery tank may be necessary
    - Temperature acclimation is necessary if the source water and the tank water differ in temperature by more that 3^{o}C. Place the collected fish in a plastic bag containing source water within the hauling tank, allowing water temperature in the bag and the tank to equilibrate

D. Arrival at hatchery

1. Acclimation (temperature, pH)
    - Temperature acclimation is the same as during collection
    - Recirculation systems often have high pH levels (approaching 9.0). If that is the case, flush recirculating systems with fresh well water.
2. Prophylactic treatment immediately, 3 consecutive days with a malachite green, nitromersal and acriflavine mixture (Ich Guard®, if available). Flush tanks with fresh water between treatments.
3. Remove charcoal filters and zeolite during treatments
4. Optional follow-up prophylactic treatments
5. Disease treatment as needed: 1-h formalin bath (100 ppm) for external parasites; 1-h chloramine-t bath (10 ppm) for external bacterial infections; oxytetracycline-medicated feeds (10 d) for systemic bacterial infections

6. Refer to "Fish Hatchery Management" (Piper et al. 1982) for more information regarding disease prevention, identification, and treatment.

E. Spawning

1. Set photoperiod to April (12.5 h L : 11.5 h D)
2. Place plexiglass plate under spawning substrates for easy transfer to hatching jars (substrate and plexiglass can be moved to hatching jars, along with any attached eggs).
3. Occasionally rest the fish by decreasing flow velocity and change to December photoperiod (10 h L : 14 h D; every third month)

F. Egg incubation

1. Hatching jars
2. Substrate and eggs inverted on top of "Bio Barrels"
3. Five d to hatch at 21 C

G. Larval rearing

1. Provide starter food immediately (ground trout starter, powdered egg yolk, and LD100)
2. *Artemia* added to diet 7 d post swim-up (10-12 d post hatch)
3. Initiate cleaning of tanks at 3 wks post hatch

H. Juveniles and Adults

1. Feed all F1 progeny a diet of Tetramin® flake and freeze-dried bloodworms when they reach approximately 30 mm in total length
2. Juveniles and adults should be fed periodically (once per month) with *Artemia* and starter trout chow, as this appears to improve overall health (A. Widmer, University of Arizona, pers. obs.)
3. Live diet items (e.g. California blackworms, can be added as desired)
4. Keep all F1 progeny separated from adult fish, to prevent backcrossing
5. Minimize simultaneous stressor factors to fish (spawning, increased flow velocities, etc.)

Addendum

Note: The following information is provided free of charge by the Arizona Game and Fish Department. It is not part of the loach minnow Cooperative Agreement.

Spikedace Reproduction

Methods–Spikedace from the upper Gila River in New Mexico (provided by J. Rinne, U.S. Forest Service, Flagstaff) and Aravaipa Creek, Arizona (provided by C. Carveth, University of Arizona, Tucson) also were held in the wetlab during the loach minnow study. New Mexico fish were held in a 185-L glass aquarium, and Aravaipa spikedace were held in a 333-L glass aquarium, both equipped with under-gravel filtration and pea gravel substrate. Spikedace were held under the same photoperiod and water temperature as loach minnow, and were fed once or twice each day *ad libitum* with Tetramin® flake food and freeze-dried bloodworms. Larvae, when found, were moved to separate 37-L aquaria, and fed as for loach minnow larvae and juveniles.

Results–Spikedace were very easy to care for in the laboratory, and the April photoperiod used to stimulate loach minnow spawning was adequate to induce spikedace reproduction as well. Spikedace spend most of their time in the water column, and feed actively on provided foods. Thus, little waste of foods occurred, and aquaria were easily kept clean. Broadcast spawning resulted in fertilized eggs settling into pea gravel, where they were protected from adult predation (adult spikedace were observed eating eggs that had not settled into pea gravel). Unlike loach minnow, however, spikedace do not appear to feed on their own young, or at least, cannibalism is far less prevalent. Larval spikedace can be left in spawning aquaria, and allowed to feed on leftover adult foods with no apparent ill effects. In addition, larval spikedace swim to the water surface when they reach swim-up, and are thus very easy to remove from holding aquaria. This species should be easy to hold in captivity and propagate for future repatriation efforts.

Discussion–Spikedace spawned prolifically in the wetlab during this study. Larvae, juveniles and adults of this species are easy to care for, and, at least during this study, minimal effort was required to induce successful spawning by adults. However, like

loach minnow, the species is short-lived. Thus, broodstock maintenance is not feasible unless large numbers of adults are replaced annually to diminish losses from senescence. Polyculture of spikedace and loach minnow might be feasible as a means to help maintain tank cleanliness, but this advantage could be offset by piscivory of larval spikedace by adult loach minnow.