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[Article]

Captive Breeding of Endangered Yaqui Topminnow and Yaqui Chub for Recovery Purposes

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Abstract.—Yaqui topminnow *Poeciliopsis occidentalis sonoriensis* and Yaqui chub *Gila purpurea*, two federally endangered fishes from the Rio Yaqui basin (Arizona, USA, and Sonora, Mexico), were propagated by various methods. Propagation of Yaqui topminnow was attempted in aquaria and wading pools; the greatest number of offspring was produced in 556-L, plastic wading pools. We added substrate, artificial plants, and a vertical mesh barrier to create refuge for offspring. Fish were stocked when water was 19°C; after 1 month, when water temperatures exceeded 21°C, Yaqui topminnow started to produce young continuously. On average, 7.4 offspring/d were captured from four pools collectively. Yaqui chub were propagated in 189-L, glass aquaria. We manipulated photoperiod, diet, and substrate to induce spawning of Yaqui chub, but success was only achieved after the water was chilled to 17°C for 30 d and then adjusted to 21°C over 14 d. After the water was maintained at 21°C for 3 d, we covered the bottom of each aquarium with glazed ceramic tiles and a raised plastic grid to protect eggs from predation. Broadcast spawning of Yaqui chub occurred at night; the next morning, we retrieved the tiles and the attached eggs and placed them in incubation tanks. Yaqui chub eggs hatched over the next 5 d, and the hatching success rate averaged 83%. After this initial spawn, manipulation of water temperature was not required to induce spawning, as Yaqui chub spawned each time tiles were placed in the tanks. These methods worked well, and we recommend them for propagation of Yaqui topminnow and Yaqui chub.

Less is known about the culture of threatened and endangered fishes than about that of fishes raised for bait and sport. As of 1999, spawning modes were known for only 13 of 43 imperiled minnow species in North America (Johnston 1999). Almost nothing is known about the spawning of the Yaqui chub *Gila purpurea* and the Yaqui topminnow *Poeciliopsis occidentalis sonoriensis*, two federally endangered fish species that are endemic to the northern Rio Yaqui basin in Arizona, USA, and Sonora, Mexico.

The Yaqui topminnow is a small poeciliid; males rarely grow larger than 25.0 mm total length (TL), and females rarely grow larger than 50.0 mm TL (Minckley 1973). The Yaqui topminnow is found in the Rio Yaqui basin only within the San Bernardino National Wildlife Refuge (SBNWR) in Arizona (AZGFD 2001a). The federal government listed this species as endangered in 1967, and the state of Arizona listed it as endangered in 1988 (USFWS 1967; AZGFD 2001a). Males are distinguished from females by the gonopodium and dark coloration exhibited in breeding season.

The Yaqui chub is a cyprinid that grows to 13.0 cm TL (Rinne and Minckley 1991). Its range in Arizona is restricted primarily to artesian ponds in the SBNWR, Leslie Canyon National Wildlife Refuge, and El Coronado Ranch (Turkey Creek and ponds) in the extreme southeastern part of the state (AZGFD 2001b). Federal endangered species listing of the Yaqui chub occurred in 1984, and state of Arizona endangered species listing occurred in 1988 (USFWS 1984; AZGFD 2001b). Researchers have reported that artificial propagation may be required for survival of this species (DeMarais and Minckley 1993).

Both species are threatened by loss of habitat through groundwater pumping, land use changes, and introductions of nonnative fishes, frogs, and parasites (DeMarais and Minckley 1993). Our objective was to develop laboratory methods for propagating these two endangered fishes to aid in conservation efforts.

Methods

Yaqui topminnow.—We used unbaited minnow traps to collect Yaqui topminnow broodstock from the SBNWR. Transport and acclimation methods were adapted from those of Widmer et al. (2005). Yaqui topminnow were transported to the laboratory in coolers fitted with a battery-operated aerator. A water conditioner (Aquarium Pharmaceuticals, Inc., Chalfont, Pennsylvania; Stress Coat) was added to the transport water. We held fish in five recirculating, 76-L aquaria filled with well water that was conditioned with AmQuel Plus (Kordon Aquarium Products, Hayward, California). Approximately 5–10 fish were stocked in each tank. Pea-sized gravel, a few broken pottery

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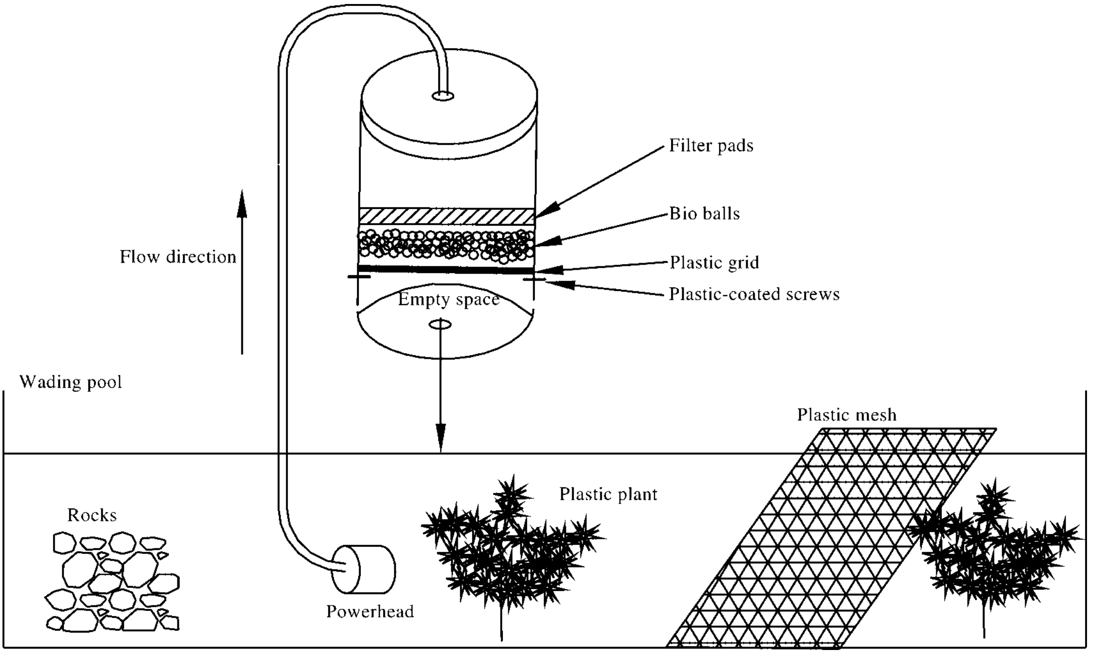

FIGURE 1.—Diagram of a 556-L, plastic wading pool used for propagation of Yaqui topminnow. The biofilter (top) was made from a 20-L plastic bucket, a Rio 1100 pump–powerhead, bioballs, filter pads, and plastic grating. The plastic mesh was a 0.32-cm rigid net that provided refuge for offspring. The mesh and plastic plants were held in place by gravel poured around the base, and the top of the mesh was secured to the edge of the pool. The entire pool was constructed for approximately US$100.

shards, and plastic submersed aquarium plants were added to each tank. Water was filtered with a power filter (Hagen, Inc., Montreal, Quebec; Aquaclear 200), and tanks were aerated with a Rena Model 200 air pump (Rena, Inc., Chalfont, Pennsylvania) and a 2.5-cm air stone. At 2 d postcapture, Yaqui topminnow were prophylactically treated for protozoan parasites *Ichthyophthirius* by use of Quickcure (Aquarium Products, Inc., Glen Burnie, Maryland). Starting at 3 d postcapture, fish were fed twice daily with a combination of three-fourths tropical flakes (Aquatic Eco-Systems, Inc., Apopka, Florida) and one-fourth frozen bloodworms (Hikari Aquatic Diets, Hayward California); both diet components contained at least 50% protein. We also held fish in four 556-L, plastic wading pools, each fitted with a 20-L bucket biofilter and recirculating pump (Taam, Inc., Camarillo, California; Rio 1100), as designed by Widmer et al. (2005; Figure 1). We aerated pools with a Rena 400 air pump connected to 5-cm air stones. For structure, we added four 20-cm-long, plastic plants (simulated hornworts *Ceratophyllum* spp.) and five to seven pieces of cobble (diameter = 20 cm). We placed a rigid plastic panel with 0.32-cm mesh across one-quarter of the pool to provide refuge for any offspring that were produced. The mesh panel and plastic plants were anchored in place by gravel (diameter = 2–6 mm) piled along their bases. We stocked pools with adult fish (2 males/pool; 5–7 females/pool). We held Yaqui topminnow in a greenhouse in early spring, when ambient temperature was below 20°C. As the temperature increased, we maintained pools at approximately 25°C by adjusting the air temperature with an evaporative cooler. We also placed shade cloth over the entire greenhouse and stretched a 1.82- × 2.43-m tarpaulin over the pools inside the greenhouse to keep them from overheating.

Yaqui chub.—We used hoop nets to capture adult Yaqui chub broodstock from Tennis Court Pond at the El Coronado (U.S. Fish and Wildlife Service [USFWS] Permit Number TE676811–0). We used the same transport and acclimation methods employed for Yaqui topminnow. We housed the fish in four 189-L, recirculating aquaria; each aquarium had two power filters (Aquaclear 500) and a Rena Model 400 air pump attached to two 2.5-cm air stones. We stocked each tank with six to nine adult fish; however, sex was unknown because Yaqui chub are not sexually dimorphic unless in spawning condition. Male Yaqui chub exhibit a distinctive bluish sheen over the body when in mating condition (Minckley 1973).

We tried a variety of methods to induce spawning in Yaqui chub over an 11-month period. Yaqui chub

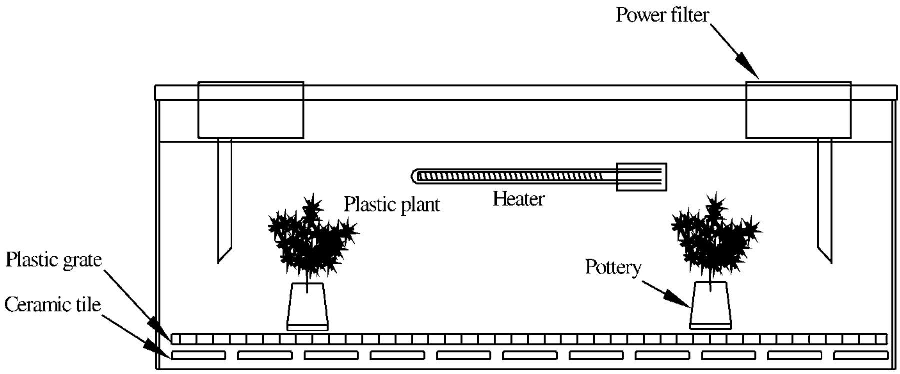

FIGURE 2.—Diagram of a tank used for propagation of Yaqui chub. The bottom of the 189-L aquarium was lined with ceramic tiles (10 × 10 cm). Eggs adhered to the tiles and were protected by a raised plastic grating made from a light diffuser panel. Pottery and plastic plants provided cover.

exhibited nervous behavior when in bare aquaria; they bumped into corners and tried to leap from the tanks. We added two 30-cm, terra-cotta flowerpots (each with a 2–5-cm access hole) and anchored two artificial plants (simulated hornworts) to the pots to increase cover. Next, photoperiod was varied from 12 h light : 12 h dark to 14 h light : 10 h dark over a period of 30 d. A 1-week period of 13 h light : 11 h dark was used to transition between the two cycles. After photoperiod manipulation was unsuccessful, we changed food from pellets to a combination of one-half high-protein finfish starter pellets (Aquatic Eco-Systems; 50% protein diet) and one-half frozen bloodworms and shrimp *Mysis* spp. (Hikari Aquatic Diets), because a high-protein diet is thought to aid in the propagation of *Gila* spp. (A. Schultz, University of Arizona, personal communication). Finally, during winter, we allowed tanks to cool to approximately 17°C for 30 d. We then added a 200-W tank heater (Eheim, Inc., Montreal, Quebec; Ebo-jager) to each tank and gradually increased the temperature to 21°C over 14 d. We kept the photoperiod at 12 h light : 12 h dark and maintained a constant feeding routine, substrate, and cover throughout the entire period of temperature increase.

To collect fish eggs, we lined the bottom of each tank with ceramic tiles (10 × 10 cm) and covered the tiles with plastic, "egg crate" light diffuser panels, which are normally used to cover fluorescent lighting fixtures in drop ceilings (Schultz and Bonar 2007; Figure 2). The width of the spaces in the diffusers (1.2 cm) was small enough to prevent adult Yaqui chub from preying on newly laid eggs that were adhered to tiles. After fish spawned, we moved the tiles with attached eggs to an incubation tank. The incubation tank consisted of a 75-L aquarium (long model) with a plastic-coated metal rack to hold tiles vertically and a 200-W Ebo-jager heater to maintain the temperature at 21°C. We used an Aquaclear 200 power filter fitted with a FilterMax II prefilter (Aquarium Technologies, Inc., Decatur, Georgia) to keep larval fish out of the filter and to provide water movement and filtration (Figure 3). Incubation tanks were kept clean, and eggs were well aerated to inhibit growth of fungus. Hatch rates were calculated by dividing the number of hatched eggs by the number of eggs laid. After swim-up, fry were moved to 38-L rearing aquaria; each tank had an Aquaclear 200 biofilter fitted with a FilterMax II prefilter over the intake to protect fry from becoming trapped in the filter. Fry were delicate, and gentle suction into a large pipette was the best method of transporting fry from incubation tanks to rearing tanks. Even specially designed soft nets proved to be too stressful for fry. As Yaqui chub grew, we separated them into 76-L tanks to reduce stress and competition. The stocking rate was dependent on fish size. Yaqui chub smaller than 10 mm were stocked at 8 fish/L, those larger than 10 mm but smaller than 20 mm were stocked at 3 fish/L, and those larger than 20 mm were stocked at 1 fish/L. Daily cleaning resulted in a 10% water change in all tanks; this was sufficient unless a water quality problem occurred, in which case a 50% water change was used. We kept all fish in accordance with protocols approved by the University of Arizona's Institutional Animal Care and Use Committee.

Results

Yaqui Topminnow

Yaqui topminnow held in aquaria produced only a few offspring (<20 offspring/tank), so we designed larger propagation pools. Once acclimated to propagation pools, Yaqui topminnow produced large numbers

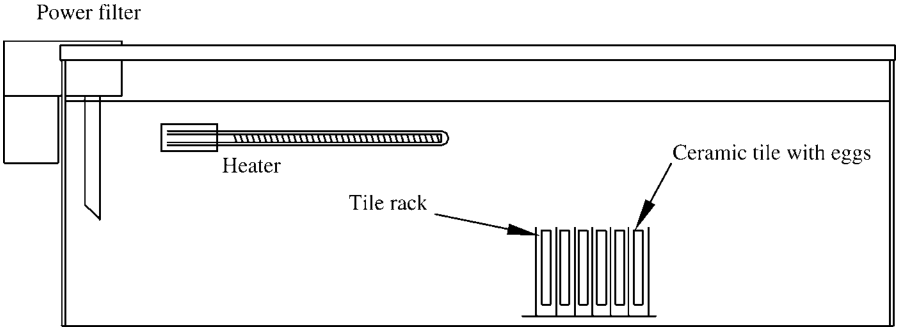

FIGURE 3.—Diagram of a tank used for incubating Yaqui chub eggs. The 75-L tank had a heater to maintain the water temperature at 21°C. Tiles with attached eggs were removed from the propagation tank (Figure 2) and placed in a rack to keep them horizontal; a power filter provided water movement and filtration.

of offspring as long as the temperature in pools remained above 21°C. Within 1 month, offspring were visible in pools, often within the screened refuge. Young fish were removed daily by netting and were placed in separate pools with the same filtration and aeration set-up used in propagation pools. The young were fed to satiation with crushed tropical flake food (Aquatic Eco-Systems), which contained 45% protein. The numbers of offspring produced were highly variable; the total number captured from the four pools in 1 d ranged from 0 to 71 (Table 1). The daily average from the four pools was 7.4 offspring. It was not possible to see and remove all juvenile Yaqui topminnow every day, so a few juveniles collected on a given day may have been produced on previous days. We propagated Yaqui topminnow for 4 months, the time required to produce sufficient numbers for another experiment, and we produced multiple generations throughout our research.

Yaqui Chub

Twenty-five Yaqui chub were held for 11 months before temperature manipulation induced the fish to spawn. Preliminary trials in which cover, photoperiod, and diet were manipulated did not successfully induce spawning. The first spawn of Yaqui chub occurred after the 30-d cooling period and 14-d heating period. After the initial spawning event, propagation of Yaqui chub could be induced without the need for temperature manipulation. Once the Yaqui chub were acclimated to the spawning set-up, placement of tiles in the aquarium during the afternoon usually resulted in spawning by the next morning. Small Yaqui chub did consume eggs, as demonstrated by one small individual that swam under the grating to access the eggs.

Each spawn occurred between 2200 hours and 0600 hours the morning after tile placement. Eggs adhered to the tiles and were an average of 1.76 mm in diameter (n = 15). Yaqui chub produced an average of 378 eggs/spawn (range = 150–734 eggs/spawn; Table 2). Fertilized eggs were a yellowish color, whereas unfertilized eggs remained clear. Eggs hatched starting on the fourth day, and fry remained at the bottom of the tank for 24–48 h while they absorbed their yolk sacs. Fry averaged 4.55 mm TL (range = 4.1–4.9 mm TL; n = 5 fish) upon hatching and 6.5 mm TL (range = 6.0–6.9 mm TL; n = 10 fish) on day 3. We observed swim-up fry after 5 d and moved them to rearing tanks (38-L aquaria) after 7 d. The average hatching success rate for seven spawns was 83.13% (Table 2). Fry were given First Bites food (Hikari Aquatic Diets) and frozen nauplii of brine shrimp *Artemia* spp. (Hikari Aquatic Diets) for the first 10 d, and larger food sizes were provided as the fry grew. After 10 d, we administered high-protein finfish starter food (Aquatic Eco-Systems) in the following sequence: meal (0.4–0.6 mm) for approximately 1 week, Number 1 crumble (0.60–0.85 mm) for 2 weeks, and Number 2 crumble (0.85–1.20 mm) for 2 weeks. Fish were fed four times daily for the first month and twice daily thereafter. After this point, Yaqui chub were given pellet-sized (1.5-mm) food and frozen bloodworms (Hikari Aquatic Diets) to satiation

TABLE 1.—Total number of Yaqui topminnow offspring collected from four 556-L propagation pools at the University of Arizona, Tucson, during June 2005. Each pool was stocked with two adult males and five to seven adult females.

Date	Offspring (N)	Date	Offspring (N)	Date	Offspring (N)
Jun 1	9	Jun 10	9	Jun 19	0
Jun 2	8	Jun 11	16	Jun 20	0
Jun 3	2	Jun 12	43	Jun 21	6
Jun 4	11	Jun 13	16	Jun 22	71
Jun 5	18	Jun 14	13	Jun 23	40
Jun 6	23	Jun 15	15	Jun 24	0
Jun 7	13	Jun 16	20	Jun 25	44
Jun 8	7	Jun 17	8	Jun 27	33
Jun 9	8	Jun 18	0	Jun 29	36

TABLE 2.—Spawning dates and number of eggs produced by Yaqui chub after ceramic tiles (10 × 10 cm) were placed in four 189-L propagation tanks at the University of Arizona, Tucson, during March–October 2005. Tiles and attached eggs were transferred to 75-L incubation tanks, where hatching success and number of fry were determined (average hatching success = 83%). Eggs began hatching after 4 d; some took up to 6 d to hatch.

Tile placement date	Spawn date	Eggs (*N*)	Hatch date	Hatch success (%)	Fry (*N*)
Mar 21	Mar 22	387	Mar 26	82	319
Apr 6	Apr 7	150	Apr 12	89	134
Apr 19	Apr 20	195	Apr 25	86	168
May 11	May 15	619	May 19	76	471
Jun 14	Jun 17	734	Jun 20	79	581
Jun 22	Jun 23	169	Jun 27	85	143
Oct 3	Oct 4	392	Oct 9	84	329

three times daily for the remainder of the trials. We raised fry to approximately 25 mm TL, which was the size needed for another experiment.

Discussion

Yaqui Topminnow

Yaqui topminnow are livebearers and have the ability to store sperm and to simultaneously carry two broods totaling 6–49 young (Minckley 1973). We thought that only food, cover, and the proper temperature would be required to induce spawning in fish with these characteristics. However, we found that Yaqui topminnow held in aquaria cannibalized most of the offspring produced, even when cover and other food were provided. We believe conditions were too crowded in the 76-L aquaria for successful propagation, so we designed 556-L propagation pools and achieved the desired results. We did not test whether successful spawning could be achieved in larger tanks (i.e., between 76 and 556 L).

In our study, temperature appeared to be the most important factor for triggering spawning. In nature, reproductive activity of Yaqui topminnow was low or nonexistent at water temperatures of 12–16°C, but fish were reproductively active in water that reached 19–22°C (Galat and Robertson 1992). Furthermore, photoperiod may be another important driver of reproductive activity in nature, especially in spring heads of constant water temperature (Galat and Robertson 1992). Our fish reproduced at 21°C in the laboratory and did not reproduce in the greenhouse until the temperature exceeded 21°C. The first spawning event in the greenhouse occurred 1 month after fish were introduced. The delay in breeding may have been due to the stress of translocation and acclimation to captive conditions but was more likely caused by the lower winter temperatures in the greenhouse. To enhance the success of the propagation methods described here, we recommend further research to find the precise breeding temperature requirements of Yaqui topminnow.

Yaqui Chub

Induction of spawning was more difficult in Yaqui chub than in Yaqui topminnow. The mating behavior of Yaqui chub or similar species has not been adequately described in the literature. Yaqui chub have reproduced when stocked into small ponds (DeMarais and Minckley 1993). However, we are unaware of aquarium programs for breeding this fish.

Temperature manipulation was required to induce spawning in Yaqui chub. No breeding behavior was observed until fish were subjected to the 30-d cooling period and 14-d heating period.

Variation in the numbers of eggs produced per spawn may have been due to variation in the number of females that were spawning. Because males and females could not be definitively distinguished, the ratio of males to females was unknown. Yaqui chub were sensitive to any changes in the tank environment. The terra-cotta pots and plastic plants in tanks provided necessary cover for Yaqui chub. However, these structures did not seem to influence where the fish spawned, as eggs were found throughout the bottom of tanks. Yaqui chub in this study were observed to be broadcast spawners, as many other *Gila* spp. are thought to be (Johnston 1999). The tiles were necessary as a substrate for adherence of eggs, and the plastic grating protected eggs from predation by adults.

Fry were reared successfully by administering feedings three times daily. Growth of fish was dependent on stocking density, feeding rates, and temperature; however, further research is required to identify the mix of factors that will produce optimal results. We successfully propagated and reared Yaqui chub and Yaqui topminnow in a laboratory, and these methods have helped conserve these species by securing genetic stocks for future re-introductions of these endangered fishes within their native range.

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