Developing Propagation and Culture Protocols for the Cahaba Shiner, *Notropis cahabae*, and the Goldline Darter, *Percina aurolineata*

Patrick L. Rakes and J. R. Shute

Conservation Fisheries, Inc., 3709 N. Broadway, Knoxville, TN 37917
Photographs by the author except where noted.

The goal of this project was to develop methods for stimulating captive reproduction and rearing of two rare fish, the Cahaba shiner, *Notropis cahabae*, and the goldline darter, *Percina aurolineata*. Successful production would then supply larval and juvenile individuals for the Environmental Protection Agency's toxicity research as part of efforts to evaluate the adequacy of water quality criteria to protect sensitive species in the Cahaba River.

Because habitat requirements and early life history of these species are poorly known, observations of aquarium-held fish, particularly behaviors associated with reproduction and early life history, could also provide information critical for future conservation and management strategies.

The attempts to spawn the two species in this study followed our successful propagation of blue shiners, *Cyprinella caerulea*, in 1997 and 1998 as part of the initial Cahaba River toxicity studies project (Rakes, 1998). We then began working with goldline darters in 1998. Our earlier project to develop propagation protocols for the channel darter, *Percina copelandi* (as a surrogate for the imperiled Pearl darter, *Percina aurora*; see Ross et al., 1998, and Schofield et al., 1999) provided us with experience that we used for the goldline darter work. The reproductive and early life history characteristics are apparently very similar for these *Percina* species. The results of three years of captive spawning and propagation efforts, 1999-2001, are described below.

Methods

Adult Cahaba shiners were collected from the Cahaba River on 29 April 1999 just downstream of the Bibb County Road 26 Bridge. Despite considerable effort with a seine (1.25 x 3 m or 2.5 x 6 m, both with 4.8 mm mesh), only nine individuals were collected. The shiners were held for a year in a 76-liter aquarium, measuring 76 x 30 x 30 cm, part of a 1200-liter, multi-aquarium, centralized system. The tank was provided with a mixed sand and fine gravel substrate, 3-5 cm deep. Water flowed into the tank, from above, at a rate of approximately 400 liters/hour. An airstone provided additional aeration. Yarn spawning mops (both floating and sinking) were provided for cover and as potential spawning substrate.

Goldline darters were collected from the Cahaba River on 28 August 1998, at the same site and with the same methods as described above for the shiners. These were held in a ~370 liter tank, with dimensions 240 x 56 x 25 cm. Two large pumps provided approximately 7500 liters/hour of flow through the aquarium, creating a riffle/run about 15 cm deep with swirling eddies and slack water areas. At one end, water exited this stream tank through a large, screened overflow constructed of acrylic and plastic mesh with 2 x 4 mm openings. The water leaving the tank then fell through plastic gutter pipes to a central sump for the 1200-liter, multi-aquarium, system. Substrate in the riffle complex consisted of a sand, gravel,

cobble, slab, and boulder mixture. Although 12 goldline darters were initially placed in the tank, mortality (various reasons) resulted in only seven fish remaining for our project. Only two of these were females.

The entire system housing both species was maintained at a photoperiod of 16 hours of light during breeding periods, alternating with 11 hours during non-breeding periods. Water temperatures were maintained at about 13-16°C during "winter" and then raised to 20-22°C when we were attempting to stimulate spawning. Some fish were moved to other systems or individual aquaria for conditioning at even lower temperatures (to 10°C) during the winter months. Water chemistry was maintained at a pH of about 7.0 and gH about 40 ppm, with no measurable ammonia or nitrite, and nitrate nitrogen less than 50 ppm.

Shiner eggs were removed from the aquaria by gently lifting mops out of the tanks and placing them in incubation containers. Darter eggs and yolk-sac larvae were recovered directly from aquaria by using an aquarium-cleaning siphon to vacuum the substrate into a 19-liter bucket. Larvae that swam up in the bucket were then collected by pipette and moved to incubation/rearing trays (30 x 15 x 8 cm) with clean system water. Any larvae or eggs remaining in the bucket were collected by pouring off most of the water, then swirling the bucket and gently pouring the remainder into one of the trays. A light, set up below the translucent tray, permitted us to remove the eggs and larvae from the debris with a pipette and isolate them in a clean tray for incubation.

Eggs and larvae of both species were incubated and reared in a number of containers and set-ups. Some were held in the trays described above, suspended in a tank that was isolated from the rest of the multi-aquarium system. These tanks had airstone aeration or internal sponge filtration, and some had a thin layer of fine substrate. Some trays were set up with a screened overflow "window" in one side, and supplied with a slow inflow of water from the main system. Occasionally, 19-liter plastic buckets with airstones or sponge filters were situated above the system aquaria, and were also used for incubation. Some of these had overflow screens nested into the 76-liter aquaria for occasional flushing water changes or continuous flow into the aquarium below. Some eggs were incubated in buckets by placing them in a Petri dish suspended on or under the water. Black or other dark, opaque versions of all these setups as well as various types of lighting and light filters were used to test the effect of background color and illumination on larval feeding ability and survivorship.

Fig. 1.
Cahaba shiner, *Notropis cahabae*. Courtesy: Rhett Johnson and Brett Wehrle, Private Forest Management Team (www.pfmt.org).

Adult fish were fed a variety of live foods including blackworms (oligochaetes), *Daphnia*, and *Artemia* nauplii. Frozen *Daphnia*, chironomids (bloodworms) and adult *Artemia* were also used. As much food as the fish could consume in a few minutes was offered 1-3 or more times daily.

Larvae were fed a variety of minute foods that included live *Artemia* nauplii, rotifers (mostly *Brachionus*), copepods, and other infusoria. They were also fed dry, commercially prepared larval food 100-350 microns in size (OSI, Ziegler, and Florida Aqua Farms). Foods of different sizes were offered simultaneously to allow for the variety of sizes of the larvae. Larval foods were provided by hand (with pipettes) 2-5 times daily. Phytoplankton (greenwater) was also added directly to larval rearing containers. Zooplankton cultures were fed phytoplankton (*Chlorella* or *Nanochlorella*, Florida Aqua Farms) and/or liquid Roti-Rich (Florida Aqua Farms).

Results and Discussion:
Cahaba Shiner

Reproductive behavior Eggs were discovered in the mops from the aquarium containing the nine adult Cahaba shiners on 6 May 1999, only a week following their capture. The small group continued to spawn, as evidenced by egg recoveries, through 29 October, although production declined at several intervals. A total of 1700-1800 larvae were produced through this period with 27 collections of eggs.

At no time during this or either of the two subsequent years was any hint of reproductive behavior observed. No spawning color changes or activities were exhibited. We could only speculate on behaviors based upon the size and placement of egg masses and the behaviors of other, similar cyprinids. Given the similarity of the spawning sites to what we had observed for Cape Fear shiners, *Notropis mekistocholas* (Rakes, 2000), we hypothesized that pairs, or small groups, of Cahaba shiners spawned in and on the yarn mops. These spawning sites were probably not defended with any form of male territoriality, but were likely selected by the females as appropriate

sites for egg deposition. Because numbers of eggs collected were variable, it was impossible to tell whether more than one female contributed to egg masses, or whether one female spawned all the eggs in one act or repeatedly added eggs to a spawning site.

In 2000, 40 propagated one-year-old fish were induced to spawn on 1 June, indicating that the species is capable of spawning at one year of age. These fish, along with the eight surviving wild-collected adults, continued to spawn through 12 September, producing at least 2000 and possibly more than 3000 young. All of the eight older fish died during this time, presumably due to old age. Eleven of the younger one-year-old captively produced individuals also died, leaving 29 to spawn in the 2001 breeding season. Because the eight wild-collected older fish died at the estimated age of two years suggests a maximum lifespan for the species of only two years. However, all of the captively propagated individuals spawning in 2001 survived through October 2001, indicating that maximum lifespan may be greater than two years, at least under favorable conditions.

Eggs and larvae Cahaba shiner eggs were found to be unlike any other fish eggs we had ever observed. Most of the eggs were deposited in and attached to spawning mops in large clusters of 20 to more than 100 eggs. They formed a gelatinous mass very similar in appearance to amphibian eggs when they were lifted from the water. Eggs were found in floating and bottom mops, but the most preferred sites appeared to be floating mops in the most shallow areas provided (~20 cm deep) where there was little or no current.

Individual eggs, teased out of the mops, or recovered loose in aquaria, were very similar in outward appearance to Cape Fear shiner eggs. They were relatively large in size (nearly 3 mm diameter), with a thin, delicate, completely translucent chorion enclosing a relatively small yolk and/or embryo. The embryo occupied only about 1/4 of the central volume of the egg. However, the eggs were much 'softer,' collapsing like a water balloon when they were removed from the water due to the greater elasticity of the chorion. The chorions were also much more adhesive, at least when the eggs were first spawned. This resulted in the eggs either adhering to each other in masses, to the spawning substrate, or collecting a coating of loose, fine materials. They were generally found in large clusters, unlike Cape Fear shiner eggs, which were always found individually scattered in, around, and under spawning mops.

The Cahaba shiner eggs contrasted even more extremely with Mobile mimic shiner eggs, which we had recovered and reared from captive fish the previous year. The mimic shiner eggs were tiny—only 1.1-1.2 mm in diameter—and were broadcast spawned over a sand and gravel substrate. Chorions were translucent, but hard, with a few short adhesive fibrils. Yolks and embryos occupied a minimum of 2/3 the volume of the egg, and embryos near hatch were tightly wrapped around the yolk, filling the entire egg volume. Thus, although adult Cahaba shiners and mobile mimic shiners are so similar in appearance as to be nearly indistinguishable to the untrained eye, they have markedly different spawning sites with associated differences in egg morphology.

While the eggs of the three cyprinids described above exhibited significant differences, the larvae and larval development of all three were surprisingly similar in many respects. In all three species, eggs hatched in less than three days at 20-22°C, producing poorly developed larvae entirely lacking in pigment. These "altricious" larvae consisted of visibly little more than a bundle of myomeres with an attached yolk sack, eyes and brain, and heart and circulatory system. Gill arches and a mouth were poorly developed and apparently not functional at hatch. These early larvae were essentially mobile, nearly invisible embryos, no longer imprisoned in the confines of an egg (and probably less susceptible to predation as a result). For the first two days after hatching the larvae tended to lie motionless on the bottom or buried in debris, only moving if disturbed, at which they would either bury deeper into cover or swim up in a darting, spiraling flight followed by inactivity, sinking motionless to the bottom.

After approximately three days, the larvae began to swim up and fill their air bladders. By this time, they were beginning to develop some pigmentation, particularly on the eyes and midlines of the body. Cahaba shiner larvae were extremely small at this stage, measuring less than 4.0 mm TL (Cape Fear shiners were ~6 mm, Mobile mimic shiners ~5 mm). The larvae apparently subsisted on yolk-sac reserves, as they did not feed until the fifth or sixth day after hatching. Cahaba shiner larvae were unable to consume prey items larger than about 200-250 microns in size (versus Cape Fear shiners' ability to ingest *Artemia* nauplii, 350-450 microns). Therefore, live rotifers or similar sized powdered preparations were required as first foods for Cahaba shiners. Early growth was relatively slow, with the larvae requiring 14 or more days to attain sufficient size to be able to consume *Artemia* nauplii.

Culture results Early attempts to rear Cahaba shiner larvae in 2-liter plastic trays resulted in extremely variable survivorship as providing sufficient food density without causing excessive declines in water quality was a problem.

However, of an estimated 1700-1800 eggs hatched, approximately 850 juveniles were reared in 1999, the bulk of which were provided to EPA for toxicity tests. In 2000, the 40 oldest one-year-olds and eight surviving wild-collected adults produced an estimated minimum of 2000 eggs, and possibly more than 3000 eggs (eggs in masses could not be accurately counted). Unfortunately, of this large number of eggs, only about 120 juveniles were produced. This was again a result of water quality problems resulting from various experimental protocols aimed at maintaining sufficient food density. An additional problem was the lack of a reliable supply of live rotifers for early feeding.

In 2001, in order to avoid the water quality problems described above, eggs were incubated and larvae reared directly in 76-liter aquaria instead of the 2-l containers used previously. These aquaria were provided with internal sponge filters to augment biological filtration during the first 2-4 weeks of larval development, when flow-thru and turnover rates were kept low to maintain high food densities. This protocol, in combination with heavy feeding with live rotifers (*Brachionus*) during the first 2-3 weeks, resulted in greatly improved survivorship. However, precise calculation of survivorship was even more difficult than the previous year, since the number of larvae that hatched was nearly impossible to estimate in the larger containers. Maintaining sufficiently high food density with an adequate number of feedings per day was probably the primary remaining obstacle to highly successful production. A total of 1330 larvae were produced and provided to EPA for water toxicity tests.

Approximately 300 juvenile Cahaba shiners survived the first round of toxicity tests and were returned to Cahaba River collection site on 20 May 2000. Another 200 survived testing in 2001, and about half of these were returned to the river on 7 November 2001.

Results and Discussion:
Goldline Darter

Reproductive behavior Goldline darter reproductive behavior was not observed in sufficient detail to be fully described during the first year (1999) of this project. However, although spawning substrates selected were very similar, there appeared to be a number of aspects where the goldline darters differed substantially from what we had previously noted for our earlier experiences (Ross et al., 1998; Schofield et al., 1999) with captive channel darters. Unlike the channel darters, which were gregarious except for dominant
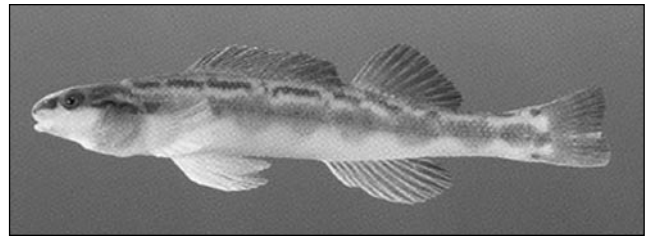

Fig. 2.
Goldline darter, *Percina aurolineata*. Courtesy: Rhett Johnson and Brett Wehrle, Private Forest Management Team (www.pfmt.org).

males, goldline darters were solitary. Male goldline darters, nearly twice as large as females, generally remained hidden under cover (tilted slab rocks in our aquaria) while females mostly stayed out in the open areas of the aquarium. These cover rocks were defended against other males, but not females. Male goldline darters were observed approaching, and closely accompanying females that entered open areas near their cover rock, but even though we discovered eggs, actual spawning was never observed. Eggs were recovered from areas of fine sand and gravel in lee and eddy areas. The most preferred substrate size for spawning appeared to be about 1.5-2.0 mm coarse sand.

On two occasions a male was observed to exhibit what appeared to be a form of advertisement behavior in these areas. The display consisted of a rapid, jerky darting movement over a 15-20 cm distance during which the substrate was "kicked up" into the water column at several points. Males also exhibited the temporary darkening of pigmentation as described above for channel darters .

In 2000 and 2001, we noted markedly different behaviors from that described above. Breeding groups consisted of six of the adults collected in 1998 and 40 propagated adults in 2000 (one-year-olds), and 35 propagated fish in 2001 (two-year-olds). Groups of males aggregated and remained in the open over preferred spawning substrates. There were very few individual aggressive interactions between the males, but groups frequently swam in parallel just off the bottom around a few large rocks in the center of the tank. These males displayed sometimes nearly black blushing pigmentation, similar to that observed in channel darters. However, this pigmentation involved their entire bodies, pelvic fins and a black band in the first dorsal fin. At times a group of these "blushing" darters would make several uninterrupted circuits almost like a living merry-go-round! The high visibility of these displays strongly suggested that they might be attractors to females or other males, with the aggregations being similar to lekking behavior. Males that participated in spawning were

typically not darkly pigmented, suggesting that this temporary "blushing" was more a function of agonistic interactions than courtship behavior. Spawning males were, however, readily distinguished from the females by the dark pigment in their fins and the soft diffusion of all lateral pigmentation on the body; females exhibited sharply contrasting light and dark blotches and speckles.

During the 2001 breeding period, spawning behavior described above was videotaped. Between the male-aggregating episodes described above, individual females would move into areas of the aquarium where there was suitable spawning substrate. Spawning clasps generally took place near the base of a large rock and seemed to result by the female selecting the site. The only apparent behavioral cue by the female inviting a spawning clasp was when she placed her head and entire ventral surface in close contact with the substrate. Males were typically perched high on their pelvic fins at all times, with heads held high. During spawning clasps, the male grasped the female with his pelvic fins on her nape, and his anal fin and posterior ventrum curved down one side of her, which pinned her to the substrate. They then could be observed simultaneously quivering, presumably releasing eggs and sperm. This was never clearly observed, however. Eggs recovered from other areas of the spawning substrate similarly suggested that females tended to select spots near vertical objects—similar sites in streams and rivers would be areas with fine substrates in the lees of cobbles and boulders in areas with current.

The duration of the spawning season was remarkably consistent for all three years of our project, lasting each time approximately six weeks (2/22-4/2/99, 4/3-5/15/00, 3/21-5/7/01). In 2001, spawning was initiated at only 13 hours of daylight and 17°C (this was the only year when increases were implemented gradually).

Eggs and larvae   Goldline darter eggs and larvae were tiny, translucent, and nearly identical to those we had observed in our earlier experiences with channel darters. However, goldline darter eggs were larger in size (eggs 1.7-1.8 mm diameter, larvae 6.0-6.5 mm TL at hatch; compared to 1.0 mm and 5.0 mm for channel darters). Goldline darters also differed in the presence of fine melanocytes scattered on the yolk-sacs of embryos near hatching. Larval development was visible through the clear chorion when viewed under a dissecting microscope. The eggs were nearly invisible with the naked eye when illuminated from above, but in a translucent tray, light from below made them contrast with associated debris, appearing to glow a pale orange or amber color in the early developmental stages. Eggs were fairly adhesive if recently spawned, so that sand and debris attached to the chorion, or two to four eggs attached to each other. Older eggs nearing hatch, however, were no longer adhesive, resulting in a clean chorion and rarely were clumps of eggs attached to each other.

Embryonic development was rapid. Although no eggs were individually monitored from an early stage until hatch, development appeared to require only about three days at water temperatures of about 19°C. Embryos were very thin, with a streamlined, elongate yolk-sac and a single large oil droplet visible inside. The only pigmentation was the eyes (black) and a fine line of melanophores along the yolk-sac.

Early larvae tended to lay motionless on their sides at first hatching, but were observed to swim in an erratic rapid spiral if disturbed. Within a few hours the larvae were pelagic and actively cruising the sides, bottom, and open water of rearing containers. They were also observed to be strongly phototropic, tending to stay on the lightest sides of containers or near reflections in corners or on the bottom of containers. The addition of foods and especially of green water (phytoplankton) to the containers caused larvae to immediately become more active, swimming more rapidly in open water, presumably searching for food. Initially, it was not clear whether this change in behavior was due to the visual or chemical stimulus provided by the food or simply the darkening of the water, which perhaps made food more readily visible, or a combination of factors. However, when larvae were transferred from a white bucket, where they tended to stay pushing against the sides, to a black bucket, they immediately swam out into and remained in open water. Because a similar response was also noted when greenwater was added to the white bucket, it appears that dark(er) surroundings may have been the most important factor.

Culture results   Initial attempts to rear the *Percina* larvae in 2-liter plastic trays resulted in 100% mortality. However, those larvae placed in white buckets survived longer and behaved more naturally, swimming more in the open and spending less time "pacing" or scraping along the sides of their containers than those in trays. This was especially so when greenwater was added to the bucket in sufficient quantity to color the water. We hypothesized that the gentle currents in a larger container facilitated pelagic positioning of the larvae and that darker surroundings somehow made the larvae more "comfortable" or less stressed. Another possibility was that the darker surroundings made food items more readily visible. When we placed the young in black buckets, survivorship increased.

Another advantage of the buckets was the ability to suspend sufficient quantities of food in the water column to permit the larvae to feed for longer periods between feeding periods. As few as two feedings per day were found to be sufficient if the foods remained in suspension. Even better results were observed in 2001, when larvae were transferred directly into 76-liter aquaria with dark sides. This permitted easier maintenance and visual monitoring of the larvae, with the added benefits of improved water quality maintenance in established aquaria (with biological filtration) that were part of the larger, multi-aquarium system.

Recently hatched larvae appeared to mostly hover motionless in the current for a few hours, possibly filter-feeding. After that, they darted throughout the water column and could be observed taking food items such as rotifers and dry food particles. Although the larvae would attack *Artemia* nauplii as soon as they started sight-feeding, their mouths were generally too small to consume nauplii for at least three to five days at water temperatures of 21-22°C, and longer at cooler temperatures.

In comparison with channel darters, goldline darter larvae exhibited a markedly longer pelagic stage. Although fin folds were absorbed and juvenile fin characteristics developed while the fish were pelagic, they did not settle to a benthic stage until around age 30 days. As with the channel darters, the pelagic goldline darter larvae were a conspicuous, translucent orange color, perhaps resulting from (orange) *Artemia* nauplii as their primary food. At transformation, the juveniles measured nearly 20 mm TL and were beginning to exhibit faintly adult pigmentation such as darker lateral bands and the dorsolateral gold bands above them.

During the first year of reproduction, only two females were present in the tank, but it was not determined whether both spawned the eggs that were recovered. If our fecundity estimates for channel darters were correct, and since the goldline darter females were about the same size as the channel darters, it seemed likely that all young were produced by only one female, since a total of only 98 eggs were recovered in that first spawning season. Alternatively, the goldline darters may have only produced half as many eggs as the channel darters.

Of the 77 goldline darter eggs and larvae collected subsequent to the first two batches in 1999, 35 fish survived (45%). In 2000, ~150 juveniles were produced from nearly 600 eggs and larvae recovered, for a survivorship of around 25%. Most losses were eggs that developed fungal infections during incubation or early larval mortality. Larval mortality was thought to be related to the difficulty of maintaining

sufficient food density while simultaneously preserving water quality. In December 2000, 130 of the 150 juveniles produced were provided to EPA for toxicity testing. In 2001, nearly 4700 eggs and larvae were collected from the breeding darters! The dramatic production increase resulted from more frequent egg collections and a larger group of two-year-old fish. If around 15 females contributed, and if most of the eggs and larvae were collected, fecundity could be estimated at around 300 eggs per female.

Unfortunately, survivorship of eggs and larvae in 2001 was extremely variable for a number of reasons. One of the worst was fungal or bacterial egg infections. This was partly due to the inevitable damage resulting from handling, and partly due to incubation techniques. We eventually resolved this problem by nesting 2-liter trays, treated with acriflavin, in the water over the larval rearing tanks (to stabilize temperature). Eggs were incubated in these trays with an airstone for aeration. Dead eggs were removed from these trays at least once daily, and larvae were transferred at least twice daily into the rearing tanks.

A second major problem was early larval feeding. We had no supply of live foods for the first three weeks of production, and were able to feed only dry foods—larval survivorship improved when live rotifers were provided thereafter. Another significant problem that developed late in the production season was the appearance of hydra in the larval rearing tanks. These predators proved to be a major threat to the tiny darter larvae, causing considerable mortality. Treatments aimed at eliminating them also eliminated nitrifying bacteria and consequently produced lethal nitrite concentrations, which resulted in the loss of most of one tank of larvae.

Finally, the goal of physically providing the darter larvae to the EPA's testing facility proved to be the greatest survivorship barrier of all. We didn't even attempt to ship larvae that were still too small to feed on *Artemia* nauplii, anticipating the great difficulties of maintaining larvae at this stage. But even 10+ day-old larvae proved to be virtually impossible to ship or transport. Nearly none survived our attempts! In our many experiences with similarly sized cyprinid larvae (such as Cahaba shiners), there has been insignificant mortality with the same handling procedures. Therefore, we initially concluded that the darter larvae have very strict microhabitat requirements, perhaps involving water movement. However, our attempts to improve water movement by adding an airstone during transport was also less than successful, even though shipping time was only approximately four hours driving time between facilities. Approximately 250 larvae were lost in these

failed shipping attempts, with the cause of the mortality still unknown. At this time, about 90 juveniles survive from the 2001 production.

Conclusions

Although unanswered questions remain as a result of this project, many others have been at least partially resolved, and culture techniques refined. Following initial problems with incubating darter eggs in 2001, we settled on a productive technique with relatively large water volumes (2-liter trays) with continuous, isolated acriflavin baths. Early larval food requirements for both the shiners and the darters did not appear to be as specific as first suspected, but ultrafine powdered dry foods were a poor "bridge" to live *Artemia* nauplii. Rotifers combined with the dry foods produced greater and more consistent survivorship. Successful simulation of natural larval microhabitat, particularly water movement and ambient illumination or background, appeared to be a key requirement for survivorship of the darter larvae. Subsequent to our hypothesis that these preferred conditions were the same as those in flowing pools in streams and rivers, we have observed other Percina larvae while snorkeling in this type habitat in the Little River and Citico Creek near Knoxville. The larvae were easily recognizable not so much by their physical characteristics as much as by their habit of holding position in areas of gentle current with bodies tilted head upward at about a 30° angle.

We have also applied the findings of this study to an unrelated darter species of the genus *Etheostoma*. The boulder darter, *Etheostoma wapiti*, produces similar, small, pelagic larvae (Rakes et al., 1999). After experiencing poor boulder darter larval survivorship (in trays) in previous years, we found that survivorship was greatly improved in black bucket setups, as described here. With additional experimentation, we also found that the boulder darter larvae survived even better in 76-liter aquaria with dark sides and gentle aeration/ water movement. We suspect that these techniques will be applicable for the propagation of many, if not all, *Percina* and *Etheostoma* (*Nothonotus*) species as well as any other larvae that are too small to take *Artemia* nauplii at first feeding. Ideas for some of these techniques were also developed from experience and published findings for marine fish larval culture.

Some of the observations of early life history characteristics described above may prove to be useful for taxonomic studies of these species and their relationships to others. Because of the paucity of such information for most nongame fish species,

we compared our observations, as much as possible, with other species that we have also observed in aquaria. For example, we have recently reared cyprinids from unknown egg clusters collected from two separate localities in the Tennessee River Drainage during May and June 2001. The juveniles reared were determined to be an undescribed species, *Notropis* sp., the "sawfin shiner." This is currently the only other small minnow that we are aware of that clusters eggs like we observed for Cahaba shiners. The eggs were also provided no parental care. The eggs are similar in size, appearance, and number in the cluster, but the "sawfin shiner" eggs were much harder, and were deposited in a crevice under a rock in a midstream glide.

Finally, there are a number of implications for management and conservation of wild populations of these species, which can be derived from our aquarium observations, as well as the EPA tests that were conducted. Examples can be as basic as the realization that high turbidity might impact reproductive behavior by interfering with visual cues, or that siltation might be especially problematic for adhesive Cahaba shiner eggs spawned in vegetation in relatively calm backwaters or river edges. More subtle considerations should include the effects of water level stability on the survivorship of Cahaba shiner eggs spawned in vegetation in shallow areas, the effects of various pollutants on the extent of spawning substrate vegetation, and the general effects of all aspects of water quality on the zooplankton that seem to be required by the larvae of both of these imperiled fish.

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