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Reproductive behavior, captive breeding, and restoration ecology of endangered fishes

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Synopsis

Species recovery efforts generally focus on in situ actions such as habitat protection. However, captive breeding can also provide critical life history information, as well as helping supplement existing or restoring extirpated populations. We have successfully propagated nine species in captivity, including blackside dace, spotfin chubs, bloodfin darters, and boulder darters. Threatened blackside dace, *Phoxinus cumberlandensis*, were induced to spawn in laboratory aquaria by exposing them to milt from a reproductively mature male stoneroller, *Campostoma anomalum* or river chub, *Nocomis micropogon*. The latter are nest-building minnows, with which *Phoxinus* may spawn in nature. Eggs are broadcast among gravel and pebbles. Blackside dace individuals reared in captivity were used for translocation. Threatened spotfin chubs, *Cyprinella monacha*, fractional crevice spawners, deposited eggs in laboratory aquaria in the spaces created between stacks of ceramic tiles. Captively produced spotfin chubs were used as part of a larger stream restoration and fish reintroduction project in the Great Smoky Mountains National Park. The bloodfin darter, *Etheostoma sanguifluum*, was first used as a surrogate to develop techniques for spawning a closely related species, the endangered boulder darter, *E. wapiti*. Both darter species mated in a wedge created between two ceramic tiles. Our efforts have had variable but generally high success, with survival rates of 50–90% of eggs deposited. Captive production of nongame fishes can aid recovery of rare species or populations, aid in watershed restoration, and can help to refine water quality standards. In addition, captive breeding allows discovery of important behavioral or life history characteristics that may constrain reproduction of rare species in altered natural habitats.

Introduction

Although darters (Percidae), madtom catfishes (Ictaluridae), and minnows (Cyprinidae) dominate lists of rare fishes from the southeastern United States, public and financial support for conservation efforts for these species has been sparse. As a consequence, until recently, only a few federally listed fishes in the southeastern U.S. have been the object of active recovery efforts. Although populations of rare fishes can be restored by moving individuals from one locality to another (e.g., snail darter, *Percina tanasi*, see Etnier & Starnes 1993; watercress darter, *Etheostoma nuchale*, U.S. Fish & Wildlife Service[1]; spring pygmy sunfish, *Elassoma alabamae* Jandebeur 1982, Mayden 1993), many fishes most in need of conservation exist

[1] U.S. Fish & Wildlife Service. 1992. Watercress darter (*Etheostoma nuchale*) (second revision) recovery plan. U.S. Fish & Wildlife Service, Southeast region, Atlanta. 14 pp.

in populations that are too small to supply individuals for reintroduction or translocation projects. In addition, because most such rare species are not economically or recreationally important, few incentives exist to develop techniques for captive propagation. Recently, however, several captive propagation and reintroduction projects have been initiated and appear successful, including reintroductions of the endangered smoky madtom, *Noturus baileyi*, and duskytail darter, *Etheostoma percnurum*, and threatened yellowfin madtom, *N. flavipinnis* (Rakes et al.[2]). This paper presents preliminary results of projects to captively propagate three rare southeastern fishes with different spawning behaviors. These include a crevice spawner, nest associate, and nest guarding egg-clumper (see also Johnston 1999 this issue).

Destruction and re-establishment of southeastern fish communities

In September 1962, personnel from Utah, Wyoming, and U.S. Federal agencies removed nongame 'trash' fishes from a section of the Green River as part of a trout stocking program in the soon-to-be-created Flaming Gorge Reservoir. Removal involved poisoning a nearly 600 km stretch of the river with rotenone. In the process, they exterminated several populations of now endangered Colorado River fishes (see Pister 1999 this issue).

Similar events on a smaller scale had earlier transpired in Abrams Creek, Tennessee, when in 1957 a reclamation project (Lennon & Parker[3]) permanently eliminated nearly half of the 63 fish species (Simbeck 1990). As was an accepted practice during that time, ichthyocides were used to eliminate all fish in a reach of Abrams Creek extending 19.4 km upstream from its conjunction with the newly created Chilhowee Reservoir on the Little Tennessee River. The objective was to improve trout fishing. Some fishes have subsequently reinvaded Abrams Creek, but four species that are now federally listed as endangered or threatened have been permanently extirpated because of the impassable impoundment that separates Abrams Creek from any other stream communities in the Little Tennessee River system. These imperiled species include the endangered smoky madtom, *Noturus baileyi*, and duskytail darter, *Etheostoma percnurum*, and threatened yellowfin madtom, *N. flavipinnis*, and spotfin chub, *Cyprinella monacha*.

Federal recovery plans for these fish species called for restoring extirpated populations. Because most of the watershed of Abrams Creek is located within a national park, the stream was considered to be a good candidate for a first attempt at reintroducing all four species. Conservation Fisheries Inc. (CFI, the nonprofit organization directed by the first two authors of this paper) was contracted by the U.S. Fish & Wildlife Service (FWS) and the Tennessee Wildlife Resources Agency (TWRA) to propagate and reintroduce rare fishes into Abrams Creek.

Two additional, federally listed species have recovery plans that call for reintroductions and restoration of extirpated populations. These are the blackside dace, *Phoxinus cumberlandensis*, and boulder darter, *Etheostoma wapiti*. CFI was first contracted to develop propagation techniques for the dace in 1993 for unspecified future restoration work to aid recovery of this species, which has been impacted by coal strip mining. The boulder darter has been adversely affected by impoundments, water pollution, and agricultural impacts. Water quality improvement in a formerly occupied stream, Shoal Creek, may present an opportunity for reintroduction.

Blackside dace

The blackside dace is a schooling minnow that inhabits small streams in the upper Cumberland River drainage in southeastern Kentucky and northeastern Tennessee (Burr & Warren 1986, Etnier & Starnes 1993). Many of the streams within the species' range have been degraded by surface mining for coal (Starnes & Starnes 1978a, O'Bara 1990), which resulted in its listing as a threatened species in 1987 (Biggins 1987). Although the species is relatively prolific (females can produce more than 1500 eggs, Starnes & Starnes 1981), blackside dace spawn only in association with pebble nest building minnow species such as stonerollers, *Campostoma anomalum*, river chubs, *Nocomis micropogon*, or creek chubs, *Semotilus atromaculatus*, (Cicerello & Laudermilk 1996, Starnes & Starnes 1981). Clean, rocky substrates and nest associates are necessary for successful reproduction. Despite the protection of the

[2] Rakes, P.L., P.W. Shute, K.L. Harpster & J.R. Shute. 1996. Captive propagation and population monitoring of rare southeastern fishes by Conservation Fisheries, Inc. Unpublished 1995 final report to U.S. Forest Service, Cherokee National Forest, 7 February 1996. 33 pp.

[3] Lennon, R.E. & P.S. Parker. 1959. The reclamation of Indian and Abrams creeks, Great Smoky Mountains National Park. U.S. Fish & Wildlife Service Special Scientific Report 306. 22 pp.

Endangered Species Act (ESA), various blackside dace populations have fluctuated greatly over the past decade (Starnes & Starnes 1978b, O'Bara 1990, R. Cicerello personal communication).

Strange & Burr[4] analyzed genetic variation within and between populations of blackside dace. Their genetic information indicated that migration is important in maintaining blackside dace populations. The relative mobility and fecundity of the species allows it to recolonize formerly degraded habitats. If water quality or habitat conditions are not favorable for a time, this opportunistic species can apparently recolonize these areas when conditions improve. However, a sufficient number of protected refugia and suitable dispersal corridors must be available. Strange & Burr's[4] recommendations emphasized protecting or restoring dispersal corridors as well as protecting individual populations. They further suggested that, if natural reinvasions of restored streams are not possible, reintroductions may be necessary. They cautioned that, because of some genetic differences, particular care should be taken to manage metapopulations separately.

In 1993, in cooperation with the FWS, TWRA, and Kentucky Department of Fish & Wildlife Resources, we obtained 12 pairs of adult blackside dace from Buck Creek, Whitley County, Kentucky. The fish were part of a stock that was isolated upstream of a coal company's settling pond. Because the stock was aging and had shown no evidence of reproduction in the portion of the stream upstream of the barrier pond, the FWS was removing all individuals and translocating them to nearby streams in the same system. This provided an opportunity to obtain specimens for developing captive propagation techniques. The recovery plan for blackside dace suggested that captive propagation might be necessary (Biggins[5]). However, there had been no previous attempts to produce this species in captivity. With genetic information to guide the effort, captively-produced individuals might then be used to restore extirpated blackside dace populations in reclaimed streams.

[4] Strange, R.M. & B.M. Burr. 1995. Genetic variability and metapopulation dynamics in the federally threatened blackside dace, *Phoxinus cumberlandensis* (Pisces: Cyprinidae). Unpublished final report to Kentucky Department of Fish & Wildlife Resources, Frankfort. 21 pp.

[5] Biggins, R.G. 1988. Recovery plan for blackside dace (*Phoxinus cumberlandensis*). U.S. Fish & Wildlife Service, Southeast Region, Atlanta. 19 pp.

Spotfin chub

The spotfin chub historically occurred throughout most of the Tennessee River drainage (Jenkins & Burkhead 1984). Currently, extant populations exist in four tributary stream systems, with records of many extirpated populations, including one in Abrams Creek in the Great Smoky Mountains National Park, Blount County. The species was listed as Threatened in 1977 (U.S. Fish & Wildlife Service 1977).

In 1988 and 1989, David Etnier (University of Tennessee), FWS and National Park Service (NPS) personnel, and North Carolina and Tennessee Wildlife Resources agencies collected spotfin chubs from the Little Tennessee River in North Carolina and transported them to Abrams Creek. In 1992, this effort ceased because only one spotfin chub had been seen at any of the Abrams Creek reintroduction sites since stocking. It was decided that any further stockings would involve captively produced individuals.

Boulder and bloodfin darters

The boulder darter is presently limited to a few scattered localities over about 96 river kilometers of the mainstem Elk River in southern Tennessee and northern Alabama (Etnier & Williams 1989). Because of its currently restricted range and apparent habitat specificity, *Etheostoma wapiti* was listed as an endangered species in 1988 (Biggins 1988).

Boulder darters were historically known from Shoal Creek, which enters the Tennessee River in northern Alabama (into Wilson Reservoir) downstream from the mouth of the Elk River. The species is believed to have been extirpated from Shoal Creek because of water pollution (Etnier & Williams 1989). As a result of water pollution controls and an upgraded wastewater treatment facility in Lawrenceburg, the water quality of Shoal Creek has improved (J. Layzer personal communication). If habitat quality and stability in this stream also improve sufficiently, boulder darters could be reintroduced into Shoal Creek. Augmentation of the Elk River population, especially at sites where rock has been added to create new spawning habitat, is also planned (R. Biggins personal communication).

The boulder darter recovery plan (Biggins[6]) recommended developing techniques for captive propagation. In cooperation with TWRA, FWS, and the Tennessee

[6] Biggins, R.G. 1989. Recovery plan for boulder darter (*Etheostoma* sp.). U.S. Fish & Wildlife Service, Southeast Region, Atlanta. 15 pp.

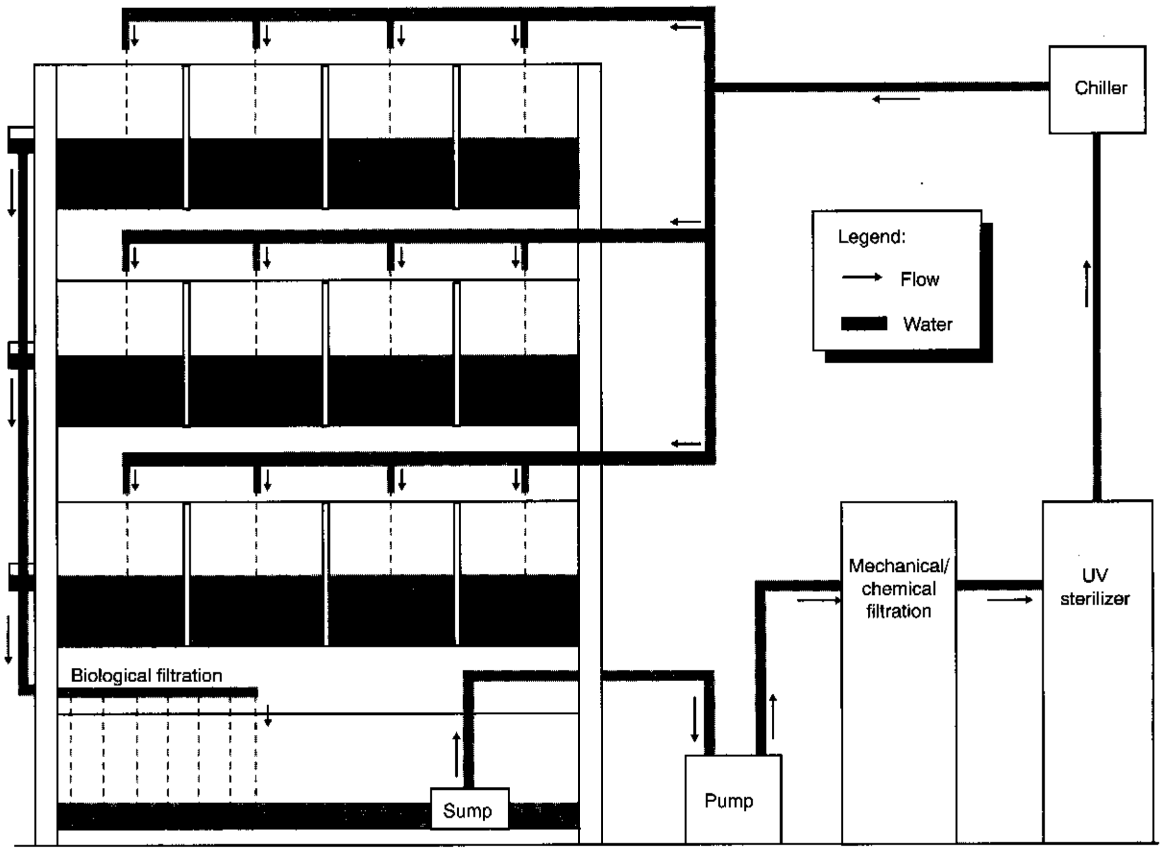

Figure 1. Annotated diagram of central aquarium system used for breeding, rearing, and maintaining rare fishes.

Valley Authority (TVA), we initiated a project to develop these techniques to support reintroduction attempts while minimizing impacts on natural populations. Because of the rarity of boulder darters, propagation techniques were developed using a closely related surrogate species, the bloodfin darter, *Etheostoma sanguifluum*. Bloodfin darters were used because they and boulder darters both belong to the *Etheostoma maculatum* group of darters (Etnier & Williams 1989), whose reproductive behavior is described as egg-clumpers (Page et al. 1982).

Materials and methods

Aquarium facility and husbandry of captive fishes

Wild fish were transported to the CFI facility and slowly acclimated to laboratory aquaria. CFI, located in Knoxville, uses deionized water produced by reverse osmosis filtration mixed with dechlorinated tap water and buffers to mimic pH and hardness of natural waters.

The aquarium systems at CFI have evolved since rare fish propagation efforts began in 1986 (Shute et al.[7], Rakes et al.[2]). Fish were kept in one of two multiple-aquaria, centralized systems of 900 or 2400 l (Figure 1). Water temperatures and photoperiod were changed gradually from a wintertime low of 12°C to a high of approximately 26°C to mimic natural conditions as closely as possible. Tanks containing breeding adults, juveniles, or older propagated fish contained

[7] Shute, P.W., P.L. Rakes & J.R. Shute. 1992. Status report and historical review of reintroduction efforts for the endangered smoky madtom (*Noturus baileyi*) and threatened yellowfin madtom (*Noturus flavipinnis*). Unpublished report to U.S. Forest Service, Cherokee National Forest, U.S. Fish & Wildlife Service, Asheville Field Office, Tennessee Wildlife Resources Agency, National Park Service, Great Smoky Mountains National Park, 15 October 1992. 51 pp.

mixed gravel substrates. In addition to spawning substrates described later, slabs of black Plexiglas, sections of PVC pipe, cobbles, and larger natural rocks (slabrocks) were provided as cover objects. Tanks with eggs or embryos had no substrate.

Eggs were placed and incubated in plastic trays (Figure 2) through which system water circulated. When hatchlings were large enough (which depended upon species and size of clutch or number of hatchlings), the box was removed and the young fish were released into the aquarium beneath or distributed to other aquaria in the same central system.

After hatching and upon absorption of yolk, larvae were fed early instar crustaceans, including copepods, ostracods, a dry artificial (rotifer size) plankton preparation by Ocean Star International®, first instar mosquito larvae, and newly hatched *Artemia* nauplii. Juveniles and adults were fed a variety of live and frozen foods, including live blackworms (aquatic oligochaetes), frozen bloodworms (chironomid larvae), frozen and live *Daphnia*, and several prepared foods. Initially, the young were fed two or three times a day. When they were able to eat whole worms or brine shrimp, they were fed once daily.

Blackside dace

Twenty-four mature adult blackside dace (12 females, 12 males) were collected by seine from Buck Creek, Hickory Creek system of the Cumberland River, Whitley County, Kentucky on 7 May 1993.

The dace were placed in two 75 l aquaria in the large system (Figure 1) and maintained at 16°C. One aquarium contained eight males and four females and the other contained four males and eight females. To simulate the pebble and cobble nests over which blackside dace mate, the aquaria contained artificial 'chub nests', consisting of a mound (approximate 20 cm wide × 30 cm long × 5 cm high) of smooth river pebbles (<3 cm diameter) and gravel. Flowing water was directed over these mounds in an attempt to mimic natural creek conditions.

Johnston (1991, 1994a,b, and personal communication) suggested that a pheromone from a reproductively active, nest-building chub or stoneroller might trigger spawning in the associating species. Therefore, when blackside dace were maintaining reproductive condition but had ceased active spawning activity, all the males were moved to the aquarium with eight females

Figure 2. Trays used for incubating blackside dace, spotfin chub and boulder darter eggs. Trays were suspended in 75 l aquaria. Note water supply to trays from overhead bars, and outflow through mesh screens in side of trays.

and milt from tuberculate male river chubs, *Nocomis micropogon*, and/or northern stonerollers, *Campostoma anomalum*, was stripped into the aquarium to induce further spawning. In addition, the stoneroller was maintained in a separate, overhead aquarium and water from this aquarium was constantly trickled into the dace tank below.

After successful spawning, eggs were removed from the tank by vacuuming through the artificial pebble nests with an aquarium-cleaning siphon so that only eggs and detritus were removed; water was siphoned into a 20 l bucket. The eggs and debris were then collected by pouring the bucket contents through a fine-mesh dipnet. Eggs were then transferred with a pipette to the incubation trays described above.

Spotfin chub

Nine immature spotfin chubs were collected in fall 1992 and summer 1993 and housed in a 380 l aquarium in the smaller central system (Figure 1). Burkhead & Jenkins (1991) and Jenkins & Burkhead (1993) described spotfin chubs as crevice spawners, eggs being deposited in crevices in rocks on the stream bottom. A variety of potential spawning substrates was provided, including natural boulders and flat rocks; broken shards of terra cotta flower pots; and stacks of eight to fifteen 15.3 × 15.3 × 0.5 cm unglazed ceramic tiles (Figure 3), modified from Snyder (1993). Projections on the tiles acted as spacers between the tiles, forming crevices approximately 2–3 mm wide. These structures were examined periodically for egg deposition.

Because the first eggs produced by captive spotfin chubs were attached to rocks too large to remove easily from the aquarium, the eggs were carefully scraped into the plastic flow-through incubation trays described above. Later egg depositions were placed between the ceramic tiles, and individual tiles with eggs attached were removed to the incubation trays. When all embryos had hatched, the tiles were removed. The embryos and larvae were maintained in these trays for approximately 7–10 days at 26°C. They were then transferred to the 75 l aquaria.

Bloodfin and boulder darters

Eleven bloodfin darters were collected from the Big South Fork of the Cumberland River at the mouth of Station Camp Creek in October 1993. Two large males (>60 mm TL) and two females (approximately 50 mm TL) were placed in a 380 l aquarium in the smaller central system (Figure 1).

Several potential spawning substrates were provided, including natural boulders and flat (slab) rocks collected from the river; broken shards of terra

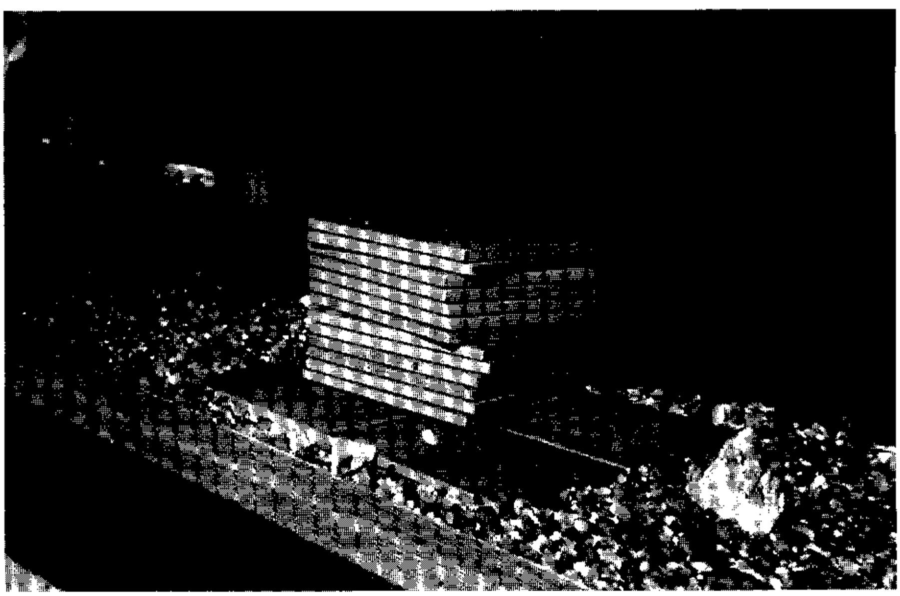

Figure 3. Stack of unglazed ceramic tiles used as spawning substrate by threatened spotfin chubs in laboratory aquaria.

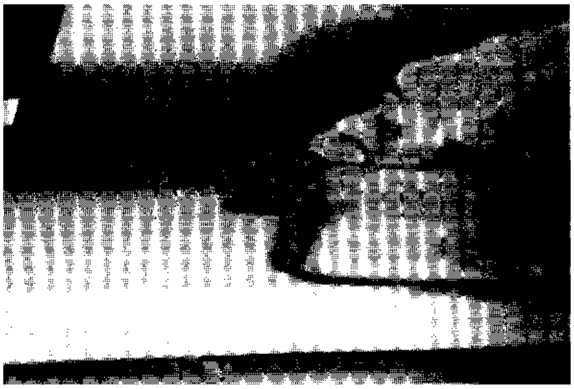

Figure 4. Unglazed ceramic tiles used as spawning substrate by endangered boulder darters in laboratory aquaria. Note male boulder darter guarding eggs in the wedge between two tiles.

cotta flower pots; and the 15.3 cm square, unglazed ceramic tiles described above, all positioned to form acute angles along upstream edges (as suggested by N. Burkhead personal communication). These structures were examined periodically for egg deposition. Eggs were placed only between tile wedges (Figure 4). These tiles were carefully removed and the entire tiles placed in trays, or eggs were scraped with a razor blade from the tile and placed in the trays.

Three boulder darters (one female and two males) were collected from the Elk River, Lincoln Co., on 20 September 1994. As described above for bloodfin darters, several potential spawning substrates were provided for the boulder darter. A pair of the unglazed ceramic tiles described above were placed together to form an acute angle (approximately 35–40°, Figure 4) near an area of current, with the opening facing downstream to mimic the spawning sites that had been selected by bloodfin darters. When eggs were discovered, the tiles with eggs attached were removed and were replaced with fresh tiles. The egg-containing tiles were then placed in incubation trays and reared as described above for bloodfin darters.

Results

Blackside dace

Approximately 300 eggs were recovered from the pebbles and gravel in the artificial nest in the aquarium containing eight males and four females three days after the fish were introduced. All appeared to have been deposited in a single, brief episode; about 230 were fertile. Only one egg was recovered from the other aquarium housing eight females and four males. Thereafter, all the males were moved to the tank with eight females.

Exposing blackside dace to milt from nest-building cyprinids, two weeks from the initial aquarium spawning, resulted in additional spawning activity. Two days after introducing milt we observed intensification of breeding colors. Four days later, we recovered 500 more eggs from a mating episode, but only about 100 of these were fertile. All the adult dace died within three days after spawning. No mortality occurred in the adjacent tank containing only females.

In October 1993, 260 individuals were released at two localities within the Buck Creek watershed (Shute et al.[8]). The success of these reintroductions has not been determined. Table 1 describes characteristics of blackside dace eggs, embryos, and larvae, and provides details on rearing and release.

Juveniles maintained as part of the captive population reached adult size of approximately 55–60 mm TL by the age of one year (spring/summer 1994) and exhibited secondary sexual characters such as spawning coloration. They were moved to an independent 380 l aquarium and exposed to water from a tank containing a male river chub. No reproductive behavior was displayed by any captive one-year-olds.

In May 1995, the now 2-year-old dace were transferred to a 380 l aquarium containing a nuptial male river chub. This aquarium was part of a 900 l multiple aquarium system (Figure 1). Current was maintained by multiple pumps, and rocky substrates of varying sizes were provided to simulate a small, flowing stream. The river chub was confined to about half of the tank by a coarse plastic grate, through which the dace could pass freely. Four days after fish introduction, we collected about 125 eggs from the rocks, of which less than half were fertile. From these, approximately 40 F_2 individuals were reared to adulthood and are now also maintained as part of the captive stock. We currently (February 1998) maintain approximately 12

[8] Shute, J.R., P.W. Shute & P.L. Rakes. 1993. Captive propagation and population monitoring of rare southeastern fishes by Conservation Fisheries, Inc. Unpublished 1993 final report to Tennessee Wildlife Resources Agency for fiscal year 1992–1993 (Contract # FA-2-09667-2-02) and first quarter report for fiscal year 1993–1994 (Contract # FA-4-10792-4-00), 29 November 1993. 27 pp.

Table 1. Characteristics of captively propagated species.

Characteristic	*Phoxinus cumberlandensis*	*Cyprinella monacha*	*Etheostoma sanguifluum*	*E. wapiti*
Egg size (diameter)	1.0 mm	1.8–2.0 mm	1.6–1.8 mm	1.6–1.8 mm
Egg characteristics	demersal, non-adhesive	adhesive	adhesive	adhesive
Deposition site	among gravel & pebbles of artificial minnow nest	basal crevice (between tile and substrate)	clumped in 30–40° wedge between tiles	clumped in 30–40° space between tiles
Incubation time	—	6 days (25°C)	—	10 days (19°C)
Hatchling size	5 mm TL	9–10 mm TL	8–9 mm TL	8–9 mm TL
Characteristics of embryos and larvae	benthic approx. 48 h	negatively buoyant, benthic approx. 30 days	positively phototactic & pelagic 10–30 days	positively phototatic & pelagic, negatively buoyant and benthic at 10–30 days
Foods used (see below) by larvae	A, B, C	B	B, C	B, C
No. fertile eggs produced	330	2,300	50–60	274
No. juveniles produced	281	2,200	15	135
% survivorship	87	95	25	49
No. released into natural stream	260	2,175	15	120
Size at release	30–40 mm TL	20–45 mm TL	55 mm TL (5 months old)	none released
No. maintained in captive stock	12	~ 25	0	7

A = live copepods; B = brine shrimp nauplii; C = commercially-prepared rotifer-sized powdered food.

F_2 offspring. The F_1 suffered senescence and mortality; the captive F_2 are approximately four years of age.

Spotfin chub

Spotfin chubs maintained in the CFI facility were not sexually mature until summer 1994, at an estimated age of two years. The first spotfin chub eggs discovered were wedged between two large, flat rocks. As described earlier, eggs were also deposited in the crevices in stacks of ceramic tiles, but exclusively in the bottom crevice adjacent to the floor formed by a larger tile or group of tiles. Male chubs seemed to select spawning sites, and would often appear to be depositing milt prior to the arrival of the females by swimming slowly and quivering along the length of the crevice. Usually, only one dominant male was active at the spawning site and the three subordinate males remained away from the spawning arena. Females would approach and either be courted or chased away by the attending male.

The actual mating appeared to last only a fraction of a second and was difficult to observe. As the female approached, the male quickly swam down beside her, quivered briefly as they both pressed their vents against the crevice, and then he swam back to his position above the spawning site. This appeared to be the time of egg deposition. The females injected the eggs far enough into the crevice to completely cover the underside of the ceramic tiles. Neither parent provided care of eggs or nest site once spawning was completed. In fact, unless they were mating, the males usually ignored female chubs or other fish that approached the nest site. If eggs were exposed, they were quickly cannibalized by both sexes. Young chubs grew rapidly, maintaining benthic behavior until about 30 days of age and 18 to 23 mm TL, when they began to shift to the water column. Table 1 provides brief descriptions of spotfin chub eggs, embryos, and larvae.

Spawning occurred from 13 July through 13 September 1994. For four months prior to spawning, the chubs were maintained at a photoperiod of 15 h daylight and water temperatures between 20 and 24°C. Immediately prior to aquarium spawning, we increased the food volume and the feeding frequency to 2–3 times per day. Spawning ceased only after photoperiod was shortened to 13 h daylight and feeding volume and frequency were decreased.

In November 1994, 700 captively produced young and the nine adults that had produced them were released into Abrams Creek. Most of the remaining 1500 were released into Abrams Creek in April 1995 (Rakes et al.[2]). Twenty-five of these young are still part of the captive stock at the CFI facility, along with nine additional wild-captured individuals collected in summer 1996 (Table 1).

Bloodfin and boulder darters

In 1994, the two female bloodfin darters mated with one male in approximately 30–40° angled crevices formed by two unglazed ceramic tiles. Table 1 describes characteristics of bloodfin darter eggs, embryos, and larvae, and provides details on rearing and release. Approximately 20 eggs were produced per mating event. The eggs were clumped together, wedged into the crevice, and attached to the tile and/or neighboring eggs. Successive depositions of eggs were placed on top of eggs produced earlier, possibly by both females. In one instance, eggs in a nest removed from the nest-guarding male had embryos hatching synchronously. All embryos hatched within a 24 h period, despite at least two days difference in mating times of two separate groups of eggs.

Hatchling bloodfin darter embryos were positively phototactic and strongly pelagic. This behavior continued for 5–21 days, after which young settled to the bottom, assumed the benthic behavior typical of the adult darters, and developed dark lateral pigment. Survivorship of captively-reared bloodfin darters was low, due to high mortality of larvae. We were unable to provide a sufficient quantity and quality of the minute food particles required by the very small gape size of these larvae at first, but later found that a commercially prepared dry, rotifer-size food was a good substitute for live foods. Bloodfin darters were 55 mm TL at the time of their release in the Big South Fork at the age of five months (Table 1). Based on our success with bloodfin darters, we recommended that an attempt be made to captively propagate boulder darters using similar techniques.

Following photoperiod and temperature acclimation, boulder darters collected in fall 1994 became reproductively active in CFI aquaria in April 1995. The one relatively small (approximately 45 mm TL) female and the largest male mated. Eggs were placed in the same type of cavity beneath unglazed ceramic tiles as described for bloodfin darters. Although 16 eggs were produced from three separate matings, only nine were viable. Boulder darter young fed at the surface or in the water column for 12–20 days, before transforming to an exclusively benthic form. The five boulder darter juveniles measured between 35 and 50 mm TL on 31 December 1995; these individuals are currently maintained as part of a captive boulder darter stock at the CFI facility.

In May 1996, the same pair of adult boulder darters again mated, this time producing four clutches of eggs over a 30 day period. Females laid 20–30 eggs per mating. The largest clutch guarded by the male consisted of about 120 eggs, deposited in at least four matings; eggs from each mating were attached in discrete aggregations, sometimes adjacent to and sometimes on top of older eggs. The male also mated with one of his female offspring to produce an additional 150 eggs. These were produced in five separate clutches over a twelve day period.

The first and last clutches of the oldest female and the last clutch of the younger female contained only infertile eggs. Each of the remaining clutches also contained from 5–10 infertile eggs; thus, the two females together produced about 265 fertile eggs of approximately 375 deposited. If left undisturbed, dead or infertile eggs were removed from the clutches, presumably by the nest-guarding male. No mortality was noted in normally developing eggs (Table 1). As observed with bloodfin darter embryos, virtually all losses occurred in the first few days after hatching. More than 50% of these larvae required 30 days to transform to benthic juveniles, and a few were pelagic for as long as 45 days. Table 1 describes bloodfin and boulder darter eggs, embryos, and larvae, and provides details on rearing and release.

Discussion

Our work has demonstrated the feasibility of captive propagation of stream fishes with divergent spawning behaviors. In addition, observations on early life history of these three species, including egg and characteristics and behaviors of embryos and larvae, have implications for conservation or management of these and similar species.

Blackside dace

We consistently found many infertile eggs after blackside dace matings. Given the high proportion of these in all clutches, we speculate that they may be selectively advantageous and not just a result of poor physiological condition. Perhaps these 'dummy' eggs dilute the total number of eggs in the nest, thereby decreasing the probability of predation on viable, fertile eggs. Johnston (1991, 1994a,b) suggested that nest associations among several fishes, including minnows, could be a mutualistic relationship that was beneficial for the associates due to a similar dilution effect. However, we are not aware of species that use infertile, possibly dummy eggs for such dilution.

We hypothesized that a chemical cue from a nest-building minnow may trigger blackside dace spawning. Our results support this hypothesis. Rice (1996) described spawning success in captive southern redbelly dace, *Phoxinus erythrogaster*, in which the spawning trigger was an abundance of high-quality food and the presence of red-colored individuals of another species. Our single observation of spawning in an aquarium with eight male dace, but not in one with four, suggests that a minimum number of reproductively active male blackside dace is necessary to induce spawning in other members of the species. It is possible that the combined effects of chemical cues from a nestbuilder plus a critical number of active males in breeding color are required to trigger normal spawning activity in this species.

Our experiences with captive blackside dace have several implications for conservation. In nature, the small, negatively buoyant eggs produced by blackside dace probably sink into gravel crevices. Our observations indicate that free embryos remain in gravel crevices for up to two days. Eggs, embryos, and larvae could therefore be highly susceptible to being smoothered by sediment. In streams that have been heavily subjected to strip mining, siltation may have led to the decline of blackside dace.

Spotfin chub

Our observations suggest similar consequences for spotfin chub. Despite relatively high reproductive output, the current distribution of the species is fragmented, occupying only a fraction of its historical range (Jenkins & Burkhead 1984). Spotfin chubs apparently prefer to mate only in the lowermost crevices, usually adjacent to the substrate, and the larvae are benthic. The current distribution of the species may be limited by the availability of suitable spawning substrates in relatively unsilted streams. For comparison, other, more abundant

Cyprinella species frequently spawn in woody debris or in crevices in other substrates that are off the bottom (Gale 1986, Snyder 1993, Vives 1993, Johnston & Shute 1997, Johnston 1999 this issue).

Bloodfin and boulder darters

Based on our aquarium observations, in the wild boulder darter embryos may drift in stream currents for many days, and be transported far downstream of their spawning sites by the time they assume their typical benthic lifestyle. The lack of suitable clean, unsilted, benthic habitats downstream of spawning areas in the Elk River could therefore have contributed to the local decline of the species. A broader, riverine ecosystem perspective may be needed to manage and insure survival of boulder darters. Freeman & Freeman (1994) reached similar conclusions with regard to conservation of the endangered amber darter, *Percina antesella*.

Conclusions and recommendations

Because many southeastern fishes are currently on the brink of extinction (Etnier 1994, Warren et al. 1994), we suggest that a proactive approach to protecting or conserving our sensitive southeastern fishes is required to offset a potentially drastic extinction crisis. As Meffe (1987) and Meffe & Vrijenhock (1988) recommended, regional facilities to preserve living and frozen gene pools are needed. In addition, captive propagation techniques need to be developed for relatively common representatives of as many taxonomic groups as possible. Common species can be used as surrogate species for closely related but rare fishes.

Life history or other ecological information essential in conserving rare species is often lacking or incomplete (Tear et al. 1995). This information is necessary to insure that habitat and community composition is appropriate at sites where reintroductions are planned. Although habitat preferences of adult boulder darters are a known limiting factor for the species, long-term survival of the only known, Elk River population of this rare fish may also hinge on behavior of embryos, larvae, or juveniles. If boulder darter embryos float downstream, as suggested by our captive rearing, current rarity of the species may result from a lack of appropriate habitat on which juveniles can settle and become benthic. Our observations indicate that spotfin chub larvae and juveniles are benthic, and that the spawning adults apparently prefer to deposit eggs in crevices on the stream bottom. Some historic populations may therefore have been extirpated because early life history intervals could be especially vulnerable to siltation. The prolific spawning capacity of the species may explain its apparent ability to reinvade suitable habitats when conditions are good, as has apparently taken place in the Holston River system (Boles[9]).

Species recovery programs are dependent on information integrated across many disciplines. Successful captive propagation efforts depend on taxonomists to produce accurate phylogenies, and field biologists to investigate and describe life history and other ecological information (Raven 1992, Noss 1994, Mayden 1992). The appropriate surrogate species for the boulder darter may not have been chosen and a spawning substrate appropriate for captive production of the crevice-spawning spotfin chub may not have been designed without accurate phylogenetic information. Similarly, the potential of sperm or pheromones from nest-building minnows may not have been suggested as a cue to trigger captive spawning of blackside dace without life history information. The current trend at universities is to de-emphasize field research and systematics in favor of more empirical laboratory research. New incentives need to be developed to attract professionals and students to more traditional research areas because systematics and field biology are so important in supporting the conservation of rare fishes.

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[9] Boles, H. 1983. Recovery plan for the spotfin chub *Hybopsis monacha*. U.S. Fish & Wildlife Service, Atlanta. 46 pp.

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