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Reproductive biology and spawning habitat supplementation of the relict darter, *Etheostoma chienense*, a federally endangered species

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Synopsis

We investigated the reproductive biology of *Etheostoma chienense* in the Bayou du Chien drainage of western Kentucky. *Etheostoma chienense* is similar in its ecology and reproductive biology to other members of the *E. squamiceps* complex. However, *E. chienense* is opportunistic in its choice of egg deposition substrates, as nests were found on a variety of natural and anthropogenic items. Due to an apparent lack of suitable spawning substrates, we added half-cylindrical ceramic tiles to several stretches of stream to increase potential nest productivity. Egg-clutches attached to artificial substrates were twice the size of egg clutches attached to naturally occurring materials. Laboratory experiments were conducted to determine nest rock size and mate choice preferences. Both the size of the male and of the nest rock appeared to be important parameters to spawning females. The life history information gathered here has significant management implications. Artificial spawning substrates should be placed in appropriate microhabitats (i.e., shallow, low-flow reaches in headwaters). Nest cavity vertical height should be about 3.0 cm and tiles should be spaced at least 0.5 m apart. We feel the use of surrogates (e.g., *E. oophlyax*) to investigate other types of spawning cover, and restoration of riparian buffer zones among other actions, would particularly benefit recovery efforts for this endangered species.

Introduction

The relict darter, *Etheostoma chienense* Page & Ceas, a recently described member of the *Etheostoma squamiceps* complex (subgenus *Catonotus*, family Percidae) (Page et al. 1992), is endemic to the Bayou du Chien River system of extreme southwestern Kentucky (Figure 1). The species is restricted to an approximately 35-km segment of the upper reaches of the system, primarily upstream of the Mississippi River floodplain (Burr & Warren 1996, Piller & Burr 1998). *Etheostoma chienense* and other species of *Catonotus* are unusual among percids in that all species engage in a complex form of reproduction referred to as egg-clustering (Page 1985). This specialization requires successful males to select appropriate nesting substrates, establish territories, court females, and fertilize and guard the eggs which are laid on the undersides of the nest substrate (usually a flat stone). Viable populations of species of *Catonotus* generally are associated with headwater reaches rich in nesting substrates. Cursory habitat assessments of Bayou du Chien indicated limited nesting resources throughout the drainage system. In a comprehensive conservation status review of *E. chienense* (Warren et al. 1994a),

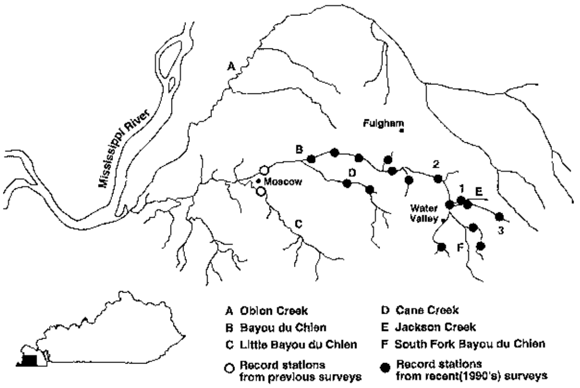

Figure 1. Historic and additional localities of occurrence sampled for nests of *Etheostoma chienense*, Bayou du Chien, western Kentucky, 1995–1996. Site numbers refer to locations of artificial spawning substrate placement. Site 1 = Jackson Creek at Lawrence Boyd Rd; Site 2 = Bayou du Chien at Rt. 1283; Site 3 = Bayou du Chien at 2422 Rd.

those cursory findings were verified and further revealed: (1) a paucity of information on the reproductive biology and ecology of the species; (2) that spawning apparently was limited to only one small reach of the system (i.e., Jackson Creek); and (3) that the addition of artificial or natural spawning substrates to selected stream reaches might enhance recruitment, survival, and dispersion. Because *E. chienense* has a localized distribution and because recruitment may be constrained by limited spawning substrates and a lack of microhabitat availability, this darter was listed as federally endangered on 27 December 1993 (Biggins 1993).

With support from the Kentucky Department of Fish and Wildlife Resources and the U. S. Fish and Wildlife Service, a research program was established to quantify population sizes at selected stream reaches and to investigate various aspects of the reproductive biology of *E. chienense*. Our specific objectives here were to: (1) determine if spawning occurs in more than one reach of Bayou du Chien; (2) investigate the efficacy of artificial spawning covers for maximizing egg numbers; and (3) conduct laboratory experiments on nest rock size and mate choice preferences in an effort to provide for informed management recommendations.

Materials and methods

Study area

The upper two-thirds of the Bayou du Chien drainage formed the study area (Figure 1). Piller & Burr (1998) found relict darters at 16 sites within the drainage, a significant increase over the 9 sites reported by Webb & Sisk (1975) and Warren et al. (1994a). In prehistoric times, *Etheostoma chienense* may have been more widespread in the Bayou du Chien drainage, but extensive channelization and removal of woody riparian vegetation in the early part of the century significantly reduced instream flows and the availability of instream cover and suitable spawning habitat. Bottom substrates today consist entirely of sand, gravel, cobble, and silt. Large slab rocks characteristically used by most species of *Catonotus* are absent from the drainage.

Reproduction: field studies

During the Spring of 1995 and 1996, we attempted to locate egg clutches by searching 200 linear meters of habitat at each known site of occurrence. Egg clutches (a cluster of eggs found on a given substrate) were found by overturning instream objects. Movable structures with egg clutches were briefly removed from the stream and eggs were either counted on site or photographed and then returned to the stream. We recorded the size of nest guarding males (mm SL), depth (cm) of the nest in the water column, distance (cm) of the nest off the bottom, nest substrate dimensions (cm^2), water velocity ($m\ sec^{-1}$) by floating and timing an object over a known distance (Orth 1983), and water temperature (°C). Substrate classifications follow Cumming (1962).

To increase potential nest sites and nest productivity, artificial spawning substrate was added to three stream reaches. Half-cylindrical ceramic tiles with a length of 14–16 cm and an inside horizontal diameter of 10 cm (Figure 2), were placed approximately 2–3 m apart on the stream bottom. Each tile was pressed one to two centimeters into the substrate and had an undersurface area of 132–150 cm^2. Sites were inspected twice a week during the spawn-

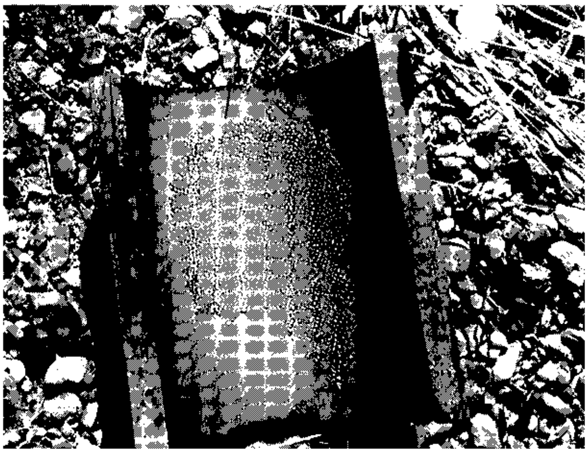

Figure 2. Ceramic tile usage as an artificial spawning substrate by *Etheostoma chienense* at Jackson Creek, 25 March 1995. Spawning substrate dimensions are 15 × 10 × 3 cm.

ing season (early March through early June). Tiles were photographed and darters beneath the tiles were captured with a hand net, sexed, measured, and released.

Seventeen to twenty tiles were placed at three separate localities (Jackson Creek at Lawrence Boyd Rd., Bayou du Chien, Rt. 1283, and Bayou du Chien 2422 Rd.) during March 1995 and 1996. If tiles became buried or washed downstream, they were replaced. Detailed descriptions of sites are provided in Piller & Burr (1998).

Egg clutches attached to artificial substrates were photographed on successive visits. This allowed a determination of both a maximum and average (mean clutch size per tile over the interval in which the tile had eggs) clutch size per tile. Maximum nest productivity (x_m) and average nest productivity (x_a) per tile were compared with the number of eggs found on natural substrates. Naturally occurring nests were photographed only once. To determine the success of the ceramic tiles for increasing nest productivity, we obtained a grand mean for each year by combining the results obtained from each site.

Reproduction: laboratory studies

Relict darters were transported from Bayou du Chien to the Vivarium at Southern Illinois University at Carbondale and were placed into one of 10 seventy-six liter aquaria. Aquaria had pea-sized gravel substrates and were maintained at 18–22 °C; photoperiod was controlled to match ambient conditions. Darters were fed a diet of frozen bloodworms (chironomids) and live California blackworms (oligochaetes). Behaviors were videotaped on several occasions. Individual darters were never used more than once in an experiment and all were returned to their streams of origin upon experiment completion.

Experiment 1 examined female choice of nest rock size. One male (57–71 mm SL) and one female (50–60 mm SL) were placed into each aquarium with two flat rocks of unequal size (mean difference = 48%). The amount of surface area of each rock was obtained with a polar planimeter. Large rocks had surface areas ranging from 206 to 230 cm^2 whereas small rocks ranged from 97 to 115 cm^2.

Experiment 2 investigated female mate choice. A large (68–72 mm SL) and a small (60–64 mm SL) male were added along with one female (48–62 mm SL) to each of 10 aquaria containing two ceramic tiles. A choice was considered to have been made when eggs were deposited under a tile and guarded by a male.

Statistical analysis

Statistical analyses were performed using the SIUC mainframe version of SAS (SAS 1985), following the procedures outlined in Zar (1996). Statistical significance was considered to be $p < 0.05$.

Results

Non-breeding habitat

Etheostoma chienense was captured in deep undercut banks with rooted vegetation in less than 0.5 m of water, but also was collected within 1–2 meters of the stream bank beneath instream objects such as leaf-litter, partially submerged logs and sticks, and human refuse. Individuals were rarely collected in mid-stream areas or riffle habitats. Apparently, the species limits its activities to undercut banks or other near-bank areas.

Sexual dimorphism

Females and non-breeding males were mottled brown and remained relatively unchanged throughout the year. Although nuptial males fail to develop bright chromatic colors characteristic of many other species of *Etheostoma*, several distinct changes occurred during the spawning season. The head and nape of territorial males became swollen and blackened. Males developed 7–8 alternating black vertical bars on the side of the body. The bars were located on the posterior half of the body beginning just below the origin of the second dorsal fin and proceeded posteriorly to the base of the caudal fin. In addition, a single intense vertical bar occurred just behind the opercular flap at the origin of the first dorsal fin. All fins with the exception of the pectorals became blackened while both the caudal and dorsal fins retained their transparent banding patterns. In early spring, males acquired swollen knobs at the tips of the second dorsal rays.

Table 1. Frequency of use of nest substrate types and mean clutch size (number of eggs/substrate) by *Etheostoma chienense*, Bayou du Chien drainage, western Kentucky, 1995–1996.

Substrate	1995		1996	
	No. of nests	Mean clutch size	No. of nests	Mean clutch size
Rock		330		189
Natural rock	25		9	
Limestone rip-rap	0		5	
Concrete		214		351
Concrete/cinder block	2		16	
Brick	1		2	
Wood		216		316
Live root	10		0	
Log/stick	14		29	
Bark	3		5	
Wooden board	0		5	
Rubber		239		498
Rubber tire	3		16	
Rubber mat	0		5	
Roofing paper/shingle	2	556	0	ND
Plastic	1	52	2	457
Blacktop aggregate	1	94	0	ND
Metal	5	445	4	289
Glass	0	ND	1	80
	67		99	

ND = No data.

Reproductive habitat and habits

Nests of *E. chienense* were found at 12 of the 16 known localities of occurrence. Six nesting localities were extremely degraded or lacked suitable habitat and yielded only 1 or 2 nests during the study. The remaining six sites, most of which have been partially modified by anthropogenic activities, contained an abundance of spawning habitat and thus yielded more nests. One site in particular, Jackson Creek, was highly productive in terms of the number of nests found. The pristine nature and plethora of spawning habitat undoubtedly makes Jackson Creek one of the primary spawning areas.

Nests were found 16 March to 8 June 1995 and 21 March to 24 May 1996 at water temperatures ranging from 11 to 22 °C. Sampling in 1996 ended 24 May. Nests were found at depths of 4.5–38 cm ($\bar{x}$ = 17) in slow to moderate flow ($\bar{x}$ = 0.40, range = 0.25–0.60 m sec^{-1}); nest cavities were 2–5 cm ($\bar{x}$ = 2.92) high. Nest-guarding males ($\bar{x}$ = 62.5, range 55–76 mm SL) were significantly larger than breeding females ($\bar{x}$ = 48.7, range 36–68 mm SL) (t-test; t = 18.74, $p < 0.0001$). In the field, nuptial males guarded eggs for 10–13 days at 20 °C and abandoned their nests soon after the embryos hatched. Freshly deposited eggs were a translucent golden-orange color and averaged 1.86 mm in diameter (1.7–2.0 mm, n = 35).

We found 166 nests on 15 different substrate types (Table 1). Small rocks, woody debris, and live tree roots were the dominant materials used. Nest sticks varied in diameter from 2–14 cm ($\bar{x}$ = 7.9 cm) (Figure 3a) and rocks had surface areas of 32–540 cm^2 ($\bar{x}$ = 166 cm^2). In addition to naturally occurring materials, 43% of *E. chienense* nests were attached to anthropogenic materials. Rubber tires were used most often, but other materials were used as spawning substrates including plastic (Figure 3b), roofing

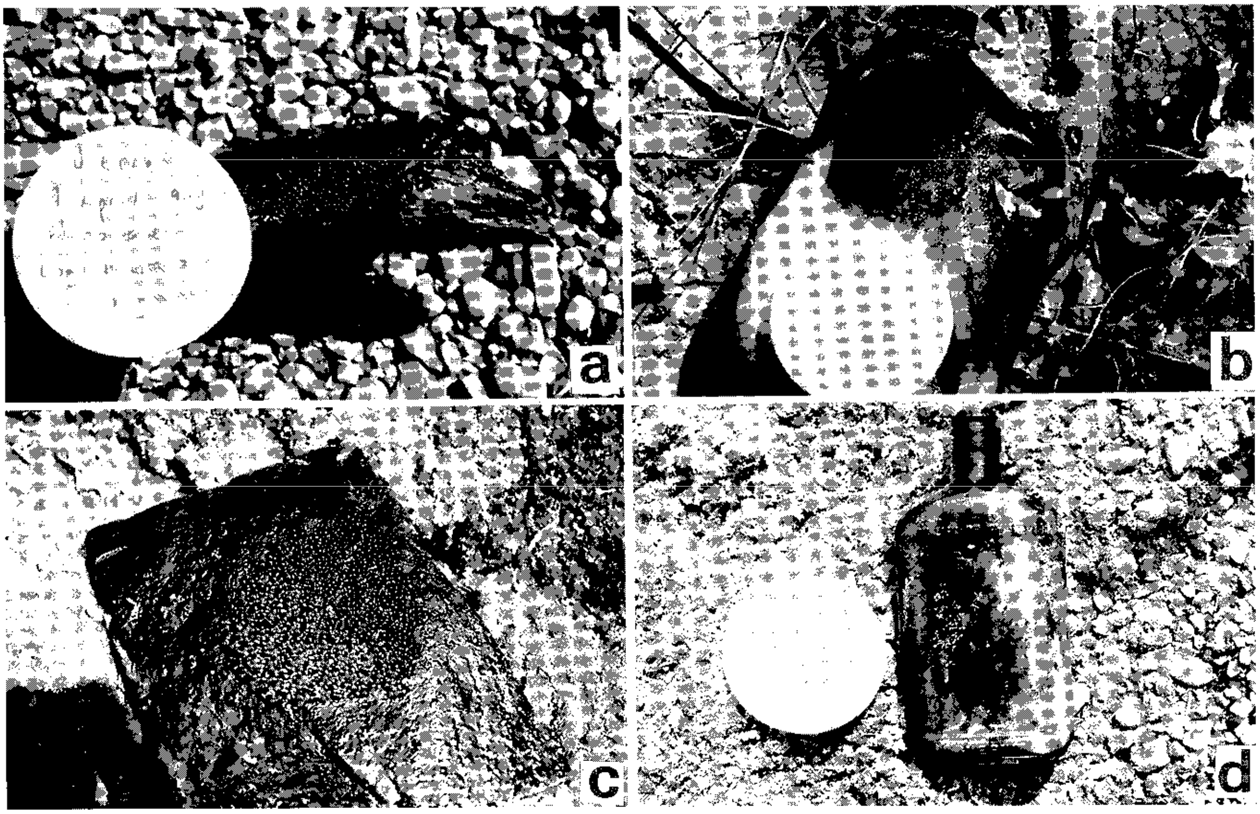

Figure 3. Spawning substrates used by *Etheostoma chienense* in the Bayou du Chien drainage, 1995–1996: a– small stick; b– plastic milk container; c– roofing shingle; d– glass bottle.

shingles (Figure 3c), glass (Figure 3d), concrete/cinder blocks, bricks, metal road signs, an aerosol can, concrete slabs, and even a discarded air conditioner.

Nests on natural materials contained 12–789 eggs ($\bar{x}$ = 255) in 1995 and from 12–1275 ($\bar{x}$ = 343) in 1996 (Table 1). In 1995, roofing shingles had the greatest mean nest clutch size among substrates ($\bar{x}$ = 556), while in 1996 the greatest mean clutch size was found on rubber materials ($\bar{x}$ = 498), including rubber tires and floor mats. No significant differences were found in mean clutch sizes among spawning substrates in 1995, but a difference was detected in 1996 (ANOVA, p = 0.01). A Tukey test indicated that rocks ($\bar{x}$ = 189) and rubber materials ($\bar{x}$ = 498) were the only two substrates that differed significantly in clutch size in 1996.

Several substrates harbored multiple nests and multiple guarding males. Many nests lacked physical barriers between clutches of eggs, and it was not unusual to find egg masses less than 10 cm apart guarded by separate males. Logs and sticks were the most common substrates containing multiple egg masses. Several of the larger-sized logs harbored three or four nests at a time, with each being guarded by a single male. Rubber tires routinely contained multiple nests. One particular tire contained five fresh nests, all greater than 10 cm apart, in addition to the remnants of four other egg masses from which embryos hatched (no guarding males were captured). At Bayou du Chien, Rt. 1283, a metal air conditioner cover harbored four nests all 20–25 cm apart from one another. One corner was firmly anchored to the stream bank while the remaining area floated on the waters surface.

As noted previously, egg-clustering is a behavior in which eggs are laid in a single layer on the underside of a substrate, but occasionally nests were found that contained eggs in double layers. Double-layered nests were found on both logs and sticks, a small piece of particle board, and occasionally on artificial tiles. Usually only a few eggs were found in this condition.

Artificial spawning substrates

The use of artificial spawning substrates varied between sites and years (Table 2). The most extensive usage of tiles occurred at Jackson Creek. Although many tiles placed at the other two sites, Bayou du Chien, Rt. 1283 and Bayou du Chien, 2422 Rd. harbored nests at least once, tile usage at these sites was much less than at Jackson Creek. During both 1995 and 1996, many of the tiles at both sites had more than one nest during the spawning season.

Clutch sizes also varied between sites and years (Table 3). In both 1995 and 1996, mean clutch size on artificial surfaces based on both the maximum and average clutch sizes observed was significantly greater (t-tests, 1995: t_m, $p < 0.0005$ and t_a, $p < 0.0003$; 1996: t_m $p < 0.0003$ and t_a $p < 0.008$) than the clutches attached to natural materials. There were no significant differences in clutch size on artificial spawning substrates between 1995 and 1996 based on both the maximum and average clutches (ANOVA, $p = 0.31$ and $p = 0.24$, respectively). The number of eggs guarded per tile increased with increasing male size for both the maximum and average clutch measurements (Figure 4).

It was a common occurrence to find large nests with clutches of eggs in four or five different stages of development, indicating that males were polygynous. In addition, on 25 April 1996, we captured a single male and three females (40, 41, 42 mm SL) beneath a single tile at Bayou du Chien, 2422 Rd.

Although we found a variety of other aquatic organisms under ceramic tiles without eggs (e.g., creek chubs, *Semotilus atromaculatus*, midland water snake, *Nerodia sipedon*, and crayfish, *Cambarus* spp. and *Orconectes* spp.), no organisms other than *E. chienense* were captured beneath tiles that harbored eggs.

Ceramic tiles were fairly stable at the Jackson Creek and Bayou du Chien, 2422 Rd. sites. Although tiles were occasionally flipped or buried during floods, most remained in the study area. Several times, *E. chienense* deposited eggs on the tops of tiles (now the undersides) that were flipped over from increased water velocity. Flooding intensity is greater in the downstream area of Bayou du Chien than in headwaters and tributaries. Several tiles were placed in the main channel of Bayou du Chien at Rt. 1283 in 1995 and even during normal flow, tiles were buried, washed downstream, or flipped over.

Laboratory observations: spawning and egg-guarding

Spawning occurred as early as three hours after introduction of adults into an aquarium, and individuals typically spawned within a few days. After acquiring a nest-rock territory, a male would periodically leave the nest to chase and court a female. A female ready to spawn followed the male beneath

Table 2. Summary of artificial spawning substrate usage by *Etheostoma chienense* in the Bayou du Chien drainage, western Kentucky, 1995–1996.

Characteristic	1995		1996	
	Jackson Creek	Bayou du Chien, Rt. 1283	Jackson Creek	Bayou du Chien, 2422 Rd.
Average number of sites available	20	20	17	20
Number of times tiles examined	15	11	14	14
% of tiles guarded at least once	100	50	88	65
% of tiles with at least one nest	70	25	88	65
Guarding males (mm SL)	58–70	58–70	55–74	56–75
Mean tile clutch size (maximum)	683	369	518	600
Mean tile clutch size (mean)	617	263	473	506
Range of clutch sizes	197–1266	93–658	11–1182	19–1132

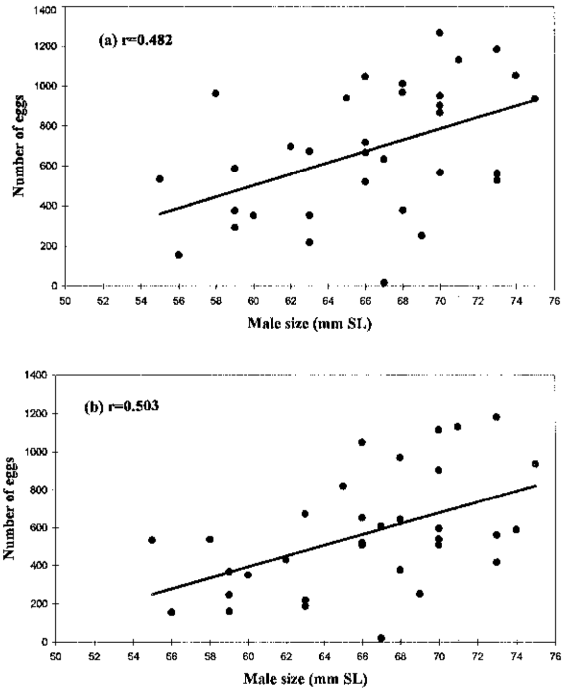

Figure 4. Effect of *Etheostoma chienense* male size (mm SL) on artificial spawning substrate use based on a– maximum number of eggs y = 28.6(x) + – 1226.0); b– average number of eggs (y = 28.6(x) + – 1323.8).

Table 3. Comparison of *Etheostoma chienense* clutch sizes (mean and range) attached to artificial and natural substrates based on: (a) maximum and (b) average clutch sizes observed. Significant differences denoted by ***.

		Artificial	Natural	t-value	p
a	1995	x̄ = 613 (93–1266)	x̄ = 254 (12–789)	4.111	p < 0.0005***
	1996	x̄ = 561 (11–1182)	x̄ = 343 (25–1275)	3.811	p < 0.0003***

		Artificial	Natural	t-value	p
b	1995	x̄ = 539 (93–1266)	x̄ = 255 (12–789)	3.32	p < 0.003***
	1996	x̄ = 490 (11–1182)	x̄ = 343 (25–1275)	2.709	p < 0.008***

the structure and immediately prior to mating, the female raised her head. Mating was initiated by the female, who inverted herself and pressed her genital papilla flat against the substrate. As soon as the female began to invert, the male positioned himself alongside the female, head-to-head, and quickly inverted. The vibrating act of egg-deposition and insemination only lasted for 1.5 to 3.0 sec ($\bar{x}$ = 2.2 sec, n = 68). It was not possible to determine the number of eggs laid during each inversion. Both sexes returned to the upright position following each mating act. Fifteen to 623 seconds ($\bar{x}$ = 84.3 sec, n = 68) elapsed between inversions. The female remained stationary until she was ready to mate again, while the male swam continually in a circle near the female's head region. During the intermating interval, the male displayed laterally to the female, showing his barred pattern and erected median fins. Occasionally a male would press his second dorsal fin against the spawning substrate and display the swollen tips of the second dorsal fin rays in an egg-mimicking type behavior (Page & Swofford 1984, Page & Bart 1989).

After completion of spawning, males aggressively guarded the egg masses by driving off intruding males. Guarding males maintained their vertical barred patterns and their swollen and blackened head and nape. Males regularly fanned the eggs with the second dorsal fin and occasionally removed dead or fungused eggs. From eggs that were not eaten by guarding males embryos hatched within 10–13 days at 20–21 °C.

Nest size and mate choice

In experiment 1 (female choice of nest rock size), eight matings occurred; six pairs mated on the large rock and two mated on the smaller rock. Although 75% of matings occurred on large rocks, no statistical preference was shown (one tailed binomial exact test, p = 0.14). In experiment 2 (female mate size choice), eight mating events occurred and females chose the larger male seven times, indicating a preference for larger mates (one tailed binomial exact test, p = 0.04).

Discussion

Reproductive habitat

Prior to our studies, spawning and nesting activities of *E. chienense* were believed to be restricted to only one stream reach in Bayou du Chien (i.e., Jackson Creek) (Page et al. 1992, Warren et al. 1994a). We found nests with guarding males or egg clutches at 12 of 16 sites of occurrence in tributaries or mainstem reaches of Bayou du Chien over a two-year period. Some of these sites dry completely or are reduced to isolated pools in the fall which may subject young-of-the-year to heavy predation from birds and water snakes. In addition, six of the 12 nesting sites yielded only one or two nests with eggs and are in areas where adequate riparian buffer zones are absent. Assuming there is moderate recruitment of juveniles from sites other than Jackson Creek bodes well for the species continued existence, especially considering that one chemical spill in Jackson Creek, the site of greatest nesting activity, could significantly affect the species. In the draft recovery plan it was suggested that before *E. chienense* could be reclassified to threatened, successful spawning must occur in at least five separate tributaries or mainstem reaches over a five-year period (Warren et al. 1994b). Moreover, it was stated that juvenile production must occur at any one site at least once in three of the five years. Our results document that successful spawning has been achieved, but more field work is necessary to document recruitment of juveniles over a several-year period before downlisting can be recommended.

Use of artificial spawning tiles as a management tool

Introduction of ceramic tiles significantly increased nest productivity in terms of both maximum and average clutch sizes. Egg-clusters on ceramic tiles were almost double the size of clutches attached to naturally occurring materials. The tiles appear to be beneficial in two ways. First, the tiles offer relatively more flat surface area for egg deposition than do small logs and sticks. During late spring 1995, a flood in Jackson Creek washed several cobble-sized (62–256 mm) rocks into the study area. Over the next several weeks, numerous egg masses were found on these relatively small cobbles. Attaching eggs to objects of this size limits the number of eggs laid and guarded, the resources are scarce and ephemeral, and cobbles are unstable. Second, although the artificial tiles are vulnerable to natural flooding, they are more stable than many of the substrates (e.g., bark, small sticks, cobbles) that were attached loosely to the stream bank. On successive visits to a site, we routinely observed the disappearance or destruction of nests attached to unstable materials.

We recommend use of artificial tiles as a cost-effective management tool for enhancing egg production and presumably recruitment. Ideal microhabitats include those in headwater reaches where flow is reduced, riparian buffer zones are at least 4–6 m wide, water is relatively shallow (to 20 cm) and clear, and clean substrates of small gravel and sand are common. Tiles placed in swift, deep water or open (i.e., non-shaded) areas were never used for spawning or guarding. Streams could be seeded with tiles prior to the spring spawning season and could conceivably be used as cover during other seasons. Lindquist et al. (1984) experimented with four artificial spawning covers (tile and slates of three sizes) in Lake Waccamaw, North Carolina, and showed that egg production in *E. (Boleosoma) longimanum* was highest under slate covers placed in 2 m of water on a mixed sand bottom.

Nest cavity height

Regardless of whether artificial tiles or natural nesting materials are used by *E. chienense*, it is essential that the cavity guarded by the male meet certain physical requirements, otherwise potential cavities will almost certainly be rejected by guarding males. Winn (1958) stated that the fantail darter, *E. (Catonotus) flabellare*, needed a vertical space of only 1.5 to 2.5 cm between the stream bottom and the substrate for spawning, just enough room for the female to remain inverted while resting on her dorsal fins with her genital papilla pressed against the substrate. Members of the *E. squamiceps* complex, including *E. chienense*, reach a larger adult size than the other two *Catonotus* species groups, and therefore presumably need a larger space for inversion. Our instream and aquarium measurements indicate that nesting cavities with egg clutches had a vertical space of between 2 and 5 cm (mode 3 cm). Cavities less than 2 cm high are simply too small to allow complete inversion of both sexes.

Opportunism and nesting constraints

Nesting on the undersides of rocks has been observed for five species in the *E. squamiceps* complex, including *E. squamiceps* (Page 1974), *E. crossopterum* (Page 1974), *E. oophylax* (Page & Mayden 1979), *E. nigripinne* (Braasch & Mayden 1985), and *E. olivaceum* (Page 1980). All of these reports indicate that slabrocks are the most common spawning material used by *Catonotus*. *Etheostoma olivaceum*, which will guard nests attached to tiles and tin cans (Page 1980), and *E. chienense*, which uses an array of nesting materials (this study), represent the only records of a species of *Catonotus* using substrates other than slabrocks. As noted earlier, lack of large rocky substrates for nesting is one of the limiting factors that resulted in listing *E. chienense* as endangered. Clearly, *E. chienense* is opportunistic and labile in its ability to utilize objects other than slabrocks as nesting sites, and anthropogenic nest material could not have been available to the species over a century ago, prior to stream modification. Some of the anthropogenic materials (e.g., concrete blocks, rubber tires) used for nesting appear to be stable, have the appropriate cavity dimensions, are situated in suitable habitats, and have probably increased egg production. Other materials, both anthropogenic and natural, may be of limited utility in increasing juvenile production because of their instability, reduced nesting surface area, and scarcity. We are not suggesting that indiscriminate dumping of human refuse or the by-products of bridge construction have indirectly improved nesting conditions of *E. chienense*, but rather that this species is capable of using an array of substrates, and those strategically selected and placed in appropriate habitats by resource managers will almost certainly enhance nest productivity.

Spawning behaviors and surrogates

Aquarium observations documented that *E. chienense* does well in captivity, and that spawning occurs under the appropriate water and nest substrate conditions. Aquarium spawnings also verified that this species mates in a head-to-head pattern, and that brief inversion by both males and females occurs. These behaviors are also documented for other, more common (i.e., non-listed) species in the *E. squamiceps* complex (e.g., Page 1974, 1980), and suggest that at least two other species might be used as surrogates for *E. chienense*, especially where large sample sizes are needed to verify experimental conditions that could have direct management applications. For example, various sized pieces of slate might be tested for nesting efficacy and compared to the ceramic tiles used in our studies. More experimentation is needed on mate choice, but those experiments could almost certainly be done with a more common close relative, for example, *E. oophylax*.

Nest size and mate choice

The two laboratory experiments provided evidence that female choice could influence nest success and ultimately population dynamics. When given a choice, females showed a tendency to spawn on the larger of the two rocks. Larger spawning substrates may provide several benefits. They are usually heavier and have a lower probability of turning over and destroying eggs, and they allow more surface area for egg deposition (Brown & Downhower 1982). As shown by Downhower & Brown (1980), aggressive male sculpins typically obtain the larger and better nest sites; larger males are usually older and generally more experienced and often control larger and superior nesting sites. Females benefit because their eggs will be protected by aggressive males which generally are the better defenders of nests. Seven of eight laboratory matings of *E. chie-*

nense occurred with larger males. By choosing a larger male, a female's own fitness may be increased via increased egg survival (Turner 1993).

Conclusions

Ecological and reproductive information gained in this study indicates that *E. chienense* is similar in its general life history to other members of the *E. squamiceps* complex. The species deviates by attaching eggs to a wide variety of both anthropogenic and naturally occurring materials, but the size of nest-guarding males, length and timing of the spawning season, spawning behavior, size of clutches, resource defense polygyny, and egg incubation periods are all analogous, and probably homologous, to what has been reported for other members in the complex (Page 1974, Page & Mayden 1979, Page 1980, Braasch & Mayden 1985). The life history traits documented here have significant management implications for recovery of the species. If artificial spawning covers are used in future recovery efforts they must be placed in microhabitats (i.e., shallow, clear, low-flow reaches in headwaters protected by adequate riparian buffer zones) as recommended here. Moreover, nest cavity vertical height should be about 3.0 cm. Tiles should be spaced at least 0.5 m from one another to reduce aggressive interactions and at the same time increase egg production. Appropriate nest materials should be distributed among several tributaries or stream reaches where nesting has been documented previously in an effort to increase the geography of egg production. Presumably, increased egg productivity at several sites will increase recruitment of juveniles and dispersion of the species.

Much research on *E. chienense* is needed, especially considering that downlisting the species to threatened or removing it from the list altogether is the desired outcome of the recovery plan (Warren et al. 1994b). Other experimental tests, perhaps using a close relative as a surrogate (e.g., *E. oophylax*), need to be conducted using other kinds of spawning covers. Larger sample sizes are desirable in future studies of mate quality and nest substrate size. Documentation of recruitment has not been achieved and is the fundamental life history aspect requiring further investigation. We recommend that management focus on habitat protection and restoration rather than culture and stocking of individuals. Restoring riparian buffer zones to headwater tributaries, reducing channelization and ditching, and educating private landowners about the needs of this species will be of much greater benefit to the future recovery of this fish than probably any other set of management tactics.

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