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Spawning and nesting behavior of the waccamaw darter, *Etheostoma perlongum*

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Synopsis

The spawning and nesting behavior of *Etheostoma* (*Boleosoma*) *perlongum* was investigated in the field and laboratory. Sexual dimorphism is highly developed in such features as genital papillae, first dorsal and paired fins, and nuptial coloration. A reproductive migration from mid-lake to shore occurs in the spring: males precede females to select nest sites under submerged sticks and other debris. The male excavates a depression beneath the submerged object. Gonad analysis indicates a single spawning season extending from March through June. Nests were found from late April to mid-June and were guarded by a single male for periods of 13 to 36 days. Males initiate courtship by lateral display, lead the female to the nest site and show the nest by inverting. The female responds by tail up, tail wag and circle; males also tail wag and circle. Spawning pairs invert, usually in unison, and orient head to head or, less often, head to tail. The female deposits eggs while holding her body in a weak 'S' or 'J' shape with the caudal peduncle held away from the spawning substrate while vibrating.

Introduction

Although darters (Percinae: Etheostomatini) are a major endemic component of North American fresh water fish communities, the spawning behavior of scarcely over one-third of the species has been investigated (L. M. Page, pers. comm.). Certain darters have become rare and subject to extirpation because of their localized distributions and the increasing amounts of man-influenced perturbations to their aquatic habitats. Those darters studied appear to be either nonguarding open-substrate spawners or guarding nest spawners (Balon 1975, Balon et al. 1977). Spawning behavior of 14 representatives of both reproductive guilds was summarized by Winn (1958a, b).

There are five species of the subgenus *Boleosoma*, to which the waccamaw darter, *Etheostoma perlongum*, belongs (Cole 1965, 1967, 1972). Reproductive behavior of two of these species, *E. nigrum* and *E. olmstedi*, has been described by Winn (1958a, b) and Atz (1940), respectively. *E. perlongum* is a fine scaled and attenuated species which presumably evolved either from an isolated remnant of an earlier *Boleosoma* stock, which has since been

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displaced by *E. olmstedi*, or has evolved from an isolated stock of *E. olmstedi* (Cole 1972). Hubbs & Raney (1946) conjectured that predation pressure in Lake Waccamaw selected for a more fusiform *Boleosoma* that could better escape predation. Frey (1951), however, presented evidence refuting their predation hypothesis. With detailed spawning information now at hand on three of the five species, it is possible to better illuminate the evolutionary relationships within the subgenus *Boleostoma* (especially the relationship between *E. perlongum* and *E. olmstedi*).

The waccamaw darter is endemic to Lake Waccamaw and the upper headwaters of the Waccamaw River in North Carolina (Hubbs & Raney 1946, Menhinick et al. 1974, Bailey 1977). Because *E. perlongum* has a localized distribution and because the lake has been threatened by increasing residential development, this darter has been proposed for protection under various endangered species programs (Williams 1977, Bailey 1977) and lists (Miller 1972, Deacon et al. 1979). However, there exists no published information on the biology of *E. perlongum* on which to base recommendations. We are gathering life history information for use in developing final recommendations on the status of this species. We report herein our findings on reproduction in *E. perlongum*.

Methods and materials

Field studies

In order to assess the presence of migratory patterns of darters to and from spawning areas, 8 shore stations and 6 mid-lake stations were sampled on a monthly basis from February to November 1979. Two of the shore stations were chosen for detailed observations of nesting and spawning activities: station A was characterized by a dense stand of introduced lotus (*Nelumbo lutea*) located between a small asphalt jetty and wooden dock; station B was vegetated by sparser stands of maidenance (*Panicum hemitomum*) and young bald cypress (*Taxodium distichum*).

We used a variety of seines ranging in size from 3 m × 1.2 m to 15.2 m × 1.8 m (mesh size typically 3 mm) to sample darters at the shore stations. Various designs of handnets were used with snorkel and faceplate to collect individuals when sighted underwater. Mid-lake stations were sampled by a small otter trawl measuring 2.8 m × 1.3 m at the mouth and lined with 3 mm mesh netting. The area seined or trawled was determined by multiplying the distance covered by the capture width of the net. Distances seined (10–50 m) were measured by a meter tape and distances trawled (250–450 m) were determined by an optical rangefinder. Thus the density of darters was computed at each of the stations sampled in this manner. Captured fish were either preserved immediately in 10% formalin or held alive in 19 l containers of aerated lake water. Living specimens were transported to laboratory aquaria in 2 to 4 hours.

Nests were marked by attaching colored nylon tape to adjacent emergent vegetation. The location of each nest was mapped. Nest-cover objects were briefly removed from the water, measured and returned to their previous positions. Egg clusters were either preserved, photographed or measured. If the nest-tending male could be captured he was marked by injecting a small amount of red acrylic paint under a scale on the caudal peduncle (Thresher & Gronnel 1978) and released on the nest.

The number of eggs in the cluster was determined by a direct count or by an estimation technique. If the entire egg cluster could be photographed, egg density was determined for a section of the cluster by examination of the transparency over a fine grid with a binocular dissecting microscope The density, multiplied by the overall size of the cluster, gave an estimate of the total number of eggs. A second estimation technique involved a direct measurement of the cluster in the field. An average density was used to arrive at the total egg estimate.

Spawning observations were made on 17 May 1979. Kodachrome photographs were taken with a Nikonos II camera fitted with a 3:1 extension tube and underwater strobe. The spawning cover, a cypress branch, was elevated 1 cm so that the fish could be brought into focus within the framing device of the extension tube.

Laboratory studies

Female gonad indices were determined by comparing the weight of the left ovary with the body weight (Schenck & Whiteside 1977). Females 29 mm SL or longer were weighed to the nearest 0.01 mg on a Mettler single pan balance after thorough blotting on paper toweling. Ovaries were then excised, blotted and also weighed to the nearest 0.01 mg. Eggs from the left ovary were then separated into various size classes and counted. The fecundity of each female was expressed first as a doubled count of total ova and then as a doubled count of mature ova. The diameter of each ovum was measured to the nearest 0.025 mm with an ocular micrometer. The mean ova diameter for each fish and the mean ova diameter for all fish for each month were then determined and also used as a gonad index.

Male gonad indices were determined by comparing the relationship between the square root of the standard length of the male with the width of the left testis (Schenck & Whiteside 1977). Males 33 mm SL or longer were measured to the nearest millimeter. The width of the left testis was measured to the nearest 0.025 mm with an ocular micrometer and then converted to a percentage of the square root of the standard length.

One 67 mm SL male and two gravid females, 50 mm and 55 mm SL, were collected and placed in a 208 l aquarium on 21 February 1979. The aquarium was filled with lake water to a depth of 30 cm. Medium coarse sand from the lake shore served as a substrate. A few plants, maidencane (*Panicum*) and hatpin (*Eriocaulon*), from the lake were established in the sand. Temperature in the aquarium was held at 20 ± 1° C. The light-dark cycle was 14:10. Fish were fed live chopped earthworms to satiation semidaily. A blind with viewing slits was placed around the tank and a small cylindrical cypress branch (65 cm long × 4 cm diam.) provided for cover on March 4.

A second aquarium, 80 l, was used as a holding tank for three females and four males. The aquarium bottom was similarly provided with coarse sand, plants, and spawning cover (a piece of wood).

Super-8 mm cine films were taken of spawning and nesting behavior. A Nikon R8 cine camera with macrolens was used in conjunction with two flood lamps placed above and to one side of the aquarium. Films were then analyzed with a standard projector at regular speed (18 fps) and slow motion (5 fps). A standard movie editor was also used for a frame by frame analysis of movements and motor patterns.

Results and discussion

Sexual dimorphism

Although Hubbs & Raney (1946) examined mature specimens, they obviously did not obtain breeding individuals in nuptial colors. Their description also did not deal with sexual dimorphism except for a brief description of genital papillae.

Genital papillae

The genital papillae of breeding individuals are illustrated in Figure 1. The male papilla is suboval and bilobed. The gonopore is located at the posterior juncture of the lobes. The outer portion of each lobe is pigmented. The papilla of the spawning male is small and inconspicuous and is not easily differentiated from the non-spawning male. The female papilla is much larger and more prominent than the papillae of the male or non-breeding female (Fig. 1). The female papilla is also a bilobed structure, as is usual in the subgenus *Boleosoma* (Cole 1967). The papilla is only lightly pigmented at the lateral edges. The gonopore in the female is located midway between the posterior end of the papilla and the anus. The genital papillae of *E. perlongum* are essentially the same morphologically as those of other *Boleosoma*. Cole (1967) described and figured the genital papillae for *E. olmstedi*. The papillae of *E. perlongum* are very similar and thus reinforce the use of the bilobed structure as a diagnostic subgeneric characteristic.

Our observations suggest that the function of the greatly enlarged papilla of the breeding female involves the tactile searching for a suitable site for individual egg attachment. Evolutionary specialization of the female genital papilla appears to have reached peak development in the subgenera *Microperca* (Burr & Page 1978) and *Hololepis* (Braasch & Smith 1967, Collette 1962). In comparison to *Boleo-*

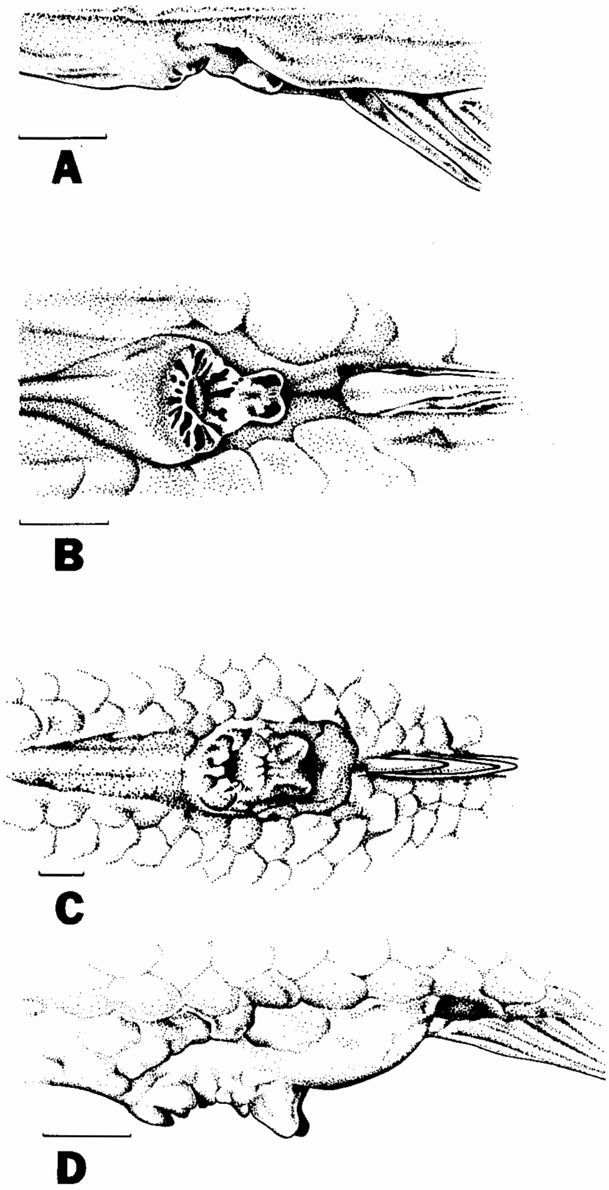

Fig. 1. Genital papillae of breeding *Etheostoma perlongum*. A and B are lateral and ventral aspects, respectively, of male, 67 mm SL taken 17 May; C and D are ventral and lateral aspects, respectively, of female, 49 mm SL taken 21 April. Scale = 1 mm.

soma, *Microperca* and *Hololepis* have longer papillae specialized seasonally as flattened and elongated tubes that function as effective ovipositors on vegetation, twigs, and sides of rocks. However, we must point out that, although the genital papillae of these two subgenera are highly modified, these fishes belong to the most primitive reproductive guild of the darters. This guild is the nonguarding, open substrate, phyto-lithophil of Balon (1975) and Balon et al. (1977). Even so, taxonomists (Bailey & Gosline 1955, Collette 1965, Page 1977, Collette & Bănărescu 1977) have indicated that *Hololepis* and *Microperca* are the most highly evolved subgenera of *Etheostoma*. Winn (1958b) pointed out apparent conflicts between this behavioral evolution and the taxonomists morphological evolution. Clearly, more behavioral and systematic studies are required to resolve these and other conflicts.

Fin rays and morphometry

The fin rays in the first dorsal, pectoral, and pelvic fins of a 69 mm SL male are modified as follows: The tips of the three most ventral pectoral fin rays are expanded as fleshy white knobs. Each spine in the pelvic fins has an enlarged knob with smaller knobs present on the three adjacent fin rays. The first five spines of the first dorsal also have knobby white tips.

Mature males are consistently larger than mature females. The proportional height of the first dorsal fin (longest spine divided by SL) in breeding individuals is greater in males ($\bar{x} = 0.147$, $N = 10$) than females ($\bar{x} = 0.112$, $N = 9$). There was no difference in the first dorsal fin height between non-breeding individuals of both sexes ($\bar{x} = 0.118$, $N = 12$).

Hubbs & Raney (1946) did not describe any modifications of the paired fins of their holotype, a 71 mm SL mature male. However, a close inspection of a photograph of the holotype, supplied with their original description, clearly reveals the presence of fleshy knobs on at least the five most ventral pectoral rays and the pelvic fin spine.

Within the subgenus *Boleosoma*, modifications of the tips of the paired fins reach extreme development in the Tar River and Neuse River populations of *Etheostoma nigrum* in North Carolina (Cole 1972). Cole (1957) gave tentative subspecific status to these populations because the lower pectoral membranes of breeding males and females are deeply indented with the tips of the rays grossly knoblike and whitened. Collette (1965) indicated that males of *E.* (*Boleosoma*) *longimanum* taken in April and June had swollen small white bulbs on the pelvic fin spines. Collette (1965) also compared a few males of *E. perlongum* taken on March 26 with

E. nigrum and *E. olmstedi* and reported that the fleshy tips of the pelvic fin rays are not as prominent in *E. perlongum*. We have compared our nuptual male of *E. perlongum* with a 60 mm SL nuptual male of *E. olmstedi* taken April 22 from the Waccamaw River in Horry Country, South Carolina. Our findings concur with those of Collette. We can add that the seven ventral soft ray tips of the pectoral fins in *E. olmstedi* have well developed fleshy knobs that are more prominent than those found on the pectoral fins of *E. perlongum*. We also found small fleshy knobs on the first five spines in the first dorsal fin of *E. olmstedi* that were not as well developed as those in the first dorsal fin of *E. perlongum*.

Winn (1958b) reported that most of the males of the fourteen darters he studied had slightly thickened outer margins of the pelvic fins and that the first four or five spines of the first dorsal fin in the males of *E. nigrum* had thickened opaque white tips. This adornment of the dorsal fin has reached utmost development in males of certain species of the subgenus *Catonotus*. Fleshy bulbs are prominent on the tips of the spines in the first dorsal fin of both sexes of *E. kennicotti* (Collette 1965), the males of *E. flabellare* and to a lesser extent the males of *E. squamiceps* (Page 1975a). Most recently, Howell & Dingerkus (1978) described a unique *Catonotus*, *E. neopterum*, that not only has large fleshy knobs on the tips of the spines in the first dorsal fin but has extraordinary development of each ray of the soft dorsal fin in males. The rays of this fin terminate in expanded, flattened flaps and serve to distinguish *E. neopterum* from all known members of *Etheostoma*.

Winn (1958b) reported that the dorsal fins of *E nigrum* and *E. flabellare* 'are used to clean an area on the underside of a rock before egg deposition and subsequently, to rub the eggs.' He further states that *E. flabellare* uses the prominent fleshy bulbs at the tips of the spines of the first dorsal and the outer edge of the second dorsal to brush the eggs. Page (1975a) suggested that those species of *Catonotus* with enlargements on the dorsal spines have undergone selection 'based on a reduction in the sharpness of the dorsal spines of nest-guarding males (which continuously brush their dorsal fins across the eggs).' Howell & Dingerkus (1978) agreed with Page adding that the unusual tips of second dorsal in *E. neopterum* would be less likely to damage or dislodge eggs when the dorsal fins are brushed across the egg mass. Speare (1965) found empty hollow eggs in the nest of *E. nigrum* that had a small hole in the egg case and suggested that anal spines or dorsal spines of the nesting male had struck the eggs and ruptured the egg envelopes. Our observations indicate that the dorsal fins of *E. perlongum* are in constant contact with the eggs during nesting activities and thus we concur with Page (1975a) that the primary function of the knobs on the dorsal spines is for a reduction in the sharpness of the spine tips. A secondary function may be for cleaning the nest site before and after egg deposition.

Cole (1957, 1972) reported that the development of the large white fleshy knobs on the exposed pectoral ray tips of *E. nigrum* coincided with the approach of the breeding season and suggested that these knobs serve to protect the rays during the spawning act and the following period of nest defense and care. Winn (1958b) reported that the elongate cup-like pelvic fins of male *E. microperca* are caused by the large fleshy flaps on the outer edge of the fin that bend inward. Winn suggested that this modification probably aids the male to maintain his position on the female during spawning. Our observations on the use of *E. perlongum's* paired fins suggest that the enlarged tips of the males fin rays serve multiple functions: 1) to assist him in maintaining an inverted position while tending the nest by allowing him to better 'grip' the nest cover; 2) to protect the eggs from being punctured by the pelvic spine; 3) to protect the tips of the soft rays from abrasion during nesting activities; and 4) to assist in cleaning the nest site before and after egg deposition.

Winn (1958b) found that in ten (including *E. nigrum*) of the fourteen species of darters studied, the male was the larger of the two sexes. Generally, in those species of darters where a territory is guarded (e.g. *Boleosoma* and *Catonotus*) the males are larger than females; males attaining a faster rate of growth in their second and third years (Raney & Lachner 1943, Page 1974, 1975b, Page & Burr 1976). Winn (1958b) also reported that eight (including *E. nigrum*) of the darters studied exhibited larger (higher) first dorsal fins in the male. Seven of these eight were species whose males are also larger.

Winn (1958b) suggested that males will have enlarged fins (anal, pelvic and first dorsal) in those species which exhibit pugnacious behavior. Our studies indicate that an important function of the enlarged first dorsal fin of the male is to enhance the lateral display of the male during courtship behavior. The dorsal fin of *E. perlongum* is brightly colored during the breeding season (Fig. 2). Brightly colored male darters also exhibit territorial behavior and males of *E. nigrum* and *E. flabellare* attack other males on the basis of color and movements (Winn 1958b).

Sexual dichromatism

The sexual dichromatism of the pair was marked (Fig. 2). The male is as follows: background body coloration pink and dusky with a series of 10 charcoal gray triangular (apex down) to rectangular bars forming the lateral body markings; top of head and snout pinkish; a charcoal bar through the eye to just behind the angle of the jaws; a charcoal stripe extending from the front of the eye to the tip of the snout and upper lip; an iridescent green spot on the opercle; caudal fin with 7 zig-zag black bars on pinkish background; anal fin orange with black margin; pelvic fins golden orange with dark interspinous membranes; pectoral fins orange at base and clear distally; first dorsal golden orange and dusky with black markings at distal margin of interspinous membranes becoming larger posteriorly and forming an ocellus-like spot; second dorsal golden orange with black distal margin and a series of 5 to 6 black zig-zag stripes extending to lower third of fin.

The female, less colorful, is as follows: background body coloration cream colored with a series of 10 'XW' blotches along midline with two zig-zag stripes and 6 dorsal blotches running below dorsal fins; top of head and snout pinkish; thin charcoal bar below eye and heavier charcoal stripe from front of eye to tip of snout and lips; iridescent green

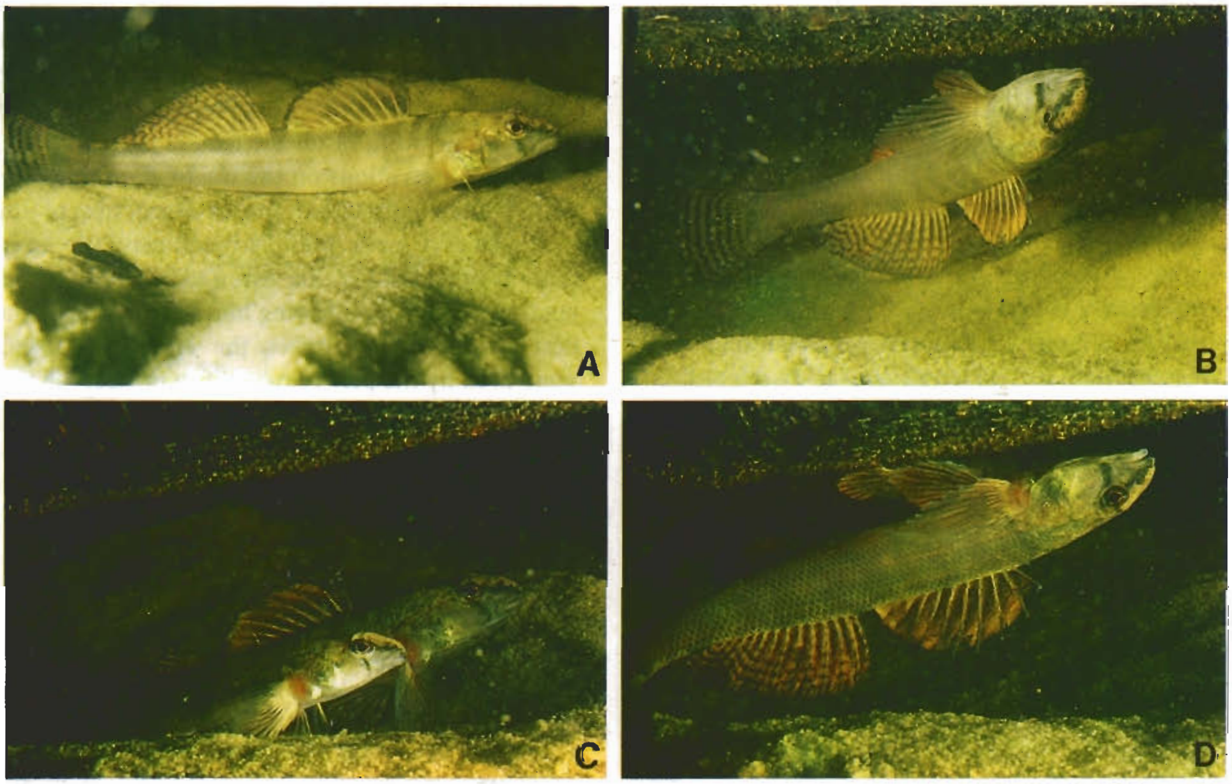

Fig. 2. Spawning activities of *Etheostoma perlongum* in nature at nest # 3 (Table 2). A. Male inverting and showing nest to female. B. Male and female (female behind male, her pelvic fin visible posterior to male's pelvic fin) inverting simultaneously immediately prior to mating. C. Female (front) joins male in nest depression. D. Male in laterial display. Nest cover stick elevated 1 cm by observers in B, C and D.

spot on opercle; caudal fin with 7 zig-zag charcoal bars on cream colored background; median fins light pink to clear; pelvic fins orange; pectoral fins clear.

Cole (1967) diagnosed the subgenus *Boleosoma* partially on the basis of a lack of bright nuptual coloration in the males. Nuptual males of *E. nigrum* and *E. olmstedi* are darker overall due to the greater development of the melanophores. Our observations of iridophores (green) and xanthophores (orange) in *E. perlongum* are unique among the species of *Boleosoma*. However, the green and orange colors are greatly subdued in comparison to the bright nuptial colors of species of the subgenera *Oligocephalus*, *Nothonotus* and *Ulocentra* (L. M. Page, pers. comm.). In this sense, it does not appear necessary to modify Cole's (1967) diagnosis of *Boleosoma*.

Reproductive migration pattern

Monthly collecting efforts at mid-lake shore stations (Fig. 3) suggest: 1) an inshore migration completed in May and June, and 2) an offshore migration from June to September. The shoreward increase corresponds to a decrease in the abundance of darters at the mid-lake stations from March through June. Conversely, as the abundance of darters tends to decrease at the shore stations from summer to fall, the abundance increases at the mid-lake stations between June and July, remaining unchanged in August. The September mid-lake samples were clogged with large amounts of green algae and the trawl did not effectively sample the benthic fishes.

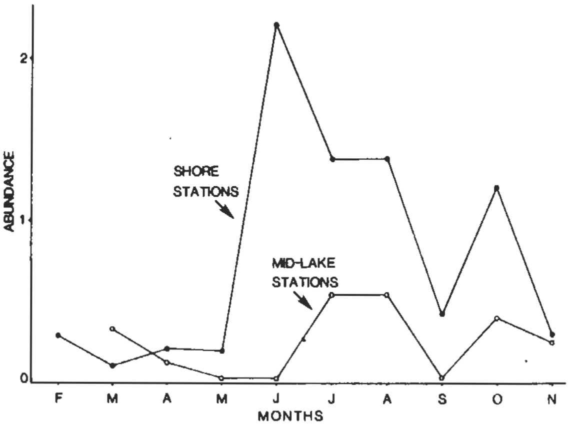

Fig. 3. Abundance of *Etheostoma perlongum* at shore and mid-lake stations during 1979. Abundance = N per 100 m^2.

An analysis of sex ratios by month (Table 1) for the shore and mid-lake samples suggests: 1) the relative number of males increases in April at the shore stations while concommitently decreasing abruptly at the mid-lake stations; 2) the relative abundance of females increases at the shore stations in May while darters apparently leave the mid-lake area in May and June. As darters return to mid-lake in July and August, females predominate the mid-lake samples. Males are then relatively more abundant than females in October at the mid-lake areas. The aforementioned account thus suggests that as darters move onshore in spring and early summer, males precede females and then as they begin returning to mid-lake, the females precede the males. The yearly totals for each location show that although the overall sex ratios favor females, they are not significantly skewed ($X^2 = 0.96$, $p > 0.50$).

Our observations indicate that suitable spawning cover is not available at the mid-lake habitats. The two inshore spawning habitats studied contained a wide variety of structures suitable for spawning cover (sticks, logs, etc.). These particular stations were also in quiet water, protected from wave action by emergent vegetation and man-made structures. Heimburger (1913) found a lake population of *E. nigrum* that was confined to quiet water less than 75 cm in depth during July and August.

Table 1. Sex ratios of *Etheostoma perlongum* from mid-lake and shore stations during 1979.

Month	Mid-lake		Shore	
	Male: female	N	Male: female	N
FEB.	–	–	0.50	3
MAR.	1.67	8	–	0
APR.	0.20	12	2.00	3
MAY	–	0	0.33	8
JUNE	–	0	1.00	4
JULY	0.33	8	4.00	5
AUG.	0.67	5	0.80	9
SEPT.	–	0*	–	1
OCT.	1.57	18	0.38	12
NOV	0.50	15	1.33	7
TOTALS	0.69	66	0.79	52

* Trawl clogged by vegetation

Hankinson (1908) suggested that there may be a migration of *E. nigrum* from lake to small creeks only during the breeding season. Winn (1958a, b) reported that a stream population of *E. nigrum* migrated several miles upstream from permanent pools to shallow spawning ground in April and that a lake population of *E. nigrum* departed from the breeding grounds and went to the deeper parts of the lake after spawning. Various other species of darters have similar reproductive migration patterns (Winn 1953, 1958a, b, Page & Smith 1970, 1971, Page 1974, Starnes 1977).

Gonad analysis

Ova maturation in *E. perlongum* occurs in spring and early summer, indicating a single spawning period during those months examined (Fig. 4). The gonad indices for males and females also indicate a single spawning period extending from March through June (Fig. 5).

The eggs from mature ovaries of preserved *E. perlongum* were separated into four size classes: 0.2–0.3 mm; 0.3–0.5 mm; 0.5–1.0 mm; and 1.0–1.2 mm. Only the 1.0–1.2 mm eggs are mature. The total numbers of eggs (all sizes) range from 180–764 with a mean of 550. The number of mature eggs range from 112–193 with a mean of 156. Total and mature egg numbers are correlated significantly with standard length (Fig. 6).

The distribution of mean ovum diameter has been demonstrated as an accurate predictor of the reproductive period in darters (Mathur 1973, Schenck & Whiteside 1977). Tsai (1972) stated that the seasonal changes in mean ovum diameter of a Maryland population of *E. olmstedi* corresponded to the seasonal changes of his gonad index. Tsai's cold reservoir tailwater population had a breeding season of April, May and June as indicated by the gonads of age class II females. Speare (1965)

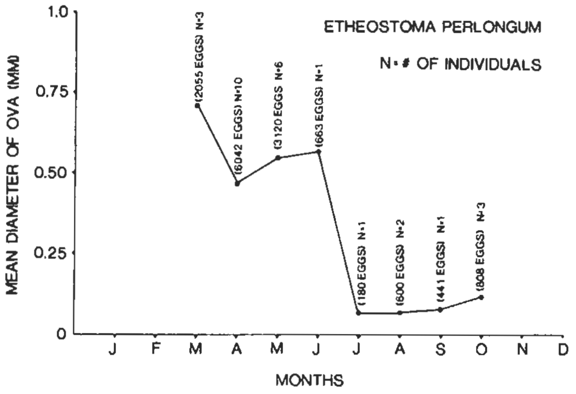

Fig. 4. Mean monthly diameter of ova from *Etheostoma perlongum* during 1979.

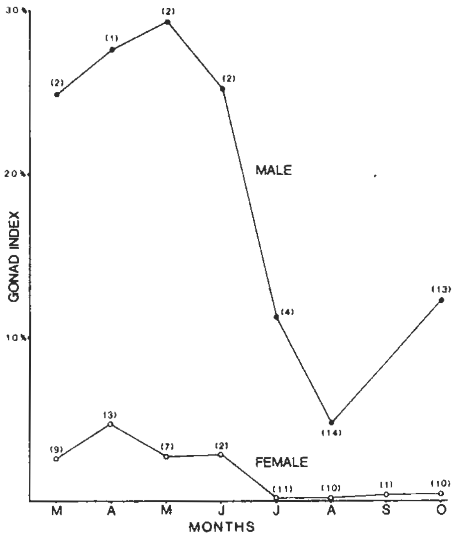

Fig. 5. Monthly changes in gonad indices for males (testes width · square root of standard length $^{-1}$ · 100) and females (left ovary weight · body weight $^{-1}$ · 100). Number of specimens examined each month is given in parentheses.

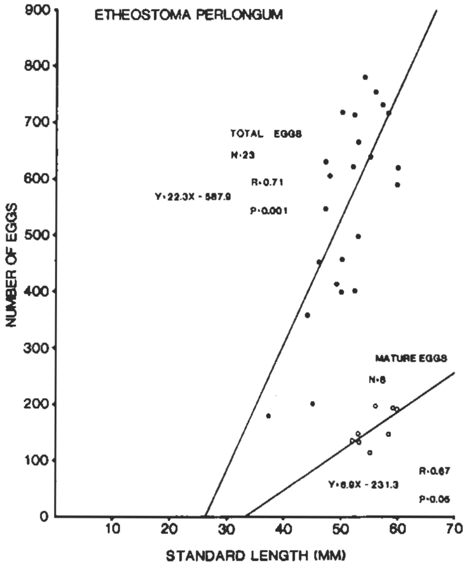

Fig. 6. Least squares regression analysis of egg numbers and standard length of female *Etheostoma perlongum*.

determined that the ovary development of a southern Michigan population of *E. nigrum* indicated that ovaries reached full maturity about 15 April and are spawned by 15 May. The reproductive period of *E. perlongum* appears quite similar to other *Boleosoma* studied despite the fact that the populations of each species are at different latitudes. Hubbs & Strawn (1957), in laboratory experiments, showed that the reproductive rate of *E. lepidum* is controlled by temperature and the condition of the fish, and not light duration and intensity. It is likely that the onset of spring warming triggers reproductive activity in *Boleosoma*. Most other darters that have been studied are spring or early summer spawners.

Hubbs et al. (1968) defined fecundity as 'the product of frequency of spawning and number of eggs produced per spawning interval.' Our observations suggest that females are capable of multiple spawnings and can lay at least 59 eggs during a spawning sequence. Our results indicate that the average number of mature eggs for females was 156, indicating approximately three spawnings for each female. However, we do not know if, after all mature eggs are laid, females produce subsequent batches of mature eggs. Fahy (1954) reported that a 62 mm *E. blennioides* spawned a total of 1478 eggs at a rate of 40 per spawning act, requiring approximately 37 acts to spawn her ova complement. Fahy suggested that female *E. blennioides* eventually mature and spawn all yolked ova larger than 0.10 mm. Winn (1958a, b) and Speare (1965) disagree as to the frequency of spawning for *E. nigrum* in Michigan. According to Winn, all eggs containing yolk are laid during a spawning season at a rate of 30 to 200 eggs during the 5 to 6 spawning acts. Speare's evidence suggested that there is but a single spawning act with a mean of 45, 82, and 112 eggs laid for age groups I, II and III, respectively. Clearly, additional observations are needed in order to resolve the question of the true fecundity in *E. perlongum* and *E. nigrum*.

Nest sites

Nesting activity, as indicated by dates on which nests were found, extends from late April to mid-June (Table 2). Lake temperatures at these stations ranged between 22 – 29° C during this period. Male *E. perlongum* select nest sites in shallow (< 1m) water generally within 10 m of the shoreline. Any type of stationary solid object, such as a tire or stone (introduced) or more often a section of waterlogged wood that lies flat on the sandy bottom can serve as cover for the nesting site. The male excavates a shallow depression in the sand below the nest cover by digging and fanning with the caudal fin. The depth of the depression is just enough to accomodate the male with raised dorsal fins.

Eggs were found clustered to the underside of the nest cover objects. The clusters always consisted of

Table 2. Descriptive characteristics of nesting sites for *Etheostoma perlongum.*

Nest No.	Station	Dates occupied (total days)	Cover		Depth (cm)	Distance From shore (m)	No. eggs (date)
			Type	Size (m)			
1	A	4–21	flat log	0.4L × 0.2W	25	2	150[a]
2	A	5–10,5–17,5–22, (13)	tire	0.65 dia.	50	14	1040[a]
3	A	5–10,5–17,5–22,6–1 (23)	stick	1.2L × 0.1D	53	10.7	2150[a] (5–17)
4	A	5–17,5–22 (6)	stick	3.4L × 0.12D	57	7	420[c] (5–17)
5	A	5–10,5–17,5–22,6–1 6–7,6–14 (36)	stick	2.8L × 0.15D	45	8.85	4700[c] (5–17)
6	A	5–17	grindstone	0.5 dia	29	1.1	1570[c] (5–17)
7	A	5–17	stick	2.7L × 0.08d	38	2	[d]
8	A	5–17,5–22 (6)	stick	1.8L × 0.15D	35	3	2350[c] (5–17)
9	A	5–17,6–1 (16)	log	3L × 0.15D	45	4.7	880[c] (5–17)
10	A	15–7	log	4.3L × 0.1D	20	1	[d]
11	A	5–17	root	1.5L × 0.2D	30	1.4	[d]
12	B	5–2,6–1 (31)	flat log	2L × 0.3W	40	21.5	3750[a] (5–2)
13	B	5–2	stick	0.5L × 0.04W	25	5	1270[b]

[a] = photographic estimate; [b] = actual count; [c] = size measurement estimate; [d] = unable to turn nest over to count eggs.

a single layer of eggs spaced somewhat evenly and tightly to one another (Fig. 7) by means of the attachment discs. Rarely were eggs laid that did not touch another egg. Nest clusters contained a range of egg numbers with a low of 150 (first nest discovered) to a high of 4700. The mean number of eggs was 1387 (N = 10, Table 2).

No direct observations of egg predation were made. However, on one occasion gut analysis of a nest guarding male revealed seven fish eggs, probably from his own nest. Also tadpole madtoms, *Noturus gyrinus*, were occasionally found underneath the same cover as nesting male darters. Inspection of the gut of one of these madtoms revealed fish eggs that probably came from the darter's nest.

The minimum number of days that nests were occupied was determined by relocating the egg clusters and nesting males (Table 2). The males of nests numbered 4, 5, 8, and 12 were tagged. Some nests could not be relocated (nests 1, 4, 7, 8, 10, 11) and another was preserved (nest 13). Of those that were relocated (nests 2, 3, 5, 9, and 12), nest occupation ranged from 13 to 36 days with a mean of 23.8 days.

The shore area searched for nests was 1820m^2 at Station A and 3300 m^2 at B. The nest densities were 6$\cdot$(100 m^2)$^{-1}$ and 0.06$\cdot$(100 m^2)$^{-1}$ for stations A and B, respectively. Nests 2, 3, and 4 were among the most closely spaced with 2.6 m between 2 and 3 and 8.5 m between 3 and 4.

The nest sites of *Etheostoma perlongum* are similar to those of the other two *Boleosoma* studied, *E. nigrum* (Winn 1958a, b, Speare 1965) and *E. olmstedi* (Atz 1940). All three species deposit their eggs on the undersides of rocks, tile, shells, and wood. However, only *E. olmstedi* and *E. perlongum* excavate depressions beneath the submerged objects. The absence of this behavior in *E. nigrum* may be due to the coarser substrate (gravel) beneath the nest sites. Other than species of *Boleosoma*, the only other darters known to spawn on the undersides of stones are five species of the subgenus *Catonotus* (Page 1975a, Page & Mayden 1979) and *E.* (*Nothonotus*) *maculatum* (Raney & Lachner 1939). Nest excavating was not reported for these species that

Fig. 7. Portion of egg cluster of nest # 3 (Table 2) of *Etheostoma perlongum*. Clutch photographed immediately after removal from water. Wood diameter = 5 cm.

are associated with coarse substrates. The nest sites of *E. flabellare* are rocks that are elevated exactly the right height above the bottom, usually 15 to 25 mm (Winn 1958a). Rocks preferred by other species of *Catonotus* are slab stones (Page 1974, 1975b, Page & Burr 1976, Page & Mayden 1979) that are elevated in a similar manner. The height of the rock above the substrate is critical to the inverted females. When resting on her dorsal fins, she can press her vent to the underside of the rock by merely erecting the dorsal fins (Winn 1958a). Nest excavaters, such as *E. perlongum*, appear to have a clear advantage because they can adjust the depth of the nest depression. This behavior appears critical for spawning success in *E. perlongum* because of the scarcity of suitable nest cover objects in Lake Waccamaw.

The number of eggs in the nests of *E. perlongum* compares well with *E. nigrum*. Winn (1958a) gave a range of 30 – 1150 and Speare (1965) stated a range of 100 – 5000 with a mean of 419. Most *Catonotus* nests, in contrast, contain fewer eggs: *E. flabellare*, 8–562 (Lake 1936); *E. squamiceps*, 8–1500 (Page 1974); *E. kennicotti* 50–400 (Page 1975b); *E. smithi*, 175–600 (Page & Burr 1976); *E. neopterum*, 577–685 (Page & Mayden 1979). Nests of *E.* (*Nothonotus*) *maculatum* contained 288–352 eggs (Raney & Lachner 1939). The smaller number of eggs in the nests of *Catonotus* and *E. maculatum* is probably due to the smaller sizes of the slab stones and the larger size of the eggs.

Spawning behavior

Seven spawnings occurred in the laboratory, four of which were observed, all in the afternoon: 6 March (1530–1625); 8 March (1245–1620); 15 March (1530–1645); 16 March (1730–1800). The three other spawnings occurred on 19 March, 4 April, and 23 April in the holding tank. The male in the large observation aquarium began preparing a nest site under the cypress stick within 24 hours of introducing the stick into the aquarium. Nest preparation consisted of fanning out a depression beneath the stick. The caudal and anal fins were used to excavate a depression deep enough to accomodate the male with erect dorsal fins. When the depression reached suitable depth, the male inverted and continued fanning while also appearing to clean the underside of the stick by nipping at its surface with his lips.

The pigmentation of the male began to intensify as nest construction commenced. The median fins darkened first, followed by the darkening of the pelvic and caudal fins. The lateral 'XW' markings on the body then formed a distinct series of 10 to 11 dark bars originating dorsally and extending and narrowing mid-ventrally. The head darkened slightly and a spot on the opercle became iridescent. The bar beneath the eye darkened.

After the nest depression was completed by the male, he periodically departed to court one of the females. The male approached a female within 10 cm of the nest and displayed the contrasting lateral body markings and the distinctly marked and darkened dorsal fins. The lateral display was often accompanied by a tail wag in the direction of the female. The female responded by returning to the nest depression area with the male. The coloration pattern of the female remained essentially undifferentiated from non-spawning females. The most noticeable aspect of the pre-spawning female was the greatly enlarged genital papilla.

After joining the male under his nest cover, the female responded to the continued lateral display of the male by circling under the nest. The male and female often exchanged tail wags in a head to head orientation. Tail wags were often followed by more circling. Circling and tail wagging also occurred in the head to tail orientation. Tail wagging by the female was usually preceded by a tail up position. Prodding or nudging of the female's flank or caudal peduncle by the male was rarely observed.

The male and female both assumed an inverted position when laying and fertilizing eggs. After tail wagging under the nest cover, the female rolled to her side that was furthest away from the nest cover and simultaneously rose to press her belly and genital area against the underside of the nest stick. Usually the male inverted simultaneously, or immediately after the female had inverted. Normally the male positioned himself parallel and slightly behind the female in an inverted head to head orientation. Occasionally the male oriented in a head to tail fashion. Part of the time the male did not invert but remained upright under the nest.

However, this did not deter the female from the egg laying process. The female held her caudal peduncle away from the spawning stick while pressing her belly and genital area against the stick. As she did so, she held her body in a weak 'S' or 'J' shape and began vibrating the trunk and tail region. The female then moved forward very slowly while vibrating and began probing the substrate with the genital papilla in search of a spot to deposit an egg. The female usually laid from one to three eggs, the male vibrated and released sperm. The vibration of the male was abbreviated and difficult to discern. Occasionally, both sexes used the erect dorsal fins to prop themselves against the substrate. Both fish simultaneously returned to an upright position under the nesting cover after the gametes had been deposited. The fish remained in an upright position for a short time before the sequence was repeated again.

A few seconds to several minutes elapsed between inverted spawning sequences. Female inversions averaged 14 seconds with a range of 0.5 – 51 sec (N=21). Male inversions averaged slightly less at 8 sec with a range of 0.5 – 14 sec (N=18). The orientation during spawning was 67% in the head to head position and 33% in the head to tail position (N=32). The vibration frequency of the female during spawning was 4.6 vibrations per second with a range of 3.6 – 5.4 (N=4).

Eggs were laid within a limited area, roughly the shape of an oblong ellipse measuring 2.5 cm by 7.5 cm. Eggs were concentrated in a single layer such that one egg usually touched another. Eggs were never laid on top of other eggs. The maximum height above the substrate at which eggs were laid was 45 mm. During the 66 female spawning attempts on 8 March, a total of 59 eggs were laid. This represents less than one egg deposited per spawning attempt.

Both females present with the male in the observation aquarium spawned. The male did not attempt to court more than one female at a time. The smaller female spawned on 6 March while the larger female spawned on March 8, 15, and 16. Eggs laid on subsequent spawnings were added to those present in the nest, thus enlarging the concentrated egg mass. Exceptions occurred on 6 March when the male apparently ate the eggs and on 8 March when all eggs were removed for developmental studies.

Spawning attempts (inversions) by both sexes and the freqency of egg deposition for the two females were compared. Individual variation in spawning behavior is indicated. A higher incidence of female attempts alone occurred on the first date (67%) as compared with the second female on the second date (27%). The male responded differently with each female. On the first date 17% of his spawning attempts were solitary while none were solitary on the second date. Actual egg deposition occurred on 84% of the spawning inversions for the first female and only 55% for the second female.

The composite results of 16 cinematographically recorded courtship and spawning sequences are shown in Figure 8 as a flow diagram. The flow diagram is based on a dyad analysis that allows the onsets of preceding and following motor patterns to be recorded as pairs. Combinations of seven female and six male motor patterns occurred at least one percent of the total preceding and following movement pairs (N=254). The movement not recorded for the male is tail up. The most frequent dyad was tail wag followed by itself in the female sequence. This dyad occurred at a frequency of 8.7% (N=22). Female tail wag was also followed by approach nest and tail wag in the male or tail up, circle, dorsal up and invert in the female. The female spawning act, which did not always result in egg deposition, most often led to inversion by the male. Sixty-four percent of the dyads were initiated by the female,

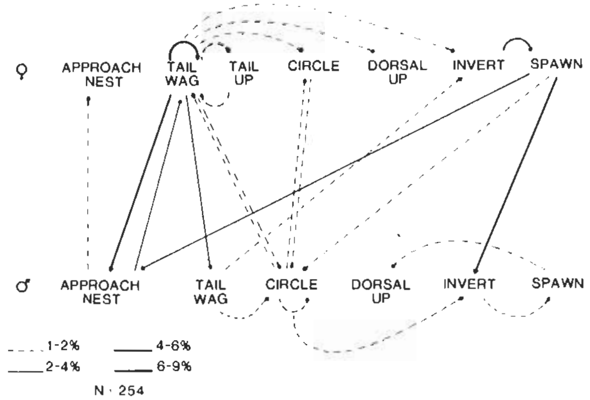

Fig. 8. Flow diagram of courtship and spawning motor patterns of *Etheostoma perlongum* based on cine analysis. Motor patterns occurring less than 1% of total are not shown.

indicating her as the more active of the pair during the filmed sequences. Furthermore, 38% of the dyads were involved in female movements followed by other female movements.

Field observations on spawning behavior were made at nest site 3 (Fig. 2, Table 2) at 1120–1150 and 1300–1400 h. Water temperature was 26° C. The nest was occupied by a 72 mm SL male who had excavated a depression 32 cm long × 2.6 cm deep below a cypress branch (Fig. 9). Approximately 2100 eggs were already present. A female, 55 mm, approached the nest and spawning activities commenced.

After the pair had completed approximately 15 or 20 spawning sequences, the female appeared to lose interest and began wandering away from the nest. The male responded by attempting to initiate spawning by inverting and moving along under the branch. As the female moved further away, the male moved toward her, displaying his dorsal fins and then moving back under the nest. It was questionable whether this display was intended to entice the female back into the nest, or whether it was an aggressive movement to run the female away from the nest. The female stopped momentarily but eventually moved beyond the male's field of view.

The spawning behavior of *E. perlongum*, *E. nigrum* and *E. olmstedi* is similar in many respects, however, some salient differences are notable. All three species show similar preliminary courtship behavior. This behavior consists of the male displaying laterally and showing the nest to the female by dashing back to the nest, inverting, and moving over the nest surface. This behavior also serves other purposes: to clean off the nest site before egg deposition; to clean and aerate the eggs after they are laid; to guard the eggs from egg predators; and to keep the female and male together for egg-laying and fertilization (Atz 1940, Winn 1958b). However, once the female joins the male under the nest cover, the males of *E. nigrum* and *E. olmstedi* both respond to the female by giving various kinds of tactile contact. The males of *E. nigrum* nudge the female prior to and during the inverted spawning activities (Winn 1958b). Atz (1940) described the male of *E. olmstedi* laying slightly over on his side with his anal fin resting against the side of the female or with his peduncle crossed over hers while both were invert-

Fig. 9. Entire nest # 3 (Table 2) of 72 mm SL male *Etheostoma perlongum*. Arrow indicates position of male at edge of nest depression. Mussels in foreground. Wood diameter = 5 cm.

ed. The function ascribed these tactile maneuvers were to prod the female *E. nigrum* to continue spawning and to press the body of the female *E. olmstedi* upward against the under surface of the nest. Body contact between male and female *E. perlongum* occurred infrequently and incidentally and thus did not appear to serve any particular function.

The orientation of the spawning partners, one to the other, was identical in the three *Boleosoma* studied. For *E. olmstedi*, Atz (1940) stated 'the partners assumed a side by side position, head to tail, or perhaps more frequently, head to head.' Winn (1958b) depicted a spawning pair of *E. nigrum* in a head to head orientation. It was also common for the female of each of the three species to invert and spawn without the inversion of the male. The male subsequently inverted and fertilized eggs deposited by the female.

Other elements of the courtship and spawning of *E. perlongum* appear to be better developed and additional in comparison to *E. nigrum* and *E. olmstedi*. The vibration of the female during spawning appeared to be ritualized since it occurred regularly, had a particular posture, and had a definable frequency range. In contrast, Atz (1940) described violent quivering during egg laying by *E. olmstedi* and Winn (1958b) stated the female *E. nigrum* usually did not vibrate but exhibited 'only a brief trembling.' We observed that *E. perlongum* frequently performed the motor patterns of tail wag, tail up and circle. These movements have not been reported for *E. nigrum* or *E. olmstedi*, which may be due to a lack of detailed observation on these species.

We agree with Winn (1958b) that the subgenus *Boleosoma* is at least as advanced, behaviorally, as *Catonotus*. However, taxonomists (e.g. Collette & Bănărescu 1977) place *Boleosoma* next to other primitive subgenera with simple non-complex spawning (non-guarding open substrate) behavior (e.g. *Ulocentra*). *Catonotus*, on the other hand, is considered by taxonomists to be one of the most advanced subgenera based on morphological criteria. Further consideration of the lack of agreement between behavior and morphological based phylogeny must await thorough analysis of all subgenera of *Etheostoma*.

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References cited

Atz, J. W. 1940. Reproductive behavior in the eastern johnny darter, *Boleosoma nigrum olmstedi* (Storer). Copeia 1940: 100–106.

Bailey, J. R. 1977. Freshwater fishes. pp. 265–98. *In*: J. E. Cooper, S. S. Robinson & J. B. Funderburg (ed.) Endangered and Threatened Plants and Animals of North Carolina, N.C. State Museum of Natural History, Raleigh.

Bailey, R. M. & W. A. Gosline. 1955. Variation and systematic significance of vertebral counts in the American fishes of the family Percidae. Misc. Publ. Univ. Mich. Mus. Zool. 93: 1–44.

Balon, E. K. 1975. Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 32: 821–864.

Balon, E. K., W. T. Momot & H. A. Regier. 1977. Reproductive guilds of percids: results of the paleogeographical history and ecological succession. J. Fish. Res. Board Can. 34: 1910–1921.

Braasch, M. E. & P. W. Smith. 1967. The life history of the slough darter, *Etheostoma gracile* (Pisces, Percidae). Illinois Nat. Hist. Surv. Biol. Notes 58: 1–12.

Burr, B. M. & L. M. Page. 1978. The life history of the cypress darter, *Etheostoma proeliare*, in Max Creek, Illinois. Illinois Nat. Hist. Surv. Biol. Notes 106: 1–15.

Cole, C. F. 1957. The taxonomy of the percid fishes of the genus *Etheostoma*, subgenus *Boleosoma*, of eastern United States. Ph.D. thesis, Cornell Univ., Ithaca. 366 pp.

Cole, C. F. 1965. Additional evidence for separation of *Etheostoma olmstedi* Storer from *Etheostoma nigrum* Rafinesque. Copeia 1965: 8–13.

Cole, C. F. 1967. A study of the eastern johnny darter, *Etheostoma olmstedi* Storer (Teleostei, Percidae). Chesapeake Sci. 8: 28–51.

Cole, C. F. 1972. Status of the darters, *Etheostoma nigrum*, *E. longimanum* and *E. podostemone* in Atlantic drainages (Teleostei, Percidae, subgenus *Boleosoma*). pp. 119–38. *In*: P. C. Holt (ed.) The Distributional History of the Biota of the Southern Appalachians Part III, Vertebrates, VA. Polytech. Inst., Blacksburg.

Collette, B. B. 1962. The swamp darters of the subgenus *Hololepis* (Pisces: Percidae). Tulane Stud. Zool. 9: 115–211.

Collette, B. B. 1965. Systematic significance of breeding tubercles in fishes of the family Percidae. Proc. U.S. Natl. Mus. 117: 567–614.

Collette, B. B. & P. Bănărescu. 1977. Systematics and zoogeography of the fishes of the family Percidae. J. Fish. Res. Board Can. 34: 1450–1463.

Deacon, J. E., G. Kobetich, J. D. Williams & S. Contreras. 1979. Fishes of North America endangered, threatened or of special concern: 1979. Fisheries 4: 29–44.

Fahy, W. E. 1954. The life history of the northern greenside darter, *Etheostoma blennioides blennioides* Rafinesque. J. Elisha Mitchell Sci. Soc. 70: 139–205.

Frey, D. G. 1951. The fishes of North Carolina's bay lakes and their intraspecific variation. J. Elisha Mitchell Sci. Soc. 67: 1–44.

Hankinson, T. L. 1908. A biological survey of Walnut Lake, Michigan. State Board Geol. Surv. Michigan Dept. 1907: 157–288.

Heimburger, H. V. 1913. The factors that determine the distribution of *Boleosoma nigrum* in Douglas Lake, Cheboygan County, Michigan. Rept. Michigan Acad. Sci. 15: 120.

Howell, W. M. & G. Dingerkus. 1978. *Etheostoma neopterum*, a new percid fish from the Tennessee River system in Alabama and Tennessee. Bull. Alabama Mus. Nat. Hist. 3: 13–26.

Hubbs, C. L. & E. C. Raney. 1946. Endemic fish fauna of Lake Waccamaw, North Carolina. Misc. Publ. Mus. Zool. Univ. Mich. 65: 1–30.

Hubbs, C., M. M. Stevenson & A. E. Peden. 1968. Fecundity and egg size in two central Texas darter populations. Southwest. Nat. 13: 301–324.

Hubbs, C. & K. Strawn. 1957. The effects of light and temperature on the fecundity of the greenthroat darter, *Etheostoma lepidum*. Ecol. 38: 596–602.

Lake, C. T. 1936. The life history of the fantailed darter *Catonotus flabellaris* (Rafinesque). Amer. Midl. Nat. 17: 816–830.

Mathur, D. 1973. Some aspects of the life history of the blackbanded darter, *Percina nigrofasciata* (Agassiz), in Halawakee Creek, Alabama. Amer. Midl. Nat. 89: 381–393.

Menhinick, E. F., T. M. Burton & J. R. Bailey. 1974. An annotated checklist of the freshwater fishes of North Carolina. J. Elisha Mitchell Sci. Soc. 90: 24–50.

Miller, R. R. 1972. Threatened freshwater fishes of the United States. Trans. Amer. Fish. Soc. 101: 239–252.

Page, L. M. 1974. The life history of the spottail darter, *Etheostoma squamiceps*, in Big Creek, Illinois, and Ferguson Creek, Kentucky. Illinois Nat. Hist. Surv. Biol. Notes 93: 1–15.

Page, L. M. 1975a. Relations among the darters of the subgenus *Catonotus* of *Etheostoma* Copeia 1975: 782–784.

Page, L. M. 1975b. The life history of the stripetail darter *Etheostoma kennicotti*, in Big Creek, Illinois. Illinois Nat. Hist. Surv. Biol. Notes 93: 1–15.

Page, L. M. 1977. The lateralis system of darters (Etheostomatini). Copeia 1977: 472–475.

Page, L. M. & B. M. Burr. 1976. The life history of the slabrock darter, *Etheostoma smithi*, in Fergusen Creek, Kentucky. Illinois Nat. Hist. Surv. Biol. Notes 99: 1–11.

Page L. M. & R. L. Mayden. 1979. Nesting site of the lollypop darter, *Etheostoma neopterum*. Trans. KY. Acad. Sci. 40: 56–57.

Page, L. M. & P. W. Smith. 1970. The life history of the dusky darter, *Percina sciera*, in the Embarras River, Illinois, Illinois Nat. Hist. Surv. Biol. Notes 69: 1–15.

Page, L. M. & P. W. Smith. 1971. The life history of the slenderhead darter, *Percina phoxocephala*, in the Embarras River, Illinois. Illinois Nat. Hist. Surv. Biol. Notes 74: 1–14.

Raney, E. C. & E. A. Lachner. 1939. Observations on the life history of the spotted darter, *Poecilichthys maculatus* (Kirtland). Copeia 1939: 157–165.

Raney, E. C. & E. A. Lachner. 1943. Age and growth of johnny dasters, *Boleosoma nigrum olmstedi* (Storer) and *Boleosoma longimanum* (Jordan). Amer. Midl. Nat. 29: 229–38.

Schenck, J. R. & B. G. Whiteside. 1977. Reproduction, fecundity, sexual dimorphism and sex ratio of *Etheostoma fonticola* (Osteichthyes: Percidae). Amer. Midl. Nat. 98: 365–75.

Speare, E. P. 1965. Fecundity and egg survival of the central johnny darter (*Etheostoma nigrum nigrum*) in southern Michigan. Copeia 1965: 308–314.

Starnes, W. C. 1977. The ecology and life history of the endangered snail darter, *Percina (Imostoma) tanasi* Etnier. Tennessee Wildlife Resources Agency Technical Report 77–52: 1–144.

Thresher, R. E. & A. M. Gronnel. 1978. Subcutaneous tagging of small reef fishes. Copeia 1978: 352–353.

Tsai, C. 1972. Life history of the eastern johnny darter, *Etheostoma olmstedi* Storer, in cold tailwater and sewage-polluted water. Trans. Amer. Fish. Soc. 101: 80–88.

Williams, J. D. 1977. Proposed endangered status and critical habitat for five fishes. Federal Register 42: 65209–65211.

Winn, H. E. 1953. Breeding habits of the percid fish *Hadropterus copelandi* in Michigan. Copeia 1953: 26–30.

Winn, H. E. 1958a. Observations on the reproductive habits of darters (Pisces - Percidae). Amer. Midl. Nat. 59: 190–212.

Winn, H. E. 1958b. Comparative reproductive behavior and ecology of fourteen species of darters (Pisces:Percidae). Ecol. Monog. 28: 155–191.