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Life Histories of the Bandfin Darter, *Etheostoma zonistium*, and the Firebelly Darter, *Etheostoma pyrrhogaster*, in Western Kentucky

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Life Histories of the Bandfin Darter, *Etheostoma zonistium*, and the Firebelly Darter, *Etheostoma pyrrhogaster*, in Western Kentucky

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Bailey and Etnier (1988) described the bandfin darter, *Etheostoma zonistium*, from Leath Creek, Chambers Creek drainage, Hardin County, Tennessee, and the firebelly darter, *E. pyrrhogaster*, from Clear Creek, North Fork Obion River drainage, Henry County, Tennessee. Both species are members of the subgenus *Nanostoma*, which encompasses *E. zonale* and all species previously referred to in the subgenus *Ulocentra* (Page 1981). *Nanostoma* includes thirteen taxonomically described species, eight of which were described as new species (Bouchard 1977; Page & Burr 1982; Bailey & Etnier 1988; Etnier & Bailey 1989) or elevated from synonymy (Etnier & Starnes 1986) in recent years. At least five species of *Nanostoma* remain taxonomically undescribed.

Substantial life history information is available for four species of *Nanostoma*: *Etheostoma zonale* (Lachner et al. 1950), *E. coosae* (O'Neil 1981), *E. simoterum* (Page & Mayden 1981), and *E. baileyi* (Clayton 1984). In addition, the reproductive behavior of *E. duryi* (Page et al. 1982) and *E. rafinesquei* and *E. barrenense* (Winn 1958a, b; Stiles 1974) has been described. Other publications discuss fecundity and spawning substrate (Weddle 1989), distributions (Bouchard 1972, 1973, 1974; Kuehne 1972; Fallo & Warren 1982; Burr & Warren 1986), interspecific interactions (Greenburg 1984, 1985), food habits (Adamson & Wissing 1977; Cordes & Page 1980), respiration and habitat selection (Ultsch et al. 1978), and evolutionary trends in reproduction, morphology, and ecology (Page & Swofford 1984; Page 1985) of species of *Nanostoma*. Finally, Kuehne and Barbour (1983) and Page (1983) provide summaries of the natural history of members of *Nanostoma*.

This paper was submitted in its original form by Douglas A. Carney in partial fulfillment of the requirements for the degree of Master of Arts in the Graduate School of Southern Illinois University at Carbondale. Mr. Carney was formerly a Technical Research Biologist with the Illinois Natural History Survey and is now a Resource Planner with the Illinois Department of Conservation. Dr. Brooks M. Burr is a Professor in the Department of Zoology at Southern Illinois University at Carbondale and an Affiliate of the Survey.

Etheostoma zonistium occurs in lower western tributaries of the Tennessee River in western Kentucky and Tennessee, barely extending into northern Mississippi and Alabama, and in the Hatchie River drainage in Tennessee and the Black Warrior River drainage in Alabama (Fig. 1). *Etheostoma pyrrhogaster* occurs in tributaries of the Mississippi River in extreme southwestern Kentucky and in western Tennessee (Fig. 1). In Tennessee (Starnes & Etnier 1980) and in Kentucky (Warren et al. 1986), *E. pyrrhogaster* is regarded as a species of special concern because of its restricted distribution and the loss of habitat resulting from the channelization of streams.

Methods

Observations and minnow-seine (1.2 m \times 3.1 m, 3.8-mm mesh) collections were made at approximately monthly intervals from 5 January to 13 November 1983. Additional observations and collections were made during spawning periods in 1983 and 1984. Data collected at West Fork Clarks River and at Terrapin Creek on each sampling date included stream conditions, darter habitat, and darter behavior. Water velocity in darter habitat was measured during spawning periods with a Teledyne-Gurley Pygmy current meter.

Fishes captured with the two darter species and the habitats in which these captures occurred were quantified in a series of three monthly samples (May–July 1983). Unit-effort sampling consisted of five seining efforts in each of four subjectively identified habitat types:

Riffles, defined as shallow areas with swift current and surface turbulence over gravel and sand substrate.

Raceways, defined as moderately shallow areas with swift current and little to no surface turbulence over gravel and sand substrate.

Stream margin zones, defined as areas of moderate depth and current next to a bank (often undercut) and primarily over sand (sometimes sand with large stones or gravel).

Pools, defined as deep areas with sluggish current over sand and detritus.

Kick-sets were used to capture fish in all habitat types except pools, where seine hauls were made. All captured fishes in each habitat type were identified and enumerated.

Unit-effort sampling comprised 60 seining efforts per study site (all habitat types combined). To facilitate discussion, an arbitrary rating of darter abundance at each study site was established for quantified sampling efforts:

Abundant, at least 60 individuals of a given species captured.

Common, 12 to 59 individuals.

Uncommon, 6 to 11 individuals.

Rare, fewer than 6 individuals.

Specimens were preserved in 10 percent formalin and stored in 70 percent ethanol. Totals of 336 *E. zonistum* from West Fork Clarks River and 164 *E.*

pyrrhogaster from Terrapin Creek were preserved and examined. Additional specimens from the ichthyological collection at Southern Illinois University at Carbondale were examined, and all darters collected during the study were deposited at that facility.

All specimens were sexed, measured, and aged. Sexing was by examination of the genital papillae or, when necessary, by examination of the gonads. Measurements were to the nearest 0.1 mm standard length (SL). Age-to-year class was determined by counting annuli on scales removed from the dorsum near the junction of the first and second dorsal fins. Year-classes were corroborated by analysis of length-frequency distribution and number of annual rings on otoliths (sagittae). Otoliths were removed by making an incision from the breast to the sacculus and bending the head back until the sagittae were exposed. Whole sagittae were immersed in glycerine, and annuli were counted with the aid of a fiber-optic light and dissecting microscope. Age to month was determined by using April, the month of greatest spawning activity, as month zero.

Seasonal variation in the reproductive conditions of males and females was determined by the gonadosomatic index (GSI). Gonad weight and adjusted body weight (weight of the specimen minus gonads, stomach, intestine, and liver) of alcohol-stored specimens (blotted dry) were measured to the nearest 0.001 gm on an analytical balance.

Ovaries of preserved specimens were dissected, and oocytes were selected at random to create a subset of total ova for diameter measurements. All fertilized eggs recovered from aquaria were measured. Fertilized eggs were incubated in an aerated malachite green solution. The larval terminology used was that of Snyder (1976). Diameters of ova and lengths of larvae and genital papillae were measured to the nearest

0.01 mm with an ocular micrometer. Ovum diameter was calculated as the mean of three measurements.

Spawning behavior was observed in the laboratory in 71-liter (30 cm high \times 25 cm wide \times 91 cm long) aquaria. Adults in spawning condition were given a choice of all known egg deposition sites used by other species of darters (Page 1983:165): vertical rock, vertical plant, raised horizontal rock, and a substrate of mixed gravel and sand.

Regression coefficients were calculated by the method of least squares using the Systems Regression procedure of the Statistical Analysis System (SAS). Correlations were Pearson product-moments. In statistical tests, differences of $P < 0.05$ were considered significant.

Study Areas

The site selected for study of *Etheostoma zonistum* was West Fork Clarks River (Tennessee River drainage) at the Kentucky Highway 121 bridge, 0.6 km east of Coldwater, Calloway County, Kentucky (Fig. 2). At this site, the stream is of moderate gradient and consists of shallow riffles with gravel and sand substrate alternating with deep, sand-bottomed pools. Widths of 2 to 6 m and depths to 1.0 m are characteristic of the study area during normal flow. Water is generally clear but becomes turbid during flooding. Agricultural lands are immediately adjacent to parts of the stream; however, a narrow margin of deciduous trees lines most of the study site. During 1983 monthly water temperature ranged from 8.5°C in January to 25°C in July.

Nineteen fish species were collected from West Fork Clarks River. Species taken, in addition to those captured in the stream margins during unit-effort sampling (Table 1), were *Notropis lutrensis*, *N. umbratilis*, *N. whipplei*, *Phenacobius mirabilis*, *Moxostoma*

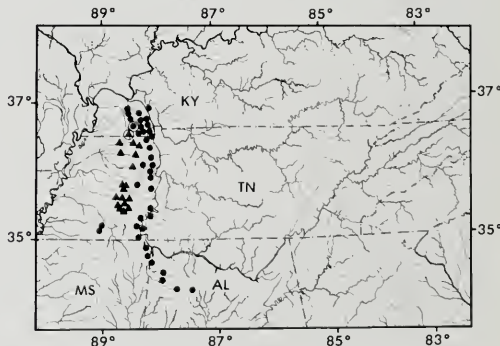


Figure 1. Ranges of *Etheostoma zonistum* (circles) and *E. pyrrhogaster* (triangles). Life history study sites are enclosed by open circles. Map modified from Bailey and Etnier (1988).

erythrum, *Ameiurus natalis*, *Noturus nocturnus*, *Lepomis cyanellus*, and *Percina maculata*.

Etheostoma pyrrhogaster was studied in Terrapin Creek (Obion River drainage) just north (upstream) of the Tennessee Highway 69 bridge at the Kentucky-Tennessee border, Graves County, Kentucky (Fig. 3). Terrapin Creek has a low-to-moderate gradient and consists of sand- and gravel-bottomed riffles alternating with sand-bottomed pools. Widths of 2 to 5 m and depths to 1.5 m are characteristic of Terrapin Creek at normal flow. The water, as in West Fork Clarks River, is turbid only during flooding. The study area is immediately bordered by deciduous forest; however, agricultural land is nearby. During 1983, monthly water temperatures ranged from 8°C in January to 20°C in June.

On 27 March and 18 April 1983, *E. pyrrhogaster* was taken for aquarium spawning observation from a backwater slough of Terrapin Creek, 0.8 km south of Bell City, Graves County, Kentucky. The slough was

1 m wide with depths to 30 cm and had water temperatures of 14°C and 17°C on the above sampling dates, respectively. A silt substrate and abundant aquatic vegetation were present in the slough, which was surrounded by deciduous forest.

Fishes collected in Terrapin Creek and the backwater slough during this study, in addition to those taken during unit-effort sampling of stream margins and pools (Table 2), include *Umbra limi*, *Notropis fumeus*, *Pimephales promelas*, *Moxostoma poecilurum*, *Noturus hildebrandi*, *Fundulus olivaceus*, *Elassoma zonatum*, *Aphredoderus sayanus*, *Etheostoma chlorosomum*, *E. gracile*, and *E. parvipinne*. Other fishes reported from the study area by Walsh and Burr (1981) but not taken during this study were *Esox americanus*, *Notemigonus*

Table 1. Relative abundance of fishes captured with *Etheostoma zonistium* in West Fork Clarks River, expressed as percent of individuals ($N = 167$) captured by unit-effort seining of stream margin habitat in May, June, and July 1983.

Species	Percentage
<i>Etheostoma zonistium</i>	44.3
<i>Campostoma oligolepis</i>	22.2
<i>Pimephales notatus</i>	17.4
<i>Semotilus atromaculatus</i>	7.8
<i>Hypentelium nigricans</i>	2.4
<i>Fundulus olivaceus</i>	1.8
<i>Erimyzon oblongus</i>	1.2
<i>Etheostoma flabellare</i>	1.2
<i>Etheostoma neopterum</i>	1.2
<i>Micropterus punctulatus</i>	0.6

Table 2. Relative abundance of fishes captured with *Etheostoma pyrrhogaster* in Terrapin Creek, expressed as percent of individuals ($N = 221$) captured by unit-effort seining of stream margin and pool habitat in May, June, and July 1983.

Species	Percentage
<i>Etheostoma nigrum</i>	31.7
<i>Semotilus atromaculatus</i>	21.7
<i>Etheostoma lyncem</i>	16.3
<i>Notropis camurus</i>	6.3
<i>Etheostoma pyrrhogaster</i>	6.3
<i>Lampetra aetoptera</i>	5.4
<i>Phenacobius mirabilis</i>	3.6
<i>Percina sciera</i>	3.2
<i>Noturus phaeus</i>	1.0
<i>Gambusia affinis</i>	1.0
<i>Lepomis marginatus</i>	1.0
<i>Etheostoma swaini</i>	1.0
<i>Erimyzon oblongus</i>	0.5
<i>Minytrema melanops</i>	0.5
<i>Lepomis cyanellus</i>	0.5
<i>Lepomis macrochirus</i>	0.5



Figure 2. Study area for *Etheostoma zonistium*, West Fork Clarks River, Calloway County, Kentucky, 18 April 1983.



Figure 3. Study area for *Etheostoma pyrrhogaster*, Terrapin Creek, Graves County, Kentucky, 22 July 1983.

crystoleucas, *Notropis umbratilis*, *Ameiurus natalis*, *A. melas*, *Lepomis gulosus*, and *L. megalotis*. A total of 34 fish species are known to occur in the Terrapin Creek study area.

Habitat and Associated Species

In West Fork Clarks River, *Etheostoma zonistium* was frequently collected over a substrate of sand or gravel and sand near the stream margin at depths of 15 to 75 cm and was often found in emergent soft rush (*Juncus effusus*). Unit-effort seining by habitat type confirmed the observation that *E. zonistium* typically inhabited the stream margin (Fig. 4). The species was abundant in West Fork Clarks River as was *E. flabellare*; however, the two were weakly associated due to the propensity of *E. flabellare* for riffles (Fig. 4). Analysis of a 2×4 contingency table of these species versus habitats by the log likelihood ratio test (Sokal & Rohlf 1981) indicated a significant association ($G = 148.65$, $P < 0.001$) between species and habitat. *Etheostoma neopterum* was rare at the study site but was found with *E. zonistium* in the stream margin (Fig. 4), a habitat commonly noted for *E. neopterum* (Howell & Dingerkus 1978; Braasch & Mayden 1985). Fishes captured in greatest numbers with *E. zonistium* were *Campostoma oligolepis*, *Pimephales notatus*, and *Semotilus atromaculatus* (Table 1).

In Terrapin Creek, *E. pyrrhogaster* typically inhabited the stream margin at depths of 30 cm to 1 m but also occurred in deeper pools, often among exposed tree roots in undercut banks over sand. Unit-effort sampling indicated an affinity of individuals ($N = 8$) for stream margin habitats; however, a number of individuals ($N = 6$) were also recovered from pools (Fig. 5). *Etheostoma pyrrhogaster* was classified as uncommon in Terrapin Creek with 18 individuals captured during the unit-effort seining (Fig. 5). Although too few individuals were captured to warrant statistical analysis of the association between species and habitat, the data represent a reliable estimate of the habitat affinity of *E. pyrrhogaster* in Terrapin Creek as judged from nonquantitative observations during the course of the study. Fishes collected in greatest numbers with *E. pyrrhogaster* were *E. nigrum*, *Semotilus atromaculatus*, and *E. lyneceum* (Table 2, Fig. 5).

Page and Mayden (1981) described characteristic *Nanostoma* habitat as clean pools with moderate current over bedrock, cobble, or gravel. The relatively fine substrates over which *E. zonistium* and *E. pyrrhogaster* were taken, in comparison with other members of *Nanostoma*, were indicative of their lowland stream habitats.

Reproductive Cycle of the Male

Male *Etheostoma zonistium* and *E. pyrrhogaster* developed brilliant nuptial coloration as described by Bailey and Etnier (1988). Color intensification began as early as

October in individuals over 1 year of age. Two-year-old males of both species collected in October were dark red above the lateral line and orange-red ventrolaterally. At this time ventrolateral coloration extended from the origin of the pelvic fin to the origin of the caudal fin in *E. zonistium* and from the origin of the pelvic fin to midway through the caudal fin in *E. pyrrhogaster*. By late March ventrolateral body coloration was bright red midway through the caudal fin in *E. zonistium* and throughout all but the margins of the caudal fin in *E. pyrrhogaster*. Commencement of spawning activity in aquaria was accompanied by a change in color of the chin and breast from blue-gray to lavender in *E. zonistium* and from gray-green to green in *E. pyrrhogaster*. By mid-July nuptial coloration in field-collected individuals had faded considerably; however, males of both species retained some red on the body and dorsal fins throughout the year.

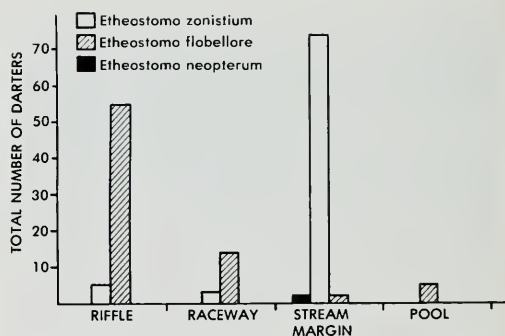


Figure 4. Number of darters captured in unit-effort seining by habitat type, West Fork Clarks River, Calloway County, Kentucky, May–July 1983.

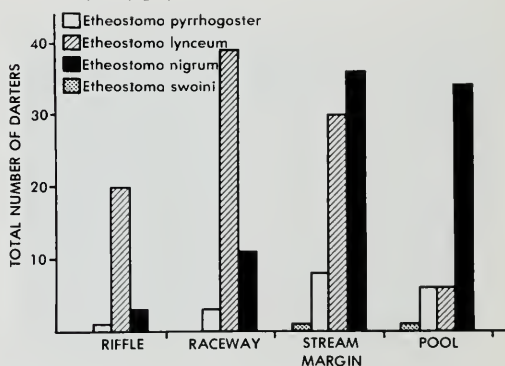


Figure 5. Number of darters captured in unit-effort seining by habitat type, Terrapin Creek, Graves County, Kentucky, May–July 1983.

The genital papilla of male *E. zonistium* was a white to cream, somewhat flattened, broad-based structure that terminated in a short tube (Fig. 6A, B). Mean papilla length was 0.8 mm (range 0.5–1.1, $sd = 0.15$) in 32 males that ranged in SL from 32 to 50 ($\bar{x} = 40.8$) mm. Genital papillae of male *E. pyrrhogaster* were identical in structure and color to those of *E. zonistium*. Mean papilla length was 0.7 mm (range 0.5–1.0, $sd = 0.11$) in 29 males that ranged in SL from 32.8 to 49.7 ($\bar{x} = 41.2$) mm.

For both species, size and weight of testes gradually increased as the spawning season approached. For male *E. zonistium*, the relationship between weight of the testes $\times 1000$ /adjusted body weight (Y) and the month (X), with October = 1 and May = 8, was $\log Y = 0.152 + 0.323X$, with $r = 0.932$ (Fig. 7). In male *E. pyrrhogaster*, the relationship, with October = 1 and June = 9, was $\log Y = 0.391 + 0.275X$, with $r = 0.852$ (Fig. 8).

All 1-year-old males collected in April ($N = 11$ *E. zonistium*, $N = 9$ *E. pyrrhogaster*) had enlarged testes and were potential spawners. The proportionally largest testes in *E. zonistium* equaled 1.6 percent of the adjusted body weight and were found in a 31-mm SL male collected on 18 April 1983. The proportionally

largest testes in *E. pyrrhogaster* equaled 1.4 percent of the adjusted body weight and were taken from a 37-mm SL male also collected on 18 April 1983.

Reproductive Cycle of the Female

Coloration of females showed no seasonal variation. Bright colors in female *Etheostoma zonistium* included a red ocellus in the first interradiar membrane and a red band in the distal one-half of the spinous dorsal fin. Female *E. pyrrhogaster* also had a red ocellus; in some individuals, a submedian red band was apparent in the second dorsal fin.

The genital papillae of female *E. zonistium* (Fig. 6C, D) and *E. pyrrhogaster* were thick, tubular structures white to cream in color. In *E. zonistium*, mean papilla length was 1.2 mm (range 0.8–1.7, $sd = 0.20$) in 47 females ranging in SL from 29.4 to 45.0 ($\bar{x} = 41.2$) mm. Mean papilla length of female *E. pyrrhogaster* was 1.3 mm (range 1.0–1.6, $sd = 0.18$) in 31 individuals ranging in SL from 30.8 to 44.5 ($\bar{x} = 39.4$) mm. As females became distended with maturing ova, the genital papillae shifted in position from lying against the abdomen to an angle varying from acute to nearly perpendicular to the abdominal wall. This change in position was accompanied by a change in the distal

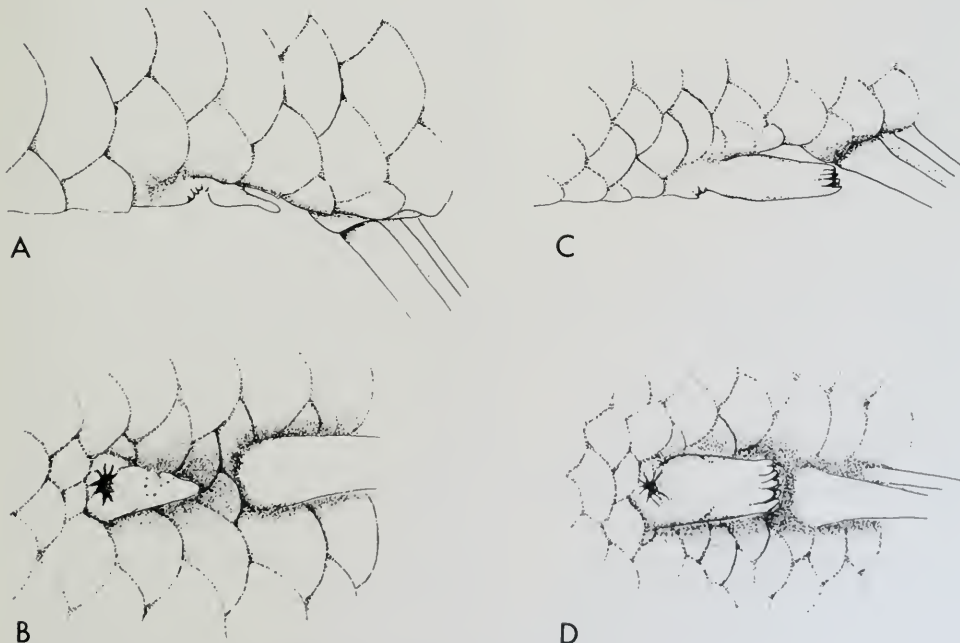


Figure 6. Genital papillae of *Etheostoma zonistium* from West Fork Clarks River, 18 April 1983: A) breeding male 43 mm SL, lateral view; B) ventral view of A; C) breeding female 33 mm SL, lateral view; D) ventral view of C.

end of the papilla from somewhat rounded to flattened, as the diameter of the gonopore increased. Increased abdominal girth and change in position of the genital papillae were first evident in late February for females of both species.

Small white ova appeared in females of both species in November. Intermediate-sized yellow ova were present in each species in February, and by late March mature ova were apparent. Mature ova were translucent with a pitlike indentation. This indentation, also present in other darters (Page 1983:162), has recently been shown to serve as a micropyle (J. M. Grady, pers. comm.).

Mean diameter of white ova in November-collected *E. zonistium* was 0.24 mm (range 0.17–0.34, $SD = 0.062$, $N = 20$). January-collected *E. pyrrhogaster* had white ova with a mean diameter of 0.26 mm (range 0.15–0.41, $SD = 0.068$, $N = 20$). Mean diameters of yellow ova in March-collected females were 0.78 mm (range 0.50–0.99, $SD = 0.180$, $N = 20$) in *E. zonistium* and 0.68 mm (range 0.54–0.86, $SD = 0.098$, $N = 20$) in *E. pyrrhogaster*. Mean diameters of mature ova in March-collected females were 1.08 mm (range 1.01–1.17, $SD = 0.038$, $N = 20$) in *E. zonistium* and 1.09 mm (range 1.02–1.18, $SD = 0.048$, $N = 20$) in *E. pyrrhogaster*.

Counts of yellow and mature ova in 1-year-old *E. zonistium* ranged from 41 to 137 ($\bar{x} = 77.0$, $SD = 25.17$, $N = 9$) (Table 3). In contrast, females of 1-year-old *E. pyrrhogaster* contained from 0 to 84 ($\bar{x} = 28.4$, $SD = 23.85$, $N = 9$) yellow and mature ova (Table 4). Similar counts of 2- to 3-year-old females ranged from 98 to 155 ($\bar{x} = 127.5$, $SD = 20.25$, $N = 4$) in *E. zonistium* and from 115 to 197 ($\bar{x} = 144.3$, $SD = 37.32$, $N = 3$) in *E. pyrrhogaster*.

Etheostoma simoterum females 12 months old contained 110 to 240 mature (orange or translucent) ova (Page & Mayden 1981). Total egg complements ranged from 288 to 496 ($\bar{x} = 382.8$) in *E. coosae* (O'Neil 1981) and 228 to 864 ($\bar{x} = 481.2$) in *E. baileyi* (Clayton 1984). Winn (1958b) estimated that 1- and 2-year-old female *E. barrenense* lay an average of 364 and 798 eggs per spawning season, respectively. His estimates are based on the premise that all ova present in the ovaries were spawned in a season.

Some darters spawn more than one egg clutch per year (Hubbs 1985), as demonstrated for *E. (Boleosoma) olmstedii* (Gale & Deutsch 1985) and *E. rafinesquei* (Weddle 1989). Both species were shown to spawn over a period of several weeks at intervals ranging from 3 to 16 days (Gale & Deutsch 1985; Weddle 1989). One

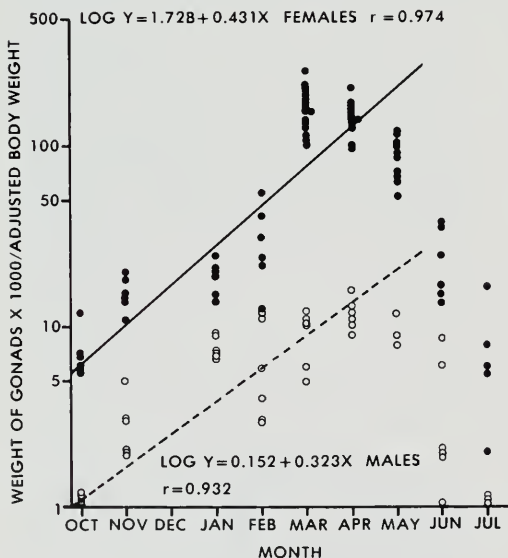


Figure 7. Monthly variation in gonad weight relative to adjusted body weight in *Etheostoma zonistium*. Dots represent individual females; circles represent individual males. Data points for June and July were not included in the analysis but are presented to illustrate the decline in relative gonad weights.

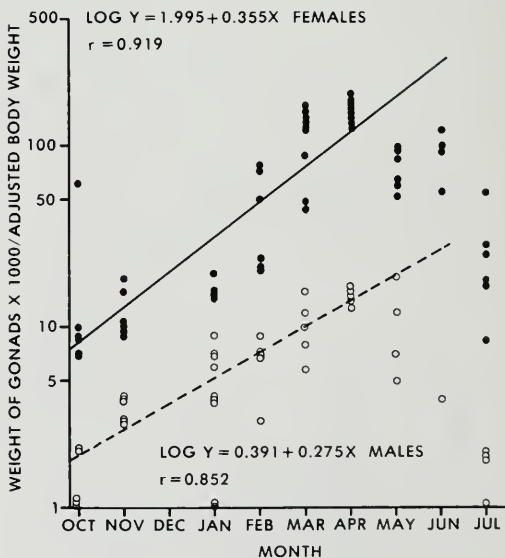


Figure 8. Monthly variation in gonad weight relative to adjusted body weight in *Etheostoma pyrrhogaster*. Dots represent individual females; circles represent individual males. Data points for July were not included in the analysis but are presented to illustrate the decline in relative gonad weight.

female *E. rafinesquei* spawned 13 clutches, a total of 780 eggs (Weddle 1989). These studies also suggest that mature ova are recruited throughout the season and that darters collected early or late in the season would contain lower numbers of mature ova than those collected during the peak spawning period. If *E. zonistium* and *E. pyrrhogaster* also produce multiple clutches, our counts of yellow and mature ova do not approximate seasonal fecundity; however, they may represent two estimates of successive clutch sizes. We agree with Gale and Deutsch (1985) and Weddle (1989) that fecundity estimates based upon single-day ova counts may grossly underestimate seasonal fecundity.

In *E. zonistium* the relationship between month (X), with October = 1 and May = 8, and weight of ovaries $\times 1000/\text{adjusted body weight}$ (Y) was $\log Y =$

$1.728 + 0.431X$, $r = 0.974$ (Fig. 7). The proportionally largest ovaries (equaling 26.3% of the adjusted body weight) were found in a 34.9-mm SL female collected on 27 March 1983. In *E. pyrrhogaster* females the relationship, with October = 1 and June = 9, was $\log Y = 1.995 + 0.355X$, $r = 0.919$ (Fig. 8). The proportionally largest ovaries (equaling 19.4% of adjusted body weight) were found in a 31.8-mm SL female collected on 18 April 1983.

All female *E. zonistium* and most female *E. pyrrhogaster* examined were sexually mature by the end of their first year of life. Individuals that failed to reach maturity within 1 year were female *E. pyrrhogaster* less than 27 mm SL (Table 4). Samples of *E. pyrrhogaster* taken on 27 March, 18 April, and 17 May 1983 yielded 29 first-year females, 7 (24%) of which were immature.

Table 3. Size, age, ovary weight, and number of ova from female *Etheostoma zonistium* collected on 27 March 1983.

Standard length	Adjusted body weight (g) ^a	Age (months)	Ovary weight (g)	Number of yellow ova	Number of mature ova	Number of yellow and mature ova
26.5	0.200	11	0.028	38	20	58
26.6	0.181	11	0.019	41	0	41
29.0	0.264	11	0.047	74	0	74
30.9	0.312	11	0.062	72	55	137
31.2	0.302	11	0.048	60	0	60
32.4	0.409	11	0.067	77	0	77
34.9	0.514	11	0.135	0	78	78
35.1	0.482	11	0.061	79	0	79
36.8	0.541	11	0.093	89	0	89
42.1	0.841	23	0.175	20	135	155
42.3	0.932	23	0.146	14	117	131
42.8	0.995	23	0.134	98	0	98
45.0	1.072	35	0.138	126	0	126
\bar{x} 35.1	0.543	16	0.089	61	31	93

^aWeight of the specimen minus gonads, stomach, intestine, and liver.

Table 4. Size, age, ovary weight, and number of ova from female *Etheostoma pyrrhogaster* collected on 27 March 1983.

Standard length	Adjusted body weight (g) ^a	Age (months)	Ovary weight (g)	Number of yellow ova	Number of mature ova	Number of yellow and mature ova
24.5	0.137	11	0.001	0	0	0
25.6	0.175	11	0.003	0	0	0
26.1	0.168	11	0.002	0	0	0
27.2	0.199	11	0.018	27	0	27
28.3	0.243	11	0.011	9	0	9
29.0	0.247	11	0.012	23	0	23
29.8	0.312	11	0.042	37	24	61
30.7	0.342	11	0.044	52	0	52
31.5	0.275	11	0.055	63	21	84
42.6	0.901	23	0.154	115	0	115
43.7	1.000	23	0.153	95	102	197
44.5	1.125	35	0.150	68	53	121
\bar{x} 29.5	0.402	14	0.050	38	15	53

^aWeight of the specimen with gonads, stomach, intestine, and liver removed.

Spawning

Observations suggested that both species began spawning in March and continued to May or June (Tables 3 & 4; Figs. 7 & 8). Spawning appeared to peak in March and April, with declines in the gonadosomatic index thereafter (Figs. 7 & 8). Low values for female *Etheostoma zonistium* collected in June (range 18–48, \bar{x} = 30.5, N = 6) and males (range 1–9, \bar{x} = 4.2, N = 6) (Fig. 7) suggested little spawning potential during that month. Female *E. pyrrhogaster* collected in June maintained relatively higher values (Fig. 8) and large yellow ova, but the four specimens captured lacked mature ova.

Water temperatures in both West Fork Clarks River and Terrapin Creek ranged from 11°C on 27 March to 17°C on 17 May 1983. On 18 June 1983, water temperature was 21°C in West Fork Clarks River and 20°C in Terrapin Creek.

Spawning occurs primarily in April among other species of *Nanostoma* with distributions limited to the southeastern United States; for example, *E. barrenense* and *E. rafinesquei* (Stiles 1974; Page & Burr 1982), *E. coosae* (O'Neil 1981), and *E. simotermum* (Page & Mayden 1981). May was reported to be the most active month of spawning for *E. baileyi* (Clayton 1984). The spawning period for *E. zonale* is May and June in Pennsylvania (Lachner et al. 1950) and April and May in Missouri (Pflieger 1975:312).

Etheostoma zonistium was removed from the study site on 27 March 1983 (N = 6) and 12 April 1984 (N = 8) for observation of spawning in aquaria. Stream temperatures were 11°C and 14°C on those dates, respectively. Darters in breeding condition were collected near the stream margin at depths of 38 to 46 cm over sand and gravel. Current speed ranged from 0.26 to 0.32 m/sec at sites of capture. Ripe individuals were most common among emergent vegetation, tree roots, and brush piles. Attempts to observe spawning in the stream were unsuccessful.

Etheostoma pyrrhogaster was removed from the backwater slough on 18 April 1983 (N = 5) and from Terrapin Creek proper on 12 April 1984 (N = 8) for observation of spawning in aquaria. Stream temperature was 14°C on both dates. Darter collection sites in Terrapin Creek on 12 April 1984 had moderate flow (0.31 to 0.40 m/sec) with depths of 27 to 38 cm over sand and stones. *Etheostoma pyrrhogaster* was captured primarily near the bank among tree roots and/or large stones (10 to 20 cm in diameter). The presence of ripe adults in the backwater slough (see Study Areas for habitat characteristics) suggests that this area may have been used for spawning.

Spawning *E. zonistium* were observed in aquaria from 28 March to 1 April 1983 and from 14 to 17 April

1984; water temperature ranged from 17 to 22°C.

Individuals of *E. pyrrhogaster* were injected with human chorionic gonadotropin (approximately 50 IU/fish) on 20 April 1983 after it appeared that an increase in water temperature was not sufficient to induce spawning in captivity. Subsequent to hormone injections, these darters spawned in aquaria on 21 April 1983 at a water temperature of 22°C. Individuals collected in April 1984 did not spawn in the aquarium before or after injection.

The spawning behaviors of *E. zonistium* and *E. pyrrhogaster* were virtually identical. A male approached a female and followed her, crossing over her dorsum in a zigzag pattern. The male often rubbed his pectoral fin, and occasionally his chin, on the female's nape. The male then followed the female to an egg deposition site. Once the site was selected, the male assumed an S-shaped posture over the female with his caudal peduncle placed adjacent to hers. While in this position, they vibrated rapidly and a single egg was deposited and fertilized. During egg deposition, the female had her mouth open. Open-mouth behavior was previously noted in spawning *Etheostoma proeliare* (Burr & Page 1978) and *E. asprigene* (Cummings et al. 1984). A period of inactivity (approximately 30 sec) followed each spawning act. Eggs were occasionally eaten by the parents. Spawning darters were promiscuous; for example, a male spawned with several females, and a female spawned with several males. Agonistic encounters between rival males occurred during and just prior to spawning. An area of approximately 15 cm in diameter was defended by males from intrusion by rivals. The largest males were generally dominant and spawned more frequently. Females congregated in a loosely knit group when not engaged in spawning. Occasionally a female would leave the group and swim in front of a male that was following another female. The male then followed the intruding female and spawned with her.

Frequency of spawning acts by males of each species was recorded during peak spawning activity. In observations of two rival males of *E. zonistium*, a 47-mm SL male spawned an average of once every 3 min and a 48.7-mm SL male spawned an average of once every 2.1 min over a 30-min period. During this time 11 aggressive encounters occurred between the two males, one encounter every 2.7 min. Similarly, a male *E. pyrrhogaster* (49.7 mm SL) was noted to spawn about every 2.5 min during 30 min of observation.

Etheostoma zonistium spawned vertically on aquarium corners and walls and on vertical rocks and plants (*Ceratophyllum demersum*). Eggs deposited on rocks were generally placed in small crevices. The species spawned horizontally on sand and gravel substrate (Fig. 9) and

on the top and the underside of raised horizontal rocks. The two most frequent spawning/egg deposition sites of *E. zonistium* were vertical corners of the aquarium and sand and gravel substrate.

Etheostoma pyrrhogaster spawned vertically on aquarium corners and on rocks. The most frequent spawning site observed in the aquarium was sand and gravel substrate at the aquarium wall interface. They spawned in a horizontal position at this site.

On 14 April 1984, an attempt was made to induce interspecific spawning between *E. zonistium* and *E. pyrrhogaster*. Male *E. pyrrhogaster* ($N = 2$) were placed with female *E. zonistium* ($N = 5$) in an aquarium. Conversely, male *E. zonistium* ($N = 2$) and female *E. pyrrhogaster* ($N = 2$) were combined. No spawning attempts were observed between male *E. pyrrhogaster* and female *E. zonistium*. One spawning attempt was observed within 1 hour of introduction between a male *E. zonistium* and a female *E. pyrrhogaster*; however, egg deposition either did not occur or the egg was quickly ingested by one of the parents. Behavior during this interspecific spawning act did not deviate from the general sequences described earlier. Agonistic behavior between males and the courting of females continued for 4 hours, but females were unreceptive. All darters in the interspecific spawning experiment were subsequently injected with human chorionic gonadotropin, but no further breeding behavior was observed.

Winn (1958a) observed spawning between a male *E. barrenense* and a female *E. rafinesquei* that resulted in fertilized eggs. Males of these two species of *Nanostoma* display dissimilar dorsal fin banding patterns (Page & Burr 1982), and these patterns are believed to be a factor in sex and/or species recognition (Page 1983: 180). Winn (1958b) concluded that characteristic movements are important in sex recognition by darters during spawning. Dorsal fin patterns of *E. zonistium* and *E. pyrrhogaster* are dissimilar yet interspecific

spawning was attempted, presumably because of the similar behavior patterns of these species.

In aquaria, *E. zonistium* and *E. pyrrhogaster* attached eggs to a site and left them unattended; that is, they were egg attachers as defined by Page (1983:165, 167; 1985). All other members of *Nanostoma* for which reproductive behaviors have been reported are also egg attachers: *E. zonale* (Pflieger 1975:312; Trautman 1957:663), *E. simoterum* (Page & Mayden 1981), *E. rafinesquei* and *E. barrenense* (Winn 1958a, b), *E. coosae* (O'Neil 1981), and *E. duryi* (Page et al. 1982). Horizontal spawning on the substrate was observed in *E. pyrrhogaster* and to a lesser extent in *E. zonistium*. When spawning on the substrate, these species placed eggs on the surface of the gravel, a behavior not associated with typical egg-buriers. Members of *Nanostoma* have most frequently been observed spawning in a vertical position on the sides of rocks; for example, *E. simoterum* (Page & Mayden 1981), *E. rafinesquei*, *E. barrenense* (Winn 1958a, b; Page & Burr 1982), and *E. duryi* (Page et al. 1982). A female *E. simoterum* occasionally buried eggs in gravel but not as deeply as more typical egg-burying species (Page & Mayden 1981). *Etheostoma coosae* spawns at angles from horizontal to vertical and in an inverted position on most inclined, creviced surfaces (O'Neil 1981). The high incidence of horizontal substrate spawning observed in *E. zonistium* and *E. pyrrhogaster* may be related to the constraints of aquarium confinement (Winn 1958b) or to their lowland stream habitat, which offers little or no bedrock and few large rock surfaces.

Aggressive behavior among male *E. zonistium* and *E. pyrrhogaster* was not considered a strict expression of territoriality because males occasionally changed locations in the aquaria and actively defended these "new" spaces. This behavior accorded with the definition of Stiles (1972), who regarded the aggressive defense of space surrounding an individual fish, no matter where that fish was located, as maintenance of individual space. Winn (1958b) referred to "moving territories," a term that apparently described the same phenomenon.

Based on field and aquaria observations, Winn (1958b) described territories of breeding male *E. barrenense* and *E. rafinesquei* as 50 to over 100 cm in diameter, centered around a large rock and apparently stationary. Field observations of these two species by Stiles (1974) indicated that breeding males were highly aggressive toward other males that invaded their individual space but were not territorial in the strict sense. Page and Mayden (1981) stated that breeding *E. simoterum* males held in aquaria did not defend stationary territories but were combative toward one another when in close proximity.

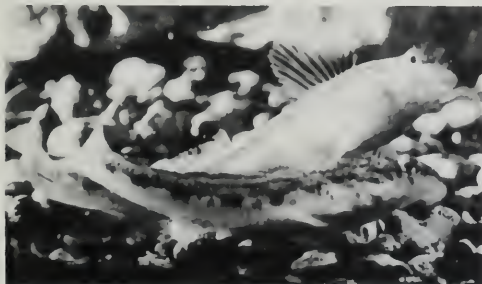


Figure 9. Spawning *Etheostoma zonistium* collected 27 March 1983 from West Fork Clarks River: horizontal position.

Development and Growth

Eggs were fertilized and larvae were reared in aquaria. Fertilized eggs of *Etheostoma zonistium* and *E. pyrrhogaster* were spherical, translucent, demersal, and adhesive. A yellow, acentric oil droplet was present in eggs of each species.

Fertilized eggs of *E. zonistium* averaged 1.7 mm in diameter (range 1.5–2.1, $SD = 0.13$, $N = 26$). Eggs incubated at $20 \pm 2^\circ C$ hatched in 163 to 170 hr (6.8 to 7.1 days). Only three embryos survived to hatching. Total lengths (TL) of two measurable *E. zonistium* protolarvae were 4.33 mm and 4.25 mm. Both specimens had 15 preanal myomeres and 21 postanal myomeres.

Fertilized ova of *E. pyrrhogaster* averaged 1.6 mm in diameter (range 1.4–1.7, $SD = 0.12$, $N = 13$). Eggs incubated at $25 \pm 3^\circ C$ hatched in 146 to 190 hrs (6.1 to 7.9 days). Protolarvae averaged 4.08 mm TL (range 3.39–4.55, $SD = 0.243$, $N = 7$). Myomere counts were as follows: 15 preanal, 21 postanal ($N = 5$), 15 preanal, 20 postanal ($N = 1$), and 16 preanal, 25 postanal ($N = 1$).

Page (1983:168–169) presented a regression of egg incubation period on temperature for all darters on which this information was available. Egg incubation in *E. zonistium* appeared to conform to Page's (1983:169) regression line; however, eggs of *E. pyrrhogaster* required an unusually long incubation time in relation to temperature.

Myomere counts were similar for *E. zonistium* (4.33 and 4.25 mm TL), and *E. pyrrhogaster* (3.39 to 4.55 mm TL): modally 15 preanal and 21 postanal. Among other members of *Nanostoma*, counts are available only

for *E. zonale*, which had 14 to 15 preanal and 22 postanal myomeres at 5 to 7 mm TL (Auer 1982).

Protolarvae of *E. zonistium* and *E. pyrrhogaster* (Figs. 10 & 11) had the following characteristics in common: mouth subterminal, well developed, and overlapped by subocular tissue (lateral view); snout rounded; eyes darkly pigmented (iris, black; pupil, olive); head sparsely pigmented dorsally; antero-ventral yolk sac with large oil droplet; single row of melanophores on each side of ventral midline (along length of anal fin); single row of melanophores ventrally along anal tube; and yolk sac heavily pigmented ventrally and lightly pigmented laterally (lower half only).

Protolarvae of *E. zonistium* ($N = 2$) (Fig. 10) lacked two to five melanophores on the lateral body region that were present in protolarvae of *E. pyrrhogaster* ($N = 7$) (Fig. 11). The mouth terminated on a horizontal plane even with the bottom of the pupil in *E. pyrrhogaster* and below the pupil in *E. zonistium*.

Growth analyses are based on specimens collected in the field throughout the study. Males of both species appeared to be larger than females of the same age, but growth curves were not calculated by sex due to the paucity of 2- and 3-year-old males. The relationship between standard length (Y) and age in months (X) for *E. zonistium* ($N = 336$) was $Y = 4.43 + 11.75 \log X$, with $r = 0.848$ (Fig. 12). At 12 months, males averaged 33.8 mm SL and were significantly ($t = 3.359$, $P < 0.01$, $df = 29$) longer than females, which averaged 31.3 mm SL. The largest specimens collected from West Fork Clarks River were a 50.4-mm SL male and a 46.7-mm SL female, both taken on 5 January 1983. The ichthyological collection at Southern Illinois University

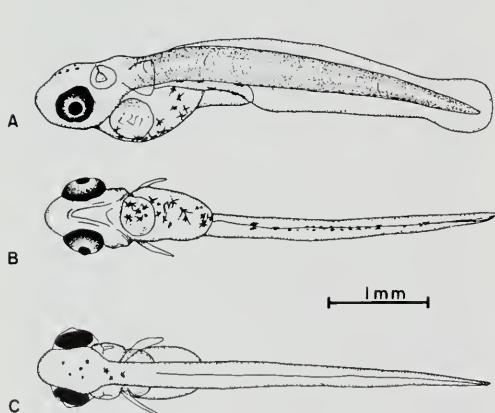


Figure 10. Protolarva of *Etheostoma zonistium*, the product of laboratory spawning by individuals collected in West Fork Clarks River: A) protolarva, 4.3 mm TL, age 170 hr, lateral view; B) ventral view of A; C) dorsal view of A.

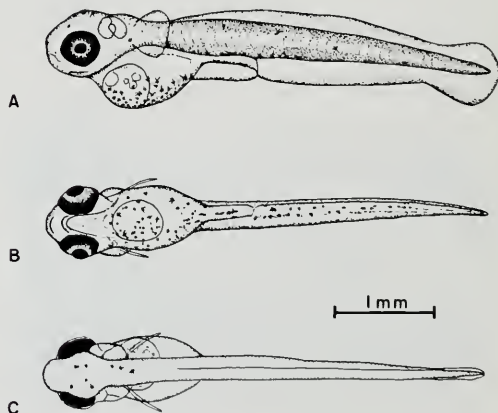


Figure 11. Protolarva of *Etheostoma pyrrhogaster*, the product of laboratory spawning by individuals collected in Terrapin Creek: A) protolarva, 4.0 mm TL, age 190 hr, lateral view; B) ventral view of A; C) dorsal view of A.

at Carbondale contains two larger males (51.6 and 53.0 mm SL) taken from Panther Creek, Graves County, Kentucky, on 16 June 1979. The oldest *E. zonistium* taken from the study area were two males and two females 35 months of age, captured on 27 March 1983. The males measured 48.0 and 49.7 mm SL and the females were 44.8 and 45.0 mm SL.

For *E. pyrrhogaster* ($N = 164$), the relationship between standard length (Y) and age in months (X) was $Y = 0.114 + 13.01 \log X$, with $r = 0.861$ (Fig. 13). At 12 months, males averaged 35.7 mm SL and were significantly ($t = 3.394$, $P < 0.01$, $df = 16$) longer than females, which averaged 32.2 mm SL. The largest and oldest male, collected from a Terrapin Creek backwater slough on 18 April 1983, was 36 months old and measured 49.7 mm SL. The two largest females (both 44.5 mm SL) were taken from Terrapin Creek on 5 January and 27 March 1983. The female collected in March was also the oldest at 35 months of age.

One-year-old males of each species were significantly longer than females at the end of their first year. Growth curves indicated that *E. zonistium* (Fig. 12) was slightly longer at 6 months of age than *E. pyrrhogaster* (Fig. 13) but had a comparatively slower growth rate thereafter.

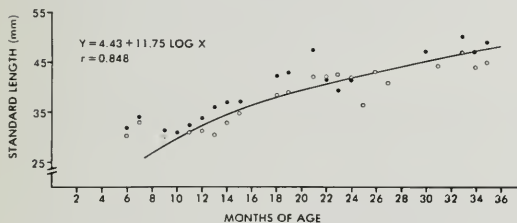


Figure 12. Size distribution by age of *Etheostoma zonistium* collected in West Fork Clarks River, Calloway County, Kentucky, between 5 January and 13 November 1983. Dots represent sample means for males; circles represent sample means for females. Regression line is for both sexes. A total of 336 specimens is represented.

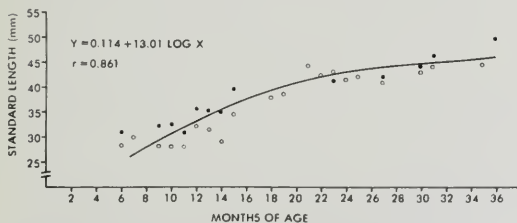


Figure 13. Size distribution by age of *Etheostoma pyrrhogaster* collected in Terrapin Creek, Graves County, Kentucky, between 5 January and 13 November 1983. Dots represent sample means for males; circles represent sample means for females. Regression line is for both sexes. A total of 164 specimens is represented.

Composition and Survival

Of 336 *Etheostoma zonistium* collected in West Fork Clarks River between 5 January and 13 November 1983, 58.6 percent were up to 1 year of age, 34.8 percent were over 1 and up to 2 years, and 6.6 percent were over 2 years (Table 5). The overall sex ratio was 1.7 females to 1 male ($\chi^2 = 23.04$, $P < 0.001$). Darters 1 year old or less ($N = 197$) were present at a female-to-male ratio of 1.4:1 ($\chi^2 = 4.88$, $P < 0.05$). Those 1+ years of age ($N = 117$) exhibited a 2.44:1 ratio of females to males ($\chi^2 = 20.52$, $P < 0.001$). At 2+ years ($N = 22$), *E. zonistium* was collected at a ratio of 2.14:1, but the ratio was insignificant, presumably a result of small sample size.

Of 164 *E. pyrrhogaster* collected in the Terrapin Creek drainage between 5 January and 13 November 1983, 59.8 percent were up to 1 year of age, 26.8 percent were over 1 and up to 2 years, and 13.4 percent were over 2 years (Table 5). The overall sex ratio was 1.3 females to 1 male, a nonsignificant deviation from 1:1. The only age class to show significantly different proportions between the sexes were 1+ darters ($N = 44$), which yielded 3.9 females to 1 male ($\chi^2 = 15.36$, $P < 0.001$).

The overall sex ratio of *E. zonistium* deviated significantly from 1:1 but that of *E. pyrrhogaster* did not. Significant deviations did occur in 1+ year classes of both species, and in each case of significantly skewed sex ratios, females outnumbered males. In general, however, darter populations do not deviate from a 1:1 sex ratio (Page 1983:169). Exceptions to this generality occur among highly territorial members of the subgenus *Catonotus*; for example, *Etheostoma flabellare* (Lake 1936), *E. squamiceps* (Page 1974), *E. kennicotti* (Page 1975), *E. smithi* (Page & Burr 1976), and *E. olivaceum* (Page 1980). Selection pressure for a sex ratio favoring females in *E. squamiceps* and *E. kennicotti* was presumably a result of the dominance of a proportionately few large males during spawning

Table 5. Distribution of sexes and age groups in samples of *Etheostoma zonistium* and *E. pyrrhogaster* collected between 5 January and 13 November 1983.

Sex	Number by age group			Total
	-1	1+	2+	
<i>E. zonistium</i>				
Male	83	34	7	124
Female	114	83	15	212
Total	197	117	22	336
<i>E. pyrrhogaster</i>				
Male	52	9	12	73
Female	46	35	10	91
Total	98	44	22	164

(Page 1974, 1975). Females significantly outnumbered males in the 1+ year class of *E. coosae* (O'Neil 1981) and in *E. simoterum* overall (Page & Mayden 1981). An *E. baileyi* population did not deviate significantly from a 1:1 sex ratio except for the 3+ year class, in which males outnumbered females (Clayton 1984). Males of *E. zonale* outnumbered females in their second and third years in western Pennsylvania (Lachner et al. 1950). Four of the six species of *Nanostoma* for which life histories have been completed indicate tendencies for sex ratios favoring females, particularly in the 1+ year class. If we assume no sampling bias, males may experience higher mortality in their first year. Both sexes may be more susceptible to large predators than shallow riffle-inhabiting darters, but predation on small, brightly colored males may be greater than predation on females and such differential predation may result in sex ratios skewed towards females.

Relative survival values for the 124 male and 212 female *E. zonistium* collected in West Fork Clarks River showed that 40.5 percent of the first-year males and 72.8 percent of the first-year females survived into their second year. Only 20.6 percent of second-year males and 18.1 percent of second-year females survived into their third year. Of the total sample of 336 *E. zonistium*, 59.4 percent of first-year individuals survived into their second year and 18.8 percent into their third year.

Relative survival values for the 73 male and 91 female *E. pyrrhogaster* from the Terrapin Creek drainage showed that 17.3 percent of the first-year males and 76.1 percent of the first-year females survived to their second year. More third-year males ($N = 12$) than second-year males ($N = 9$) were taken during the study; however, data suggested that only 28.6 percent of the second-year females survived until their third year. Of the total sample of 164 *E. pyrrhogaster*, 44.9 percent of first-year individuals survived into their second year and 50 percent into their third year.

Diet

Stomach contents of 89 *Etheostoma zonistium* and 81 *E. pyrrhogaster* were tabulated by month of collection (Tables 6 & 7). The diets of these species, similar to those of most darters (Page 1983:171), were composed primarily of microcrustaceans and aquatic insects. Dipteran larvae were reported to be a predominant food of the closely related darters *E. zonale* (Forbes & Richardson 1920; Adamson & Wissing 1977; Cordes & Page 1980; Wynne & Wissing 1982), *E. coosae* (O'Neil 1981), and *E. simoterum* (Page & Mayden 1981). Predominant food items of both *E. zonistium* and *E. pyrrhogaster* were larval chironomids, and these were consumed in greatest abundance in May, June, and July. The diet of *E. pyrrhogaster* included ephemero-

Table 6. Stomach contents of *Etheostoma zonistium* from West Fork Clarks River by month of collection. Mean number of food organisms per stomach is followed parenthetically by percentage of stomachs in which food organisms occurred.

Food organism	Jan <i>N</i> = 10	Feb <i>N</i> = 11	March <i>N</i> = 10	April <i>N</i> = 11	May <i>N</i> = 9	June <i>N</i> = 10	July <i>N</i> = 9	Oct <i>N</i> = 8	Nov <i>N</i> = 11
Gastropoda	—	—	—	—	—	—	—	0.4 (13)	—
Arachnida									
Acarina	—	—	—	0.1 (09)	—	—	—	—	—
Crustacea									
Cladocera	—	—	—	—	—	0.1 (10)	—	1.9 (50)	0.1 (9)
Ostracoda	—	—	—	0.3 (27)	0.1 (11)	0.2 (20)	—	—	—
Copepoda	—	—	0.2 (20)	—	0.7 (33)	—	0.6 (33)	0.3 (25)	—
Eubranchiopoda									
Conchostraca	—	—	—	—	—	—	—	0.2 (13)	—
Insecta									
Plecoptera	0.1 (10)	—	—	—	—	—	—	—	—
Ephemeroptera	—	0.2 (18)	0.1 (10)	—	—	—	—	0.1 (13)	0.4 (18)
Trichoptera	—	—	—	—	—	0.1 (10)	0.1 (11)	—	—
Diptera									
Tipulidae	—	—	—	—	—	0.2 (10)	—	—	—
Simuliidae	—	0.2 (18)	—	—	0.1 (11)	2.1 (20)	3.4 (11)	—	0.1 (9)
Chironomidae	8.5 (50)	23.8 (73)	15.8 (70)	11.5 (73)	31.3 (89)	109.1 (100)	31.1 (100)	9.1 (88)	2.6 (45)
Ceratopogonidae	—	—	—	—	—	0.6 (30)	0.3 (11)	—	—
Empididae	—	—	0.1 (10)	—	—	—	0.2 (11)	—	—
Unknown pupae	—	0.3 (27)	—	—	0.3 (22)	1.0 (50)	1.7 (56)	0.9 (38)	—
Teleostomi eggs	—	—	—	0.2 (18)	1.2 (33)	0.3 (20)	—	—	—
Empty	—	(40)	(18)	(30)	(9)	—	—	—	(50)

teran nymphs and trichopteran larvae throughout the year; however, *E. zonistium* fed on these larval insects less consistently. Empty stomachs were encountered in individuals of both species between January and April.

Predation

Potential predators captured in West Fork Clarks River were examined for darter remains in the stomach and gut: three *Micropterus punctulatus*, two *Lepomis cyanellus*, one *L. marginatus*, three *L. megalotis*, and two *L. gulosus*. At Terrapin Creek one *Aphredoderus sayanus*, three *Lepomis cyanellus*, one *L. macrochirus*, and two *L. marginatus* were examined. No evidence of fish predation was found.

Parasitism

None of the 89 *Etheostoma zonistium* stomachs examined contained parasites. One of 81 *E. pyrrhogaster* stomachs contained *Leptorhynchoides thecatus* (Acanthocephala) (April). Of 336 *E. zonistium* examined for external parasites, 7 harbored cysts thought to be trematode metacercaria (2 in January and 5 in March). The only external parasite on the 164 *E. pyrrhogaster* examined was a leech from a specimen collected in January.

Summary

Major aspects of the life history of *Etheostoma zonistium* in West Fork Clarks River and *E. pyrrhogaster* in Terrapin Creek are summarized in Table 8.

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Table 7. Stomach contents of *Etheostoma pyrrhogaster* from Terrapin Creek drainage by month of collection. Mean number of food organisms per stomach is followed parenthetically by percentage of stomachs in which food organisms occurred.

Food organism	Jan N = 14	Feb N = 11	March N = 10	April N = 8	May N = 7	June N = 4	July N = 9	Oct N = 8	Nov N = 10
Turbellaria	—	—	—	0.3 (13)	—	—	—	—	—
Gastropoda	—	—	—	—	0.7 (43)	—	—	0.4 (30)	—
Arachnida									
Acarina	0.1 (7)	—	—	—	—	—	—	—	—
Collembola	0.1 (7)	—	—	—	—	—	—	—	—
Crustacea									
Cladocera	—	—	—	—	0.1 (14)	—	—	—	0.3 (30)
Ostracoda	—	—	—	—	—	0.3 (25)	—	—	0.1 (10)
Copepoda	—	—	0.7 (40)	0.5 (50)	0.7 (43)	—	0.1 (11)	—	1.3 (60)
Insecta									
Plecoptera	—	0.2 (18)	0.1 (10)	—	0.1 (14)	—	—	—	—
Ephemeroptera	0.1 (7)	0.1 (9)	0.3 (30)	0.1 (13)	0.1 (14)	—	0.9 (56)	0.9 (25)	0.2 (10)
Trichoptera	0.1 (7)	0.2 (18)	0.1 (10)	—	0.3 (29)	0.5 (25)	1.0 (44)	0.1 (13)	6.6 (100)
Diptera									
Simuliidae	—	—	—	—	—	—	0.2 (11)	—	—
Chironomidae	7.9 (71)	20.5 (100)	15.6 (90)	13.0 (88)	31.1 (100)	88.8 (100)	89.1 (100)	15.3 (100)	11.4 (90)
Ceratopogonidae	—	—	—	—	—	—	0.1 (11)	—	—
Unknown pupae	—	0.2 (9)	0.4 (30)	—	0.1 (14)	0.8 (50)	2.1 (78)	1.9 (25)	0.2 (20)
Empty	(21)	—	(10)	(13)	—	—	—	—	—

Table 8. Summary of life history information on *Etheostoma zonistium* in West Fork Clarks River and *E. pyrrhogaster* in Terrapin Creek.

Characteristic	<i>Etheostoma zonistium</i>	<i>Etheostoma pyrrhogaster</i>
Principal habitat	Stream margins with sand, gravel, and emergent vegetation	Stream margins with undercut banks and exposed tree roots over sand
Age at sexual maturity	1 year	1 or 2 years
Size at sexual maturity	Females average 31 mm; males average 34 mm	Females average 32 mm; males average 36 mm
Sexual dimorphism	Males are larger than females and brightly colored	Males are larger than females and brightly colored
Breeding tubercles	Absent	Absent
Description of genital papillae	Long and tubular in females, short and flattened in males	Long and tubular in females, short and flattened in males
Number of mature ova in preserved females	20–135	21–102
Description of mature ova	1.1 mm in diameter, translucent with a pitlike indentation	1.1 mm in diameter, translucent with a pitlike indentation
Description of fertilized eggs	1.7 mm in diameter, spherical, translucent, demersal, and adhesive	1.6 mm in diameter, spherical, translucent, demersal, and adhesive
Spawning period	From March into May; water temperature 11–17°C	From March into June; water temperature 11–20°C
Spawning habitat	Unknown in nature	Unknown in nature
Egg deposition sites in aquaria	Aquarium walls, sand and gravel substrate, rocks, and plants	Sand and gravel substrate, aquarium corners, and rocks
Spawning position	Male and female in vertical or horizontal position	Male and female in vertical or horizontal position
Egg guarding	None observed	None observed
Incubation period	163–170 hrs at 20 ± 2°C	146–190 hrs at 25 ± 3°C
Size at hatching	4.3 mm TL	3.4–4.6 mm TL
Influence of sex on growth rate	At 1 year, males significantly larger than females	At 1 year, males significantly larger than females
Sex ratio	1.7 females:1 male	1.2 females:1 male
Longevity	35 months	36 months
Maximum size	50 mm SL	50 mm SL
Migrations	None observed	None observed
Territoriality	None observed	None observed
Principal diet	Immature aquatic insects and microcrustaceans	Immature aquatic insects and microcrustaceans

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