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ABSTRACT

The reproductive biology of the Frecklebelly Darter, *Percina stictogaster*, was studied in the Red River, Menifee-Powell counties, Kentucky, from 2009-2012. Males and females mature at Age II. Spawning occurs from late February to early April in water temperatures of 7-16° C in areas with strong current (0.16-0.88 m/sec) and fine gravel substrates. A 52 mm SL female collected in early March had 100 mature ova. Aquarium observations confirm this species buries its eggs in a manner similar to other *Percina* darters. Fertilized eggs were about 2.5 mm in diameter, clear, demersal, and slightly adhesive. At 10° C eggs hatched in 18-25 days (100% survival) into larvae 7-8 mm TL. Larvae were initially benthic, but became pelagic 2-3 days later. By about 11 mm TL, the yolk sac was absorbed, and the young returned to the bottom. In early June, young (about 2 months old) were 16-25 mm SL and had acquired diagnostic pigmentation. They occupied areas with *Justicia* sp. or coarse woody debris in low-velocity habitats, adjacent to riffles.

INTRODUCTION

Knowledge of the reproductive biology and early life history of fishes is vital in effective management of fishes and aquatic ecosystems. Simon and Wallus' (2006) impressive compilation of early life history information about percids is a valuable resource for aquatic biologists and allows identification of many larval percids. However, the reproductive biology and early life history of several percids, including *Percina stictogaster* Burr and Page, the Frecklebelly Darter, are still essentially unknown. *Percina stictogaster* is restricted to clear, large creeks and small rivers with silt-free substrates in the Kentucky River and Green River drainages in Kentucky and Tennessee (Burr and Warren, 1986; Etnier and Starnes, 1993). Little is known of its natural history, other than it is associated with heavy cover (live plants and root masses) in areas of low flow. Therefore, a study describing the natural history of *P. stictogaster*, including reproductive biology, is warranted. Although it is not currently protected in Kentucky or Tennessee, its restricted distribution and intolerance of habitat disturbance (Burr and Page, 1993) further support the need for natural history information.

Our objectives are to describe the reproductive biology and early life history, including spawning season, habitat, and behavior, growth and age at maturity, embryology, and larval development of *P. stictogaster* in the Red River, Kentucky.

METHODS

This study was conducted in the Red River (Kentucky River drainage) at the KY Hwy 77 bridge, Menifee and Powell counties, Kentucky.

There, the stream is 25 to 35 m wide with diverse, silt-free substrates, well defined riffles, raceways, and pools, abundant *Justicia* sp. beds and coarse woody debris, and a wide, forested riparian zone. Much of its watershed (483 km^2) is protected within the Daniel Boone National Forest and supports a rich fish community, including 12 darter species at out study site (Burr and Warren, 1986; Thomas, 2000). Frecklebelly Darters were captured by seining, from February 2009 to March 2012, with most effort concentrated in the spring months, when spawning was presumed to occur. Most captured darters were measured (standard length (SL)) and released; a few were preserved to examine gonad development, and a few transported to lab aquaria to observe spawning.

Microhabitat variables (substrate, depth, and flow) were collected for 57 individuals in late winter-spring and 21 individuals in summer. Substrate was assessed from a 1 m grid, centered over the point of capture. At each corner and at the center of the grid, depth and flow were measured with a meter stick and a Swoffer 3000-C140 flowmeter, respectively; each of the five measurements were averaged.

Male and female *P. stictogaster* brought back to the lab were placed in a 57 L aquarium located in a GCW15 Environmental Growth Chamber set on a 13 hour day-11 hour night light cycle and 10° C. To provide a diversity of spawning substrates, the aquarium was provided with live plants (*Justicia* sp.), flat cobbles overlaying cavities, coarse woody debris, and areas of sand, pea gravel (4-10 mm), and coarse gravel (10-30 mm). A large filter provided current (up to 0.16 m/sec) on one side of the tank. Darters were periodically observed to document spawning behavior. Fertilized eggs were removed from the aquarium and placed in a 1-liter jar with aeration and a fungicide, nitrofurazone. Developing eggs and larvae were observed daily; periodically a few embryos or larvae were fixed in 5% formalin and vouchered in the Morehead State University Fish Collection (MOSU) for further study.

Ages of darters were determined by length-frequency analysis and for 27 preserved specimens, examination of scale annuli. Gonadosomatic indices (GSI) were calculated from 20 preserved specimens 48-66 mm SL collected 1999-2012 from the study site. Eviscerated specimens and gonads were blotted dry and weighed to the nearest 0.001 g. Gonadosomatic indices were calculated as gonad weight divided by eviscerated weight X 100 for females and testis width divided by the square root of standard length X 100 for males (Layman, 1991).

RESULTS

Growth

At least three age groups were present throughout the year (Fig. 1). The smallest size class was distinct throughout the year, but the boundary between the upper two groups was less distinct. At one year of life (March-April) *P. stictogaster* were 38-45 mm SL (mean = 41.8). Individuals 48-58 mm SL were likely Age II and those 59-66 mm SL were likely Age III. Juveniles first appeared in seine (3.2 mm mesh) collections in late May or early June. By July, *P. stictogaster* averaged 32.1 mm SL at Age 0, 50.9 mm SL at Age I, and 61.5 mm SL at Age II.

Time and place of spawning

Darters collected 7 January to 1 March (2009-2011), in water 2-3° C, were not spawning. Males had dark vertical bars and a metallic green or golden sheen and females were visibly gravid, but neither expelled gametes under slight pressure of the abdomen nor exhibited spawning behavior when transferred to a lab aquarium. During 2009-2011, years which featured relatively cool winters and springs, males collected 7 March to 5 April (7-16° C) and one female collected 5 April extruded gametes under slight pressure to the abdomen. These individuals, with other gravid females collected at these times, exhibited spawning behavior within 24 hrs of being transferred to the lab aquarium. The ripe female and two males were collected from a raceway with pea gravel substrates and strong current (0.53-0.88 m/sec), in water 45 cm deep. Other mature males and females also were captured in strong current (0.16-0.88 m/sec) and moderate depths (21-59 cm) (Fig. 2). They occupied a variety of substrates, but usually occurred over sand and fine gravel with vegetation (dormant *Justicia* sp. roots) or coarse woody debris. Juveniles occupied

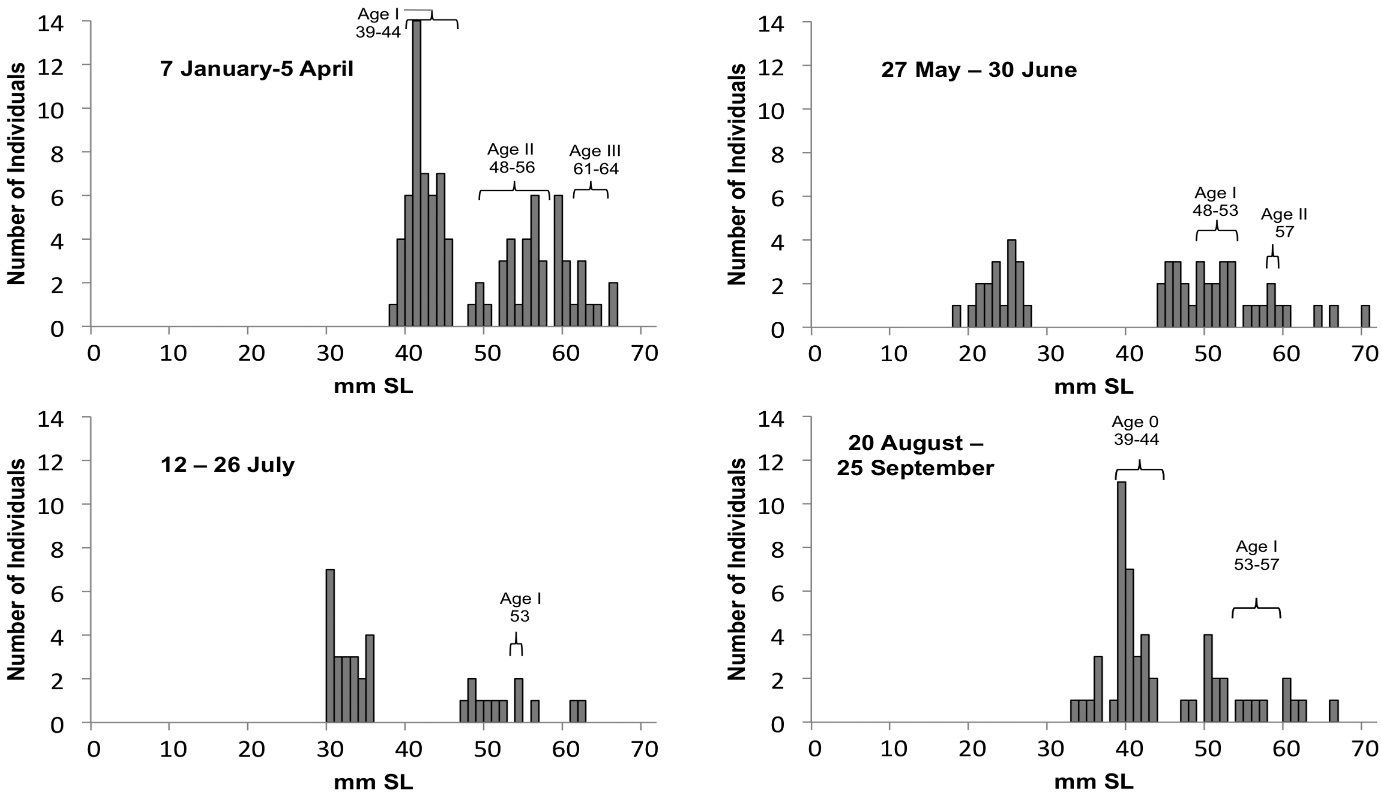

Figure 1. Age and growth of *Percina stictogaster* in the Red River, Kentucky. Brackets above bars indicate ages of 27 specimens as indicated by examination of scale annuli.

similar substrates and depths, but in slower current (Fig. 2). Males and females collected 31 March 2012 (15° C) were not gravid, lacked breeding coloration, and for males, had barely discernible caudal keels. The winter and spring of 2012 were unusually warm; spawning during 2012 appeared to occur earlier than in 2009-2011.

Gravid and ripe females were 48-64 mm SL, Age II-III; mature males were 52-66 mm SL, Age II-III (Fig. 1). Secondary sexual characteristics (e.g., caudal keel, chromatic coloration, distended abdomen) were not observed in Age I fish. Mature males had large, white testes which became enlarged by September (Fig. 3). Ovarian enlargement and development apparently occurs later, during late fall and winter (Fig. 3). A female collected in early March had the largest ovaries (GSI = 29.4), and was the only female with mature ova (Table 1). She had three sizes of ova (Table 1): mature ova were yellow-orange, 1.6-1.8 mm in diameter, maturing ova were pale yellow, 1.0-1.3 mm in diameter, and immature ova were white, 0.5-0.8 mm in diameter. Only immature ova were present in females in September; maturing ova were also present by January. The ovaries of females collected 31 March 2012 appear to be in a post-spawn condition; all ova were white, 0.8-1.5 mm in diameter, and not in multiple distinct size classes.

Young-of-the-year (YOY) from September through January had tiny, clear, poorly differentiated gonads suggesting that few, if any, one-year-old fish spawn. Although sample size was small (n=5), female length was highly correlated with numbers of mature + maturing eggs ($r = 0.9569$, $P = 0.0101$) The logarithmic regression which described this relation was: log M (mature + maturing ova) = -3.4321 + 3.2222 log SL).

Spawning behavior

In 2009, one ripe male and one gravid female were placed in an aquarium 7 March, and three more gravid females were added 22 March. In 2010, one ripe female and one gravid male were placed in an aquarium 2 March, one ripe male and

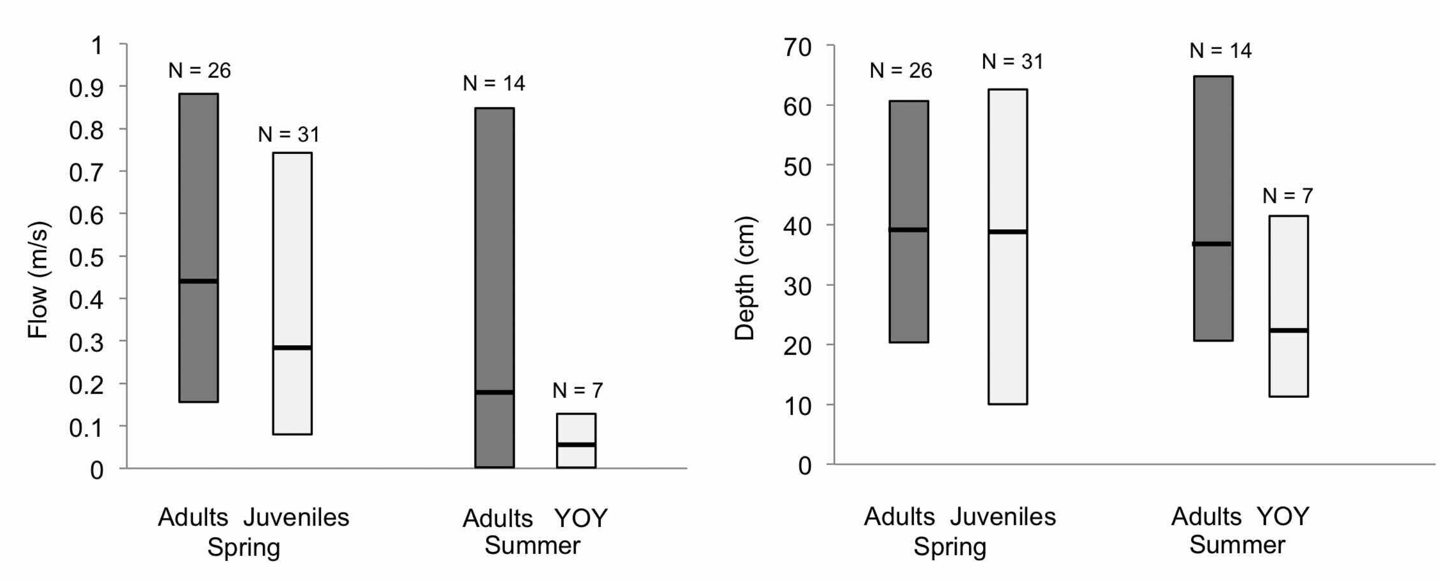

Figure 2. Flow and depth where *Percina stictogaster* were captured in the Red River, Kentucky. Bars show ranges and means of observations.

one gravid female were added 20 March and one ripe female was added 5 April. In 2011 one ripe male and one gravid female were added 29 March. Courtship was observed in March and early April in 2009 and 2010. A male displayed to a female by moving in front of the female, propping up on the tip of his pelvic fins, erecting all fins, and occasionally bobbing his head. Males would often chase females. While displaying, males became shiny green-gold and the lateral stripe became indistinct, replaced by dark vertical bars or rectangles. Females typically retained their dark lateral stripe, but often had a gold background. When not engaged in courtship or spawning behavior, all individuals typically occupied the same cover space (a rock cavity); no antagonistic male-male or female-female behavior was observed.

Spawning was observed 23-24 March 2010. The spawning act is similar to that of *P. cymatotaenia* (Gilbert and Meek) (Pflieger, 1984; Page and Sabaj, 1994). During spawning a male followed a female as she swam to an area of pea gravel and current below a filter outflow. The male mounted the female, curved his caudal peduncle around hers, and the pair started vibrating rapidly as they moved forward about 6-8 cm through the gravel. During the movement, the female's caudal peduncle became completely buried in the substrate and the male's caudal peduncle became partly buried. The spawning act lasted about 5 seconds; the pair exited the spawning area at the end of the run. We did not observe repeated spawning, as has been reported for its sister species, *P. cymatotaenia* (Pflieger, 1984), but *P. stictogaster* was very timid and difficult to observe without disturbing. On 13 March 2009 and 31 March 2011 males were observed to mount females, as described above, but the pair became startled and did not complete spawning. Eggs were not observed in 2009 and 2011, but successful spawning must have occurred because larvae were discovered in the aquarium 3-4 weeks later.

Early development

Immediately following the observed spawning on 23 March 2010, 46 eggs were removed from the substrate and transferred to a separate container. Twenty eggs were clear and fertilized and the remaining 26 were opaque white and either dead or unfertilized. Eggs were in clumps of 2-4. Fertilized eggs were 2.3-2.5 mm in diameter, spherical to slightly oblong, and slightly adhesive to each other and gravel. The yolk sac included a spherical oil globule 0.5-0.7 mm in diameter. At three days post-fertilization, the embryo was an elongate, opaque white mass about 1.4 mm TL on the surface of the yolk sac (Fig. 4). Myomeres began to appear at 6 days, eyes were distinct at 7 days, and the first movements were observed at 9 days. At 11 days, embryos were wrapped about ¾ around the yolk sac and eyes had pigment; about 25 indistinct myomeres were visible (Fig. 4). At 17 days, embryos were wrapped completely around the yolk sac and had retinas with a black center surrounded by gold. The otic capsules and pectoral fin buds were visible, the eyes moved, and the heart was beating.

All embryos survived until hatching, which occurred 18-25 days after fertilization at 10° C. Larvae broke through the chorion tail-first. Larvae were 6.8-8.2 mm TL (Fig. 4); larvae that hatched later were larger and more developed. Larvae had 39-41 total myomeres (18-21 preanal; 18-22 postanal). Initially, larvae remained on the bottom of the jar, but 3-6 days after hatching (25-28 days post-fertilization) young were actively swimming in the water column. At 7-10 days post-hatching, the larvae, now 9.7-9.9 mm TL (Fig. 4), became benthic again. These larvae exhibited movements of the pectoral fins, mandible, and gill arches. The yolk sac was absorbed about 10-15 days post-hatching, at 10.4 mm TL (n=1). Plankton-rich pond water was provided as a food source, but all darters died soon after the yolk sac was absorbed.

Small YOY (16-26 mm SL) were collected from the Red River 2-30 June. By about 21 mm SL, lateral pigmentation diagnostic of adults was present (Fig. 5). The YOY were collected in shallow water (< 30 cm) with little or no flow, over substrates of sand. Most were collected in areas with emergent *Justicia* sp. or coarse woody debris, sometimes with adults.

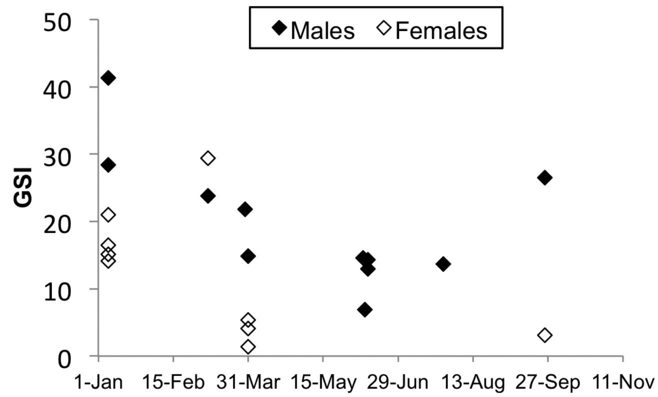

Figure 3. Seasonal changes in GSI of *P. stictogaster*, 48-66 mm SL. For females, GSI is (gonad weight/eviscerated body weight) X 100. For males, GSI is (testis width/standard length$^{1/2}$) X 100. Points on the graph indicate individuals, collected 1999-2012.

DISCUSSION

Identification of young

Larvae are most similar to those of *P. maculata* (Girard), the Blackside Darter and *P. sciera* (Swain), the Dusky Darter. Postanal myomere counts are higher in *P. sciera* (22-24) than in *P. stictogaster* (18-22) but myomere counts are highly overlapping between *P. maculata* and *P. stictogaster* (Simon and Wallus, 2006). However, these (and other syntopic darters) usually spawn later (April-June) than *P. stictogaster* (Petravicz, 1938; Page and Smith, 1970). Larvae of *P. stictogaster* are likely to be present from late March to late April, before most other darters have hatched or even spawned. Distinctive pigmentation of *P. stictogaster*, in the form of a black, sharply defined caudal spot, surrounded by a pale orange halo, is present by about 21 mm SL (Fig. 5).

Comparison to *P. cymatotaenia*

Many reproductive and early life history aspects of *P. stictogaster*, including spawning behavior and spawning dates, are similar to those of the only other member of subgenus *Odontopholis*, *P. cymatotaenia* (Pflieger, 1984), except that Pflieger observed antagonistic male interactions, which we did not observe. Male aggression may have been more pronounced in Pflieger's aquarium study because more males were used (3-6) than in our study (usually 1-2). Although we did not observe repeated spawning by a female (as was observed in *P. cymatotaenia* (Pflieger, 1984)), this behavior seems likely in *P. stictogaster* because the number of mature ova observed was much higher than the number of eggs recovered from the spawning event.

In *P. cymatotaenia*, hatching occurred in only 9-10 days, but eggs were incubated at a higher temperature (12-20° C) than in our study (10° C) (Pflieger, 1984). Egg mortality was high in Pflieger's study (over 50%), but zero in our study, suggesting that embryos may be sensitive to high temperatures. Unlike our study, Pflieger (1984) did not detect a relationship between fecundity and female length. However, most of the female *P. cymatotaenia* were collected late in the spawning

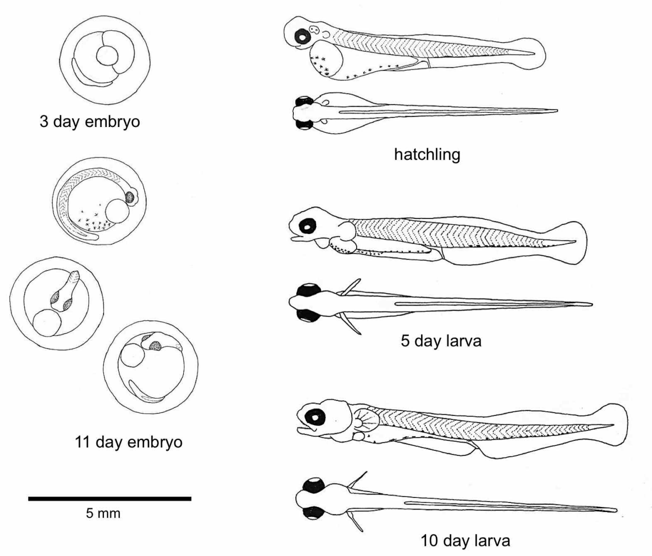

Figure 4. Embryos and larvae of *Percina stictogaster*. Drawings by DJE.

Figure 5. Juvenile *Percina stictogaster* (17-25 mm SL) from the Red River Kentucky, 11 June 2010.

Table 1. Numbers of ova in female *Percina stictogaster*. No examined females 43 mm SL or smaller had identifiable ova. Ages were determined by length-frequency analysis and examination of scale annuli.

Date collected	Age (months)	Size (SL)	Immature ova	Maturing ova	Mature ova
7 March 2009	23	52	140	80	100
25 Sept 2009	18	53	312	0	0
7 Jan 2012	33	61	125	286	0
7 Jan 2012	21	56	144	182	0
7 Jan 2012	21	53	81	169	0
7 Jan 2012	21	48	74	97	0
31 Mar 2012	23	52	66[a]	0	0
31 Mar 2012	23	56	19[a]	0	0
31 Mar 2012	23	55	82[a]	0	0

[a]In these females, which were apparently post-spawn, all ova were white and 0.8-1.5 mm in diameter.

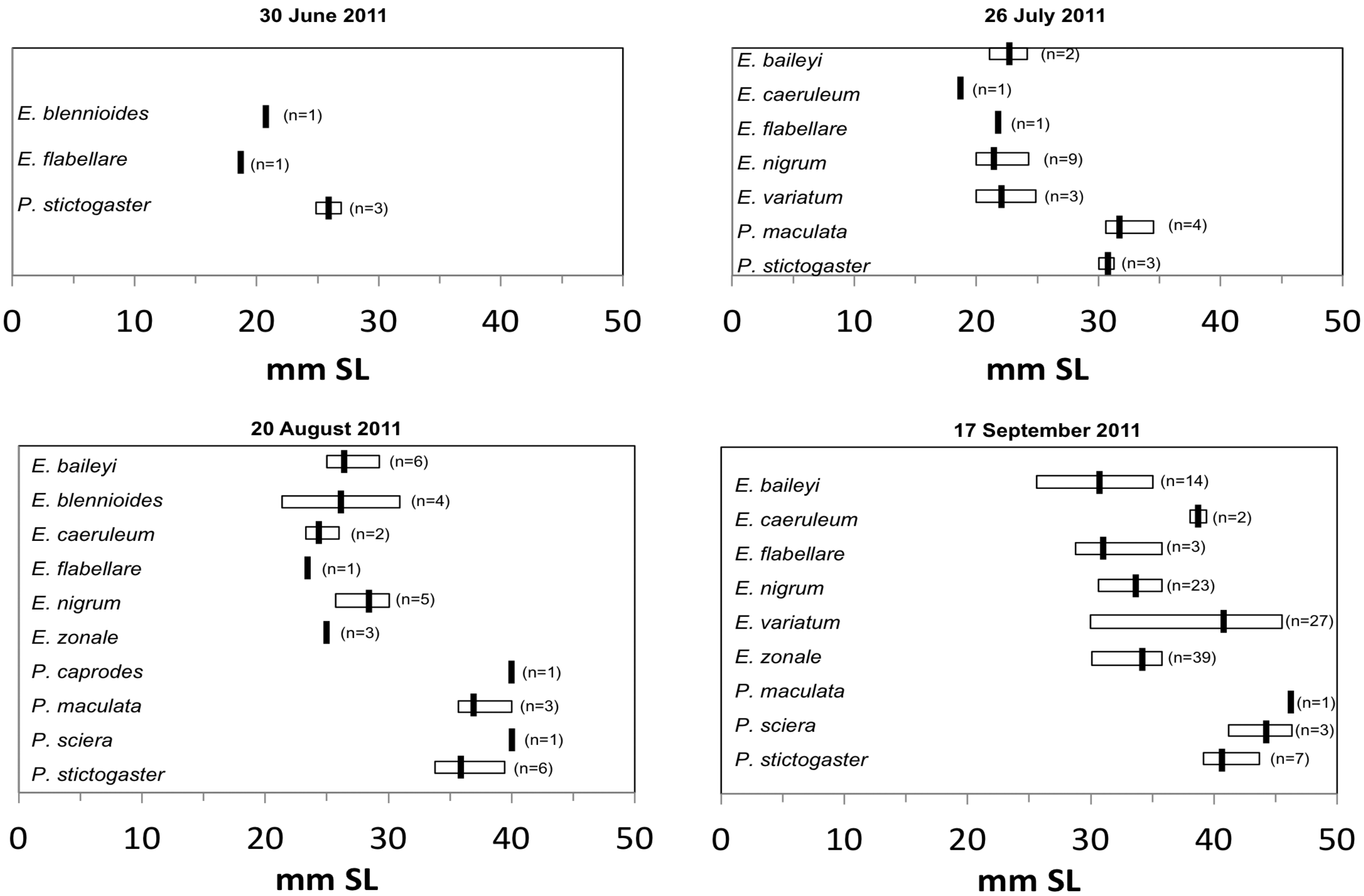

Figure 6. Sizes of young-of-the-year darters from the Red River, 2011. Bars and lines indicate ranges and means, respectively.

season (late March), so some may have already spawned. In contrast to our results, some Age I (44-46 mm SL) *P. cymatotaenia* were mature in Pflieger's study. No Age I *P. stictogaster* (39-44 mm SL) examined in our study had mature gonads.

Place and time of spawning

Percina stictogaster occupy faster water during and just before spawning than they occupy at other times of the year (t-test, P<0.001), and spawning adults occupy faster water than do juveniles (t-test, P<0.001) (Fig. 2). Despite higher flows in the spring, low-flow areas existed in our study area, but typically were not utilized by *P. stictogaster*. Utilization of faster flows during spawning may be because developing eggs and larvae require silt-free substrates or high oxygen levels.

Percina stictogaster spawns earlier (late February-early April) than any of the other 11 syntopic darters at the Red River study site (Simon and Wallus, 2006). Young of *P. stictogaster* appear earlier in summer and are larger than those of other darters, especially benthic species (Fig. 6). Larger fishes are thought to be less vulnerable to predatory fishes than are smaller ones (Schlosser, 1987; Houde 1997). Predation avoidance could be particularly important for *P. stictogaster*, which spend more time in the water column (unpubl. data) than do other syntopic darters. Two other highly pelagic *Percina* darters, *P. macrocephala* (Cope) and *P. williamsi* Page and Near (Greenberg, 1991; Eisenhour et al., 2011), also probably spawn in late winter to early spring (Etnier and Starnes, 1993; Page and Near, 2007) and share similar body pigmentation patterns with *Odontopholis* species. These two darters are not closely related to *Odontopholis*, and instead are placed in either subgenus *Alvordius* (Near, 2002) or *Pagella* (Near et al., 2011). Perhaps the unique combination of lateral pigmentation, early spawning, and pelagic habitat are correlated, but clearly this group of characters evolved independently in the two clades. This hypothesis deserves further study.

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