

1-1-2013

Number 54 (January 2013)

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Recommended Citation

(2013) "Number 54 (January 2013)," *Southeastern Fishes Council Proceedings*: No. 54.

Available at: <https://trace.tennessee.edu/sfcproceedings/vol1/iss54/1>

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Abstract

Reproductive Biology of the Frecklebelly Darter, *Percina stictogaster* (Teleostei: Percidae) by David J. Eisenhour, Audrey M. Richter, Lynn V. Eisenhour, and Carol-Rose Gingra

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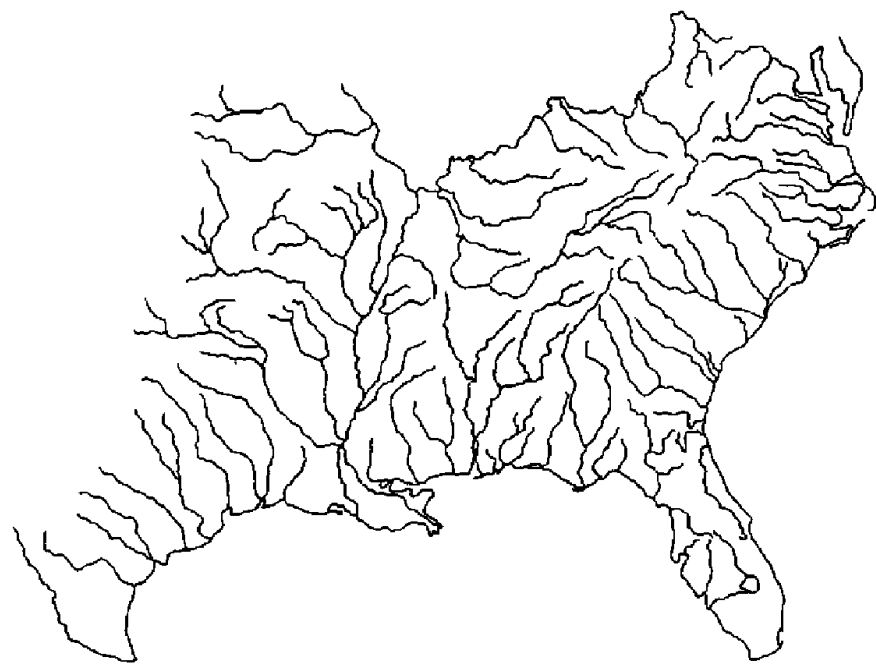
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Minutes, Business Meeting, 37th Annual Meeting, Southeastern Fishes Council

2011 Treasurer's Report for the Southeastern Fishes Council

Southeastern Fishes Council Proceedings

Dedicated to the Conservation of Southeastern Fishes



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- 2nd Place – Kristen Pilcher, Austin Peay State University. Filling the gap: a survey of the Rutherford Fork of the Obion River provides new localities for 20 species (with Rebecca Blanton Johansen).
- 3rd Place – Jared Harris, The University of Southern Mississippi. Life history trait convergence in two species of the *Fundulus notatus* species Complex (with Jake Schaefer).

Oral Presentations

- 1st Place – Jessie Green, University of Central Arkansas. Examining community level variables of fishes in relation to natural gas development (with Ginny L. Adams and Reid Adams).
- 2nd Place – Lyndon Coghill, University of New Orleans. Phylogeography and genetic structure of a genetically distinct population of *Lepomis megalotis* in Cuatro Ciénegas, Mexico (with C. Darrin Hulsey and Steven G. Johnson).
- 3rd Place – Michael Schwemm, Oklahoma State University. Demographic history and population structure of *Notropis suttkusi* in southeastern Oklahoma (with A.A. Echelle and R.A. Van Den Bussche).

39th Annual Meeting, Southeastern Fishes Council

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Reproductive Biology of the Frecklebelly Darter, *Percina stictogaster* (Teleostei: Percidae)

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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 km²) is protected within the Daniel Boone National Forest and supports a rich fish community, including 12 darter species at our 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

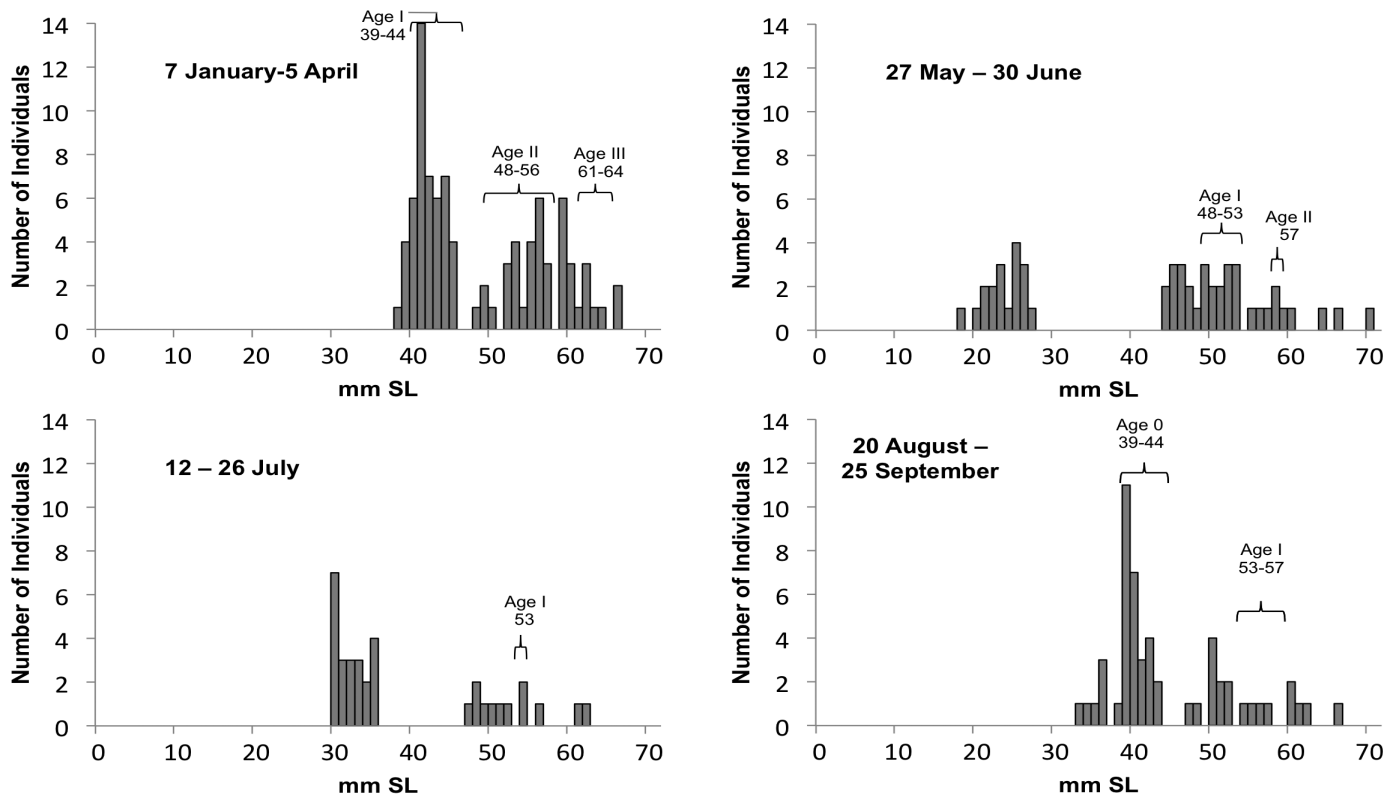


Figure 1. Age and growth of *Percina stictogaster* in the Red River, Kentucky. Brackets above bars indicate ages of 27 specimens as indicated by examination of scale annuli.

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 diam-

eter. 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

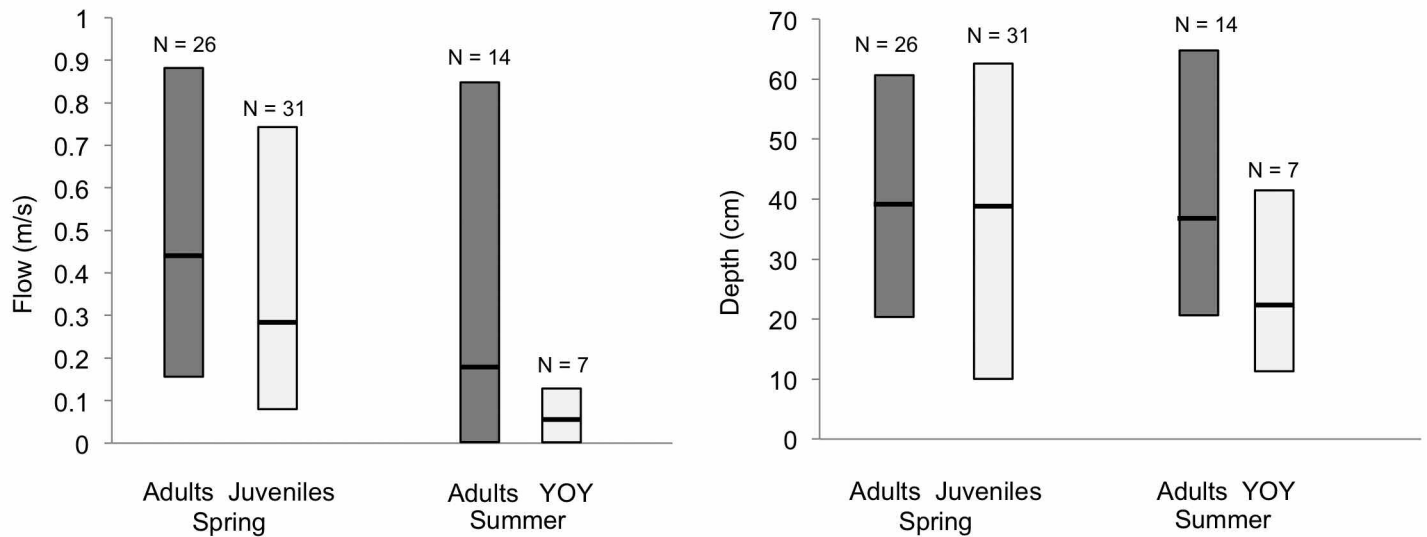


Figure 2. Flow and depth where *Percina stictogaster* were captured in the Red River, Kentucky. Bars show ranges and means of observations.

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 $\frac{3}{4}$ 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.

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, sur-

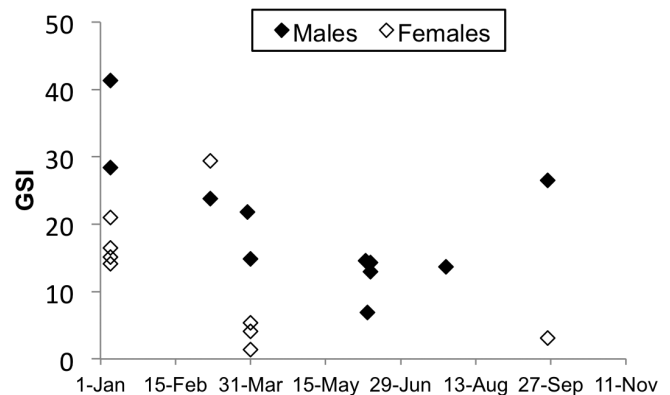


Figure 3. Seasonal changes in GSI of *P. stictogaster*, 48-66 mm SL. For females, GSI is (gonad weight/visceral body weight) X 100. For males, GSI is (testis width/standard length^{1/2}) X 100. Points on the graph indicate individuals, collected 1999-2012.

rounded 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

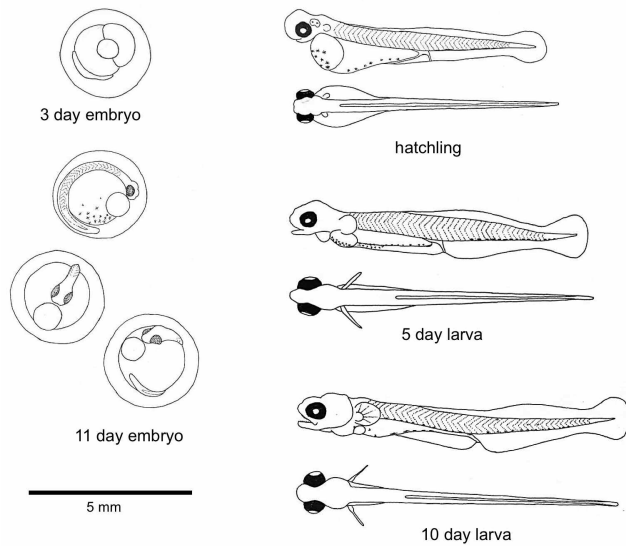


Figure 4. Embryos and larvae of *Percina stictogaster*. Drawings by DJE.



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

^aIn these females, which were apparently post-spawn, all ova were white and 0.8-1.5 mm in diameter.

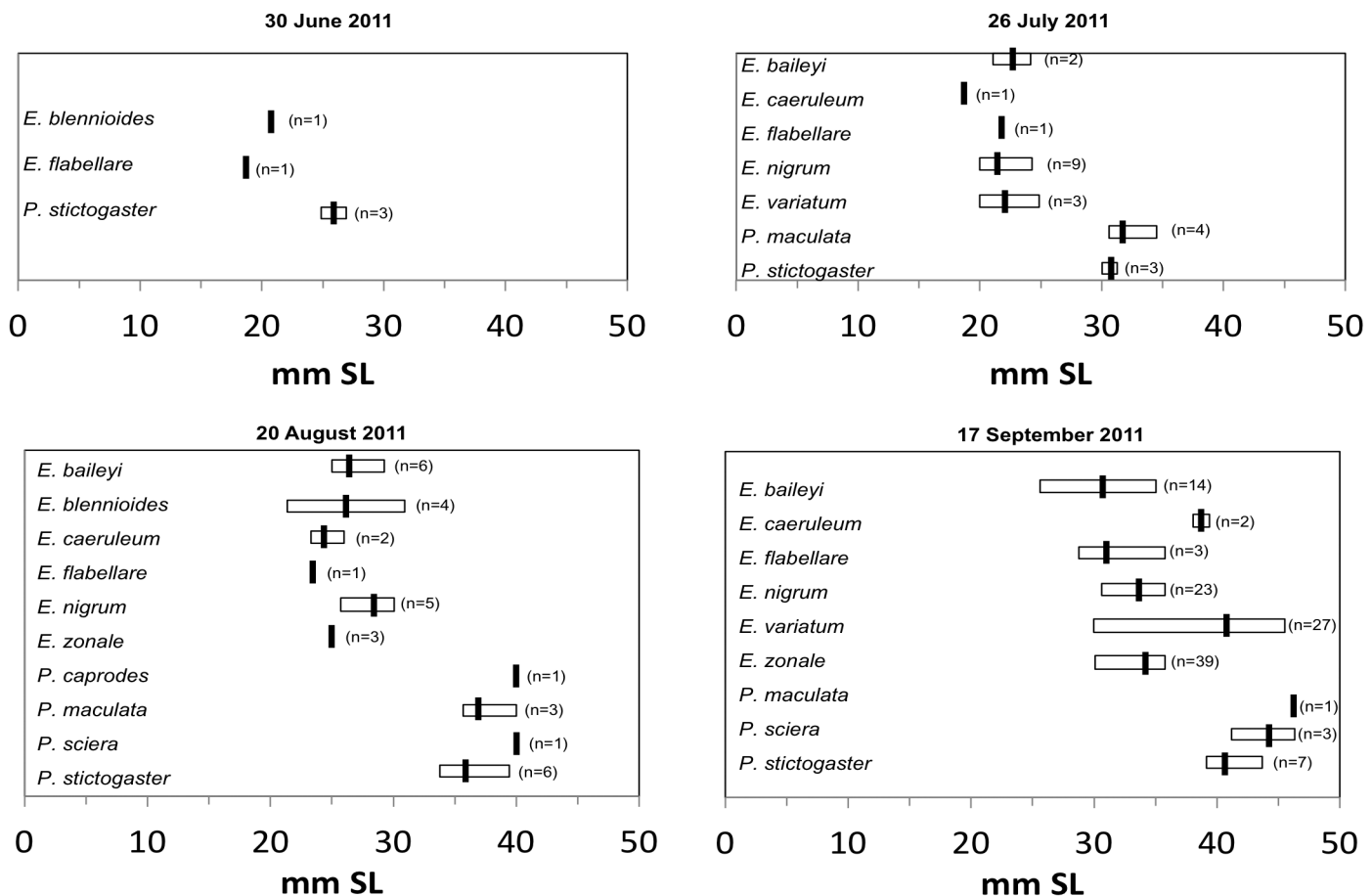


Figure 6. Sizes of young-of-the-year darters from the Red River, 2011. Bars and lines indicate ranges and means, respectively.

season (late March), so some may have already spawned. In contrast to our results, some Age I (44–46 mm SL) *P. cyematotaenia* 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.

ACKNOWLEDGMENTS

We thank R.T. Broadway, J.D. Eisenhour, K.L. Eisenhour, P.J. Eisenhour, and S. Emeterio for field assistance in collections. Partial support was provided by grants from the Department of Biology and Chemistry, Morehead State University. David Smith allowed access to Environmental Growth Chambers for observing darter spawning. Permits to collect darters and to work with live vertebrates were provided by Kentucky Department of Fish and Wildlife Resources and Morehead State University IACUC.

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Post-Hurricane Katrina Survey for the Blackmouth Shiner (*Notropis melanostomus*) at Historical Localities in Mississippi

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ABSTRACT

The Blackmouth Shiner (*Notropis melanostomus* Bortone) has a limited range, occurring only in select drainages of southern Mississippi, southern Alabama, and western Florida (AFS status = threatened; NatureServe rank = G2, "At high risk"). In southern Mississippi it is found in ephemeral ponds, oxbow lakes, backwaters, and other floodplain habitats. We surveyed 35 sites in 1995 and found eight new populations of *N. melanostomus* in the Pascagoula River floodplain. To assess possible impacts of Hurricane Katrina on these *N. melanostomus* populations and habitats, we re-surveyed the area in 2007-2008. Of the eight localities discovered in 1995, only two yielded *N. melanostomus*. Three 1995 oxbow lake sites were repeatedly sampled in 2007-2008 but no *N. melanostomus* were collected. The remaining three 1995 sites were either partially or wholly dry. Expanding our sampling efforts south in 2007, we discovered a new population of *N. melanostomus* in Luther Lake. While we are encouraged that the species is still present and that more undiscovered populations likely exist, there is concern about the decrease in *N. melanostomus* localities over the last twelve years. For example, recent clear-cutting activities adjacent to one historic site threaten the largest and most consistent population of the species in Mississippi.

INTRODUCTION

The Blackmouth Shiner (*Notropis melanostomus* Bortone) is considered imperiled globally because of its rarity (Litt et al., 2000). It is currently known only from four separate drainages: Pascagoula River Drainage, Mississippi; Blackwater River Drainage, Florida; Yellow River Drainage, Florida; and the Mobile Drainage, Alabama. A recent analysis of museum holdings and collections data suggested that there has not been enough sampling to accurately determine the distribution of *N. melanostomus* across its entire known range (O'Connell et al., 2005). Without this information the conservation status of *N. melanostomus* cannot be properly assessed.

In Mississippi, the last survey work to find populations of *N. melanostomus* was conducted in 1995 (O'Connell et al., 1998). This survey work yielded eight new localities for *N. melanostomus*, increasing the known localities for the species in the state from three to eleven. Once the habitat affinities of *N. melanostomus* were determined during this survey, sampling for the presence of the species in other localities became more effective (O'Connell et al., 1998). Unfortunately, no *N. melanostomus* were collected at these localities during subsequent, less exhaustive surveys in 1998, 1999, 2001, and 2002

(O'Connell et al., 2005). It is unknown if this failure to collect *N. melanostomus* is a result of extirpation or a reflection of the life history and habitat of the species. Many of the habitats used by *N. melanostomus* are ephemeral, including floodplain ponds and backwaters of rivers (Bortone, 1993; O'Connell et al., 1998). Of the eight new localities discovered in 1995, four were ephemeral floodplain ponds. Other historical localities for the species in Mississippi have also been documented as being temporary (Suttkus and Bailey, 1990; O'Connell et al., 1998). The use of ephemeral habitats by this short-lived species makes accurate assessment of its distribution and conservation status problematic. Further complicating the continued existence of this species in Mississippi is the possible negative impact of Hurricane Katrina which may have destroyed some of the habitats at these historical sites.

Therefore, to determine if *N. melanostomus* are extirpated from historical localities in Mississippi either through long-term habitat loss or loss due to Hurricane Katrina, we re-surveyed the area in 2007-2008 using the exact sampling methods developed for the 1995 survey. We wanted to determine if *N. melanostomus* were still present at any of the historical localities or whether there was evidence that Hurricane Katrina impacted these populations. A failure to find any populations of *N. melanostomus* would suggest it has become extirpated from historical localities in Mississippi. Finally, we also conducted some basic geographical analyses to determine if the pattern of *N. melanostomus* occurrence revealed anything about the preferred macrohabitat of this species.

METHODS

To determine if *N. melanostomus* are extirpated from historical localities in Mississippi, in 2007-2008 we sampled the prime habitats discovered in the 1995 survey. We focused on these habitats to increase the chances of determining if populations still exist. Based on our field notes from the 1995 survey, we sampled sites with prime *N. melanostomus* habitat during the following periods: 22 – 25 May 2007, 5 – 7 June 2007, 20 – 22 June 2007, 9 – 11 June 2008, 23 – 25 July 2008, and 10 – 11

October 2008.

All but two of the eleven historical sites were sampled along with other nearby sites where *N. melanostomus* had never been collected but where potentially prime habitat exists (O'Connell et al., 1995). Sampling involved either a crew of two observers in one boat or a crew of five to six observers in two boats with polarized sunglasses scanning the shoreline for schools of *N. melanostomus*. This method has proved effective for locating *N. melanostomus* in both Florida (Bortone, 1993) and Mississippi (O'Connell et al., 1998). At each historical locality, surveying continued until all suitable habitats were scanned. If a school of *N. melanostomus* was observed, we estimated school size and then attempted to capture voucher specimens using fine-mesh dipnets (as described in O'Connell et al., 1998). Fish were anesthetized in the field with sodium bicarbonate (Booke et al., 1978), fixed in 5% formalin, and preserved in 70% ethanol. Voucher specimens were archived in the Mississippi Museum of Natural Sciences (MMNS) and the University of New Orleans Vertebrate Collection (UNOVC).

In late 2007, we contacted Becky Stowe of the Mississippi Chapter of the Nature Conservancy (TNC). She granted us access to TNC property which had never been surveyed for *N. melanostomus*. We took advantage of this opportunity and surveyed oxbow lakes and ponds on TNC property in 2008. Later in 2008 we also made two trips to the locality of the first known collection of *N. melanostomus* in Mississippi: Doctor Lake. This small, isolated oxbow pond is located in the floodplain of Black Creek, a tributary of the Pascagoula River. While previous attempts post-1995 to locate Doctor Lake were unsuccessful because the lake had completely dried up, on both occasions in 2008 water was present.

We have determined that *N. melanostomus* occur in microhabitats that have submersed aquatic vegetation, sand, and some detritus (O'Connell et al., 1998). These microhabitats are also less turbid than those areas without Blackmouth Shiners (O'Connell et al., 1998). On a macrohabitat scale, lakes where *N. melanostomus* have been found do not have a specific geographic orientation and additionally this species can be found in other types of habitats (i.e., backwaters, ponds, and sloughs). For this study we

attempted to determine if any of these habitats were more important than others for this species. We characterized all sites sampled either in 1995 or 2007-08 into one of four categories: oxbow lake, pond, slough, or backwater. Sites were divided into those where *N. melanostomus* occur (i.e., presence confirmed at least once) or where *N. melanostomus* do not occur (multiple attempts have yielded none). We then conducted a chi square test followed by residual analyses (with residual values >1.96 or <-1.96 indicating significant deviations from expected) to determine which waterbody categories had significant numbers of occurrences of *N. melanostomus*.

Occurrence of *N. melanostomus* in any of these macrohabitats is likely the result of dispersion of individuals during inundation of the floodplain. If so, we expected that the proximity of a macrohabitat to the Pascagoula River could determine the likelihood of occurrence. That is, macrohabitats closer to the river would be more likely to contain *N. melanostomus*. Local topography could also determine whether *N. melanostomus* occurs in a given macrohabitat. Waterbodies at higher elevations are less likely to be inundated during flooding, precluding colonization by *N. melanostomus*. Topography can also determine whether a macrohabitat retains water during drier periods. Using Earthtools (Stott, 2011), we measured the shortest distance from the Pascagoula River for all sites surveyed, including both known *N. melanostomus* sites and non-occurrence sites. We also determined the elevation above sea level for each site. We then characterized each site based on the level of measured *N. melanostomus* occurrence:

- 1) *N. melanostomus* collected both in 1995 and 2007-2008 (highest level of occurrence);
- 2) site sampled both in 1995 and 2007-2008 but *N. melanostomus* collected only during one period OR site sampled during only one of these periods and *N. melanostomus* collected;
- 3) site sampled only in 2007-08 and no *N. melanostomus* collected;
- 4) site sampled only in 1995 and no *N. melanostomus* collected; and
- 5) site sampled both in 1995 and 2007-2008 and no *N. melanostomus* collected.

Using MANOVA (IBM SPSS Statistics 19; SPSS, Inc., 1989; 2010) we determined if distance from the Pascagoula River and elevation differed among these categories to determine if these factors play a role in determining Blackmouth Shiner occurrence in particular macrohabitats.

RESULTS

Of the eight localities that contained *N. melanostomus* populations in 1995, only two yielded *N. melanostomus* during our 2007-2008 survey (Fig. 1). The first of these two historical sites was a shallow floodplain pond located under the Route 26 bridge east of Benndale. This site is located on the Pascagoula River Wildlife Management Area (PRWMA) and is on the east bank of the Pascagoula River. The second of these two historical sites is an artificial pond located southeast of Boneyard Lake (Site #31, MT95-031 in O'Connell et al., 1995). While it was previously believed that this pond was on PRWMA property, clear-cutting in the forest surrounding the pond during the summer of 2008 indicates it is privately owned.

Of the remaining six localities that contained *N. melanostomus* populations in 1995 but did not yield the species in 2007-2008, three were oxbow lake sites. These were repeatedly sampled in 2007-2008 but no *N. melanostomus* were collected even though the habitat was good to moderate. The remaining three 1995 historical sites were either partially or wholly dry. One of these was an ephemeral pond on PRWMA property that was wholly dry for both 2007 and 2008, though it consistently had water and *N. melanostomus* in 1995. Another site was located just west of the Route 26 bridge site mentioned above. This waterbody was low, muddy, and contained neither *N. melanostomus* nor proper habitat, though both were present in 1995. The third historical site was a backwater area of an oxbow lake. No *N. melanostomus* were collected and the water was low, stagnant, and no appropriate habitat was located.

Our efforts to sample beyond the area covered in the 1995 survey resulted in the discovery of a new population of *N. melanostomus* in Luther Lake. Luther Lake is on the PRWMA and is just south of historical site #31 (Fig. 1). Luther Lake was inaccessible in 1995 and could not be surveyed.

Appropriate *N. melanostomus* habitat exists in Luther Lake at the distal ends of the lake (north and south ends) and one school was observed just across from (west of) the PRWMA ramp. Unfortunately, our other efforts beyond the historical *N. melanostomus* range yielded no more new populations. For example, none of the habitats we sampled on TNC property (e.g., see upper-most six white circles in Fig. 1) contained the species. Most of these lakes were turbid and muddy with little to no appropriate *N. melanostomus* habitat. Some of the smaller lakes and sloughs on TNC property, though, did support small areas of good habitat, but no *N. melanostomus* were collected. We also sampled McCrea Dead River on the west bank of the Pascagoula River which was also not accessible in 1995. While in the area, we briefly surveyed potential backwater habitats along the Pascagoula River itself just to the east of this large oxbow lake. Neither McCrea Dead River nor the backwater habitats had appropriate *N. melanostomus* habitat and none were collected. While both trips to Doctor Lake (not shown in Fig. 1) revealed that it was not dry and contained water, neither trip produced a collection of *N. melanostomus*.

Our geographical analyses revealed no relationships between *N. melanostomus* occurrence and the measured geographical features. According to the chi-square analysis, none of the four waterbody categories had more occurrences of *N. melanostomus* than expected (observed 2 = 2.60; $p = 0.91$). Also, neither distance to the Pascagoula River (MANOVA, $p = 0.941$) nor elevation (MANOVA, $p = 0.568$) differed among the five occurrence categories.

DISCUSSION

While our 2007-2008 survey for *N. melanostomus* in historical localities in Mississippi revealed that this species still exists in the region at two historical sites and at a newly discovered locality, we are concerned about the absence of *N. melanostomus* from other historical localities. Based on our observations of habitat conditions both in 1995 and 2007-2008, the only marked change in aquatic habitat over the last twelve years has been the drying of certain ephemeral ponds which had previously contained *N. melanostomus*. The lack of water in these habitats, though, does not appear to be a long-term condition

because many appeared still damp and muddy. We fully expect that after the dry 2007-2008 period these historical sites will again offer potential habitat to *N. melanostomus*. Even though we found fewer localities with *N. melanostomus* than in 1995, there was no evidence that Hurricane Katrina had been the cause of this decline. Except for the low water and dry conditions, the habitats and the area in general seemed mostly unchanged from 1995. It should also be noted that most of these historical sites remain protected as part of the PRWMA and the level of protection has also not changed in the last twelve years.

Our discovery of a new population of *N. melanostomus* in Luther Lake is encouraging and suggests that other undiscovered populations likely exist along the floodplain habitats of the Pascagoula River. This discovery agrees with our modeling work, which predicted that more *N. melanostomus* populations would be discovered with increased surveying efforts (O'Connell et al., 2005). The survey methods needed to find *N. melanostomus* and the habitats where they occur are atypical. The perceived rarity of this species is likely due, in part, to *N. melanostomus* not being collected during standard surveys with typical collecting gear (e.g., seines, electroshocking). As we have done with the help of TNC in the current survey, we suggest that future conservation efforts in Mississippi focus on attempting to gain access to areas that have yet to be surveyed for *N. melanostomus* (e.g., privately owned property).

These current survey results offer a mixed bag regarding the current conservation status of *N. melanostomus* in Mississippi: while finding a new population is a sign that the populations are more numerous than previously thought, the absence of the species from historical sites warrants an increased level of protection until further expanded survey work can be conducted as outlined above. We still know little about the actual ecology of *N. melanostomus*, though our recent observations during 2007-2008 have given us more insight as to how it uses these floodplain habitats. Our analyses indicate that this species is not limited to certain habitats and can occur both near and far from the Pascagoula River. In Mississippi, because *N. melanostomus* occurs more in floodplain habitats versus backwaters of the Pascagoula River, we suspected that individu-

als reach these isolated habitats during flooding events. Therefore the occurrence of *N. melanostomus* might be based on chance and the vagaries of non-directional dispersion during river inundation of the floodplain. We refer to this phenomenon as a flood-based 'lottery' delivery system where individual *N. melanostomus* are deposited either in good habitat (e.g., ponds or oxbow lakes with submersed aquatic vegetation) or bad habitats (e.g., turbid water bodies with no vegetation). The fact that we found no *N. melanostomus* in habitats that appeared good both in 1995 and 2007-2008, suggests that this type of 'lottery' delivery system is not occurring every year. That is, we would have expected to find *N. melanostomus* in at least a few of those good habitats that were not inhabited in 1995: we did not. We suggest that the dispersion might occur only during large, 'super floods' when most of the floodplain is inundated and fishes have access to all riparian ponds and oxbows. This would explain why after 12 years *N. melanostomus* was still present at the historical site #31 which is upstream of a mostly blocked culvert and likely rarely inundated. Without larger floods dispersing *N. melanostomus* to other habitats, populations such as those that existed in historic localities such as Upper and Lower Rhymes lakes and Hudson Lake in 1995 may have died off as this species is short-lived (1-2 years) and available good habitat can change on a seasonal basis.

While lower elevation could indicate the potential for a shallow area to receive and retain water after a flood (due to its closeness to the water table) and thus providing *N. melanostomus* habitat, this factor was not significant in determining *N. melanostomus* occurrence. Describing topography for a site is difficult with just one elevation value as elevation can vary greatly within a site. Even other methods of topography determination may not adequately describe potential *N. melanostomus* habitat within an area. Again this lack of relationship may indicate the vagaries of floodplain dispersion of Blackmouth Shiners. It may, though, also indicate that extensive, persistent shallow habitat is not necessarily good for this species. While the importance of submersed aquatic vegetation for this species is known, there was a lack of *N. melanostomus* in some sites where there was widespread shallow habitat with submersed aquatic vegetation. Areas with extensive submersed

aquatic vegetation may provide habitat for other species which could potentially out-compete or prey upon *N. melanostomus*. Besides the magnitude of flooding playing as a determinant of where *N. melanostomus* occur, it may be that persistent yet small amounts of clear, shallow areas with submersed aquatic vegetation offer better habitat for this species by protecting them from those species that need and can exploit larger areas with good habitat. It is important to better understand these *N. melanostomus* requirements so that essential habitat can be adequately protected.

We are also concerned about the current conservation status of *N. melanostomus* in Mississippi because historical site #31 is directly threatened by recent clear-cutting activities which extend up to the banks of this pond. Based on all of our research since 1995, we know that this site contains the largest and most consistent population of the species in Mississippi. Though we had assumed in the past that this pond was on the PRWMA, the 2008 logging activity revealed that the pond is located on private in-holdings that are being cleared for development. The loss of this large *N. melanostomus* population will eliminate one of only two populations which survived between 1995 and 2007-2008 (the other is under the Route 26 bridge). We suggest either protection of this pond (if the population has not already been eliminated) or more efforts to confirm that other large populations of *N. melanostomus* exist elsewhere in Mississippi.

ACKNOWLEDGMENTS

This research was funded by a grant from the U.S. Department of the Interior, Fish and Wildlife Survey (Agreement # 401817G022). We would like to thank Daniel Drennen and Todd Slack for their help with developing this project. Thanks also to Becky Stowe of the Mississippi Chapter of the Nature Conservancy who allowed us unprecedented access to previously un-surveyed areas. Finally, thanks to the following individuals who assisted us during the 2007-2008 field season: Sunny Brogan, Chad Ellinwood, Ashley Ferguson, Patrick O'Connell, Jeff Van Vrancken, and Jenny Wolff. This manuscript represents publication No. 11 for the Nekton Research Laboratory, Pontchartrain Institute for Environmental Sciences.

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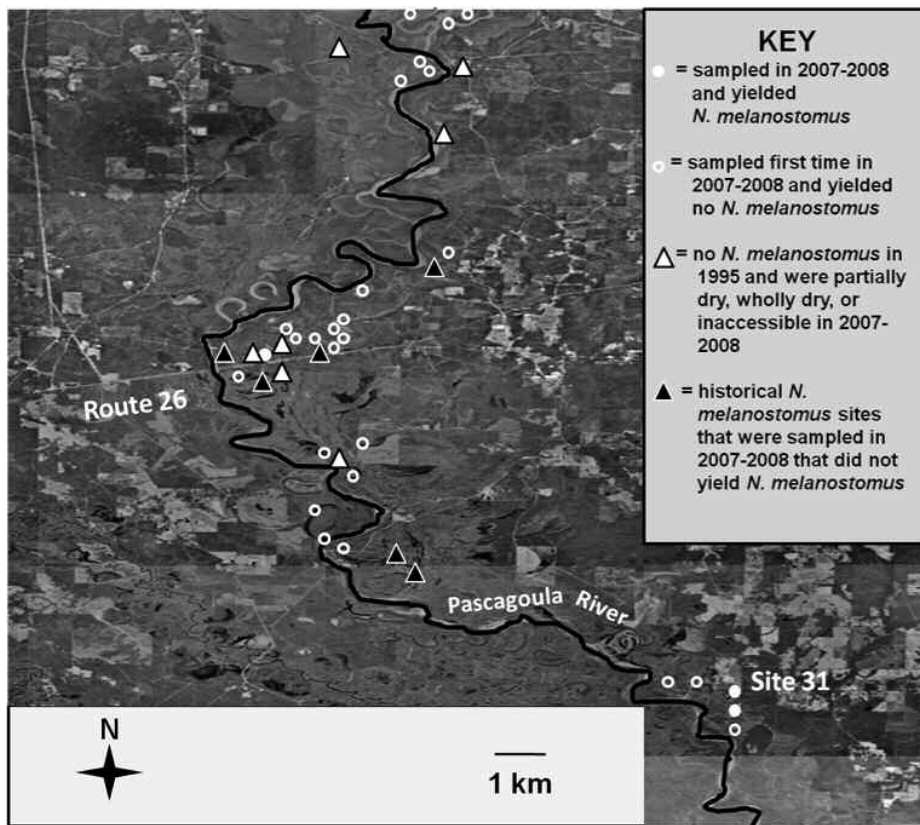


Figure 1. Select sampling sites for 1995 and 2007-2008 survey for *Notropis melanostomus* in historical and nearby localities along the upper Pascagoula River, Mississippi. Symbols represent different results of sampling (see key). Historical site 31 (MT95-031) contained the largest and most consistent population of the species in Mississippi (lower right). This site is currently threatened by riparian vegetation loss due to clear-cutting.

Rediscovery of a Lost Paratype of the Boulder Darter, *Etheostoma wapiti*

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Etheostoma wapiti Etnier and Williams, Boulder Darter, is an endemic of the Elk River and Shoal Creek (Tennessee River basin) in south central Tennessee and northwestern Alabama. It is protected as Federally Endangered (Biggins, 1988), Endangered in Tennessee (Withers, 2009), and is a species of Highest Conservation Concern in Alabama (Shute, 2004). At the time of its description only 55 specimens were known (Etnier and Williams, 1989), and it is estimated that there are presently about 80 wild-caught specimens cataloged in natural history collections (Boschung and Mayden, 2004). Etnier and Williams (1989) designated 48 specimens in 16 lots as paratypes. Two of the paratype lots are cataloged in the Royal D. Suttkus Fish Collection at the Tulane University Museum of Natural History (TU). However, it was reported in the most recent account of the TU type specimens that TU 30271 was missing (Bart and Taylor, 1993). Consequently, this paratype lot was excluded from the printed *Catalog of Fishes* (Eschmeyer, 1998) and the online version (Eschmeyer, 2012). The other lot of paratypes at Tulane, TU 148010, has two specimens and was accounted for by Bart and Taylor (1993).

During ongoing recuration at TU, the lost paratype TU 30271 was rediscovered. The single specimen is 28.37 mm SL and its sex is undetermined. R.D. Suttkus, J.S. Ramsey, and M.D. Dahlberg collected this specimen in 1963, from the Elk River at mile 89.7, Fayetteville, Lincoln County, Tennessee. This locality is at the known upstream limit of *E. wapiti* in the Tennessee River

basin (Etnier and Williams, 1989; Etnier and Starnes, 1993). The condition of the specimen is relatively poor as it is soft and pigmentation of the fins is lacking. A note written on the original catalog label stated that the dorsal fin margin had orange pigment. This anecdotal note is somewhat contradictory to the description by Etnier and Williams (1989) that the submarginal band is pale or yellowish. The original TU label tentatively identified the specimen as *Etheostoma camurum* (Cope) and later the identification was changed to *Etheostoma* sp. David A. Etnier examined the specimen and recognized that it was a new species of the *Etheostoma maculatum* Kirkland species group. After publication of the description of *E. wapiti* and designation of TU 30271 as a paratype, it appears that the lot remained shelved with approximately 220 other lots identified as "*Etheostoma* sp." rather than being moved to the separate type room at TU.

Justin G. Mann brought my attention to the unidentified lots of darters at TU and helped in locating and recurating the specimen. I thank Henry L. Bart, Jr. for permission to publish this correspondence.

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Business Meeting

37th Annual Meeting Southeastern Fishes Council

10th-11th November, 2011

Chattanooga, TN

The 2011 business meeting of the Southeastern Fishes Council was called to order by Chair Gerry Dinkins at 4:31 p.m. Seventy-six people were in attendance.

Report of the Secretary

Secretary Rebecca Blanton reported that the 2010 minutes were unanimously accepted by e-mail vote; 26 members voted. She noted that the 2010 minutes would be posted on the SFC website.

Secretary Blanton also provided a summary of the current and past membership, noting the following for 2011 and overall for the history of the society:

191 total members including:

5 life members (2%)

121 regular members (65%), including 8 family memberships (8%) and 3 library memberships (2%)

62 student members (33%)

the expectation that expenses of the current meeting would be offset by income from registration.

George noted that the 2011 ending budget presented did not include expenses for the 2011 meeting (\$9850) or the printing and shipping for the 2011 Proceedings (\$2000). She reported the total expenses and revenue for the year at \$14,000 (expenses)/\$16500 (revenue). Chair Dinkins called for a vote to approve the Treasurer's Report, the motion was seconded and the report was unanimously approved.

Report of the Committees

Nominating Committee – Carol Johnston.

Candidates for Chair, Secretary, and Treasurer were previously identified by the Chair of the nominating committee, Carol Johnston. Election of new officers was held by ballot vote. Candidates for the Chair-elect were Ginny Adams (University of Central Arkansas) and Cathy Phillips (U.S. Fish and Wildlife Service), for Secretary, Mollie Cashner (Southeastern Louisiana University) and Will Duncan (U.S. Fish and Wildlife Service), and for Treasurer, Steve Ryder (Alabama Fish and Wildlife Service), and Benjamin Keck (University of Tennessee). New officers elected were: Ginny Adams, Chair-elect; Mollie Cashner, Secretary, and Steve Ryder, Treasurer.



Report of the Treasurer

Treasurer Anna George presented the society's overall financial standing, including a summary of the expenses related to the 2011 meeting. She noted the overall good financial standing of the society and

Program Committee – Mary Freeman, Anna George and Jim Williams.

Nothing to report.

Constitution Committee – Bernie Kuhajda

Nothing to report.

Proceedings Committee – David Neely and Chris Skelton

Editor Neely reported that the 2011 issue of the Proceedings was complete and would soon be mailed to the membership. He noted that only one volume would be produced in the current year and that the state reports would be included in that volume.

Awards Committee – Anna George

The executive committee and membership acknowledged the service of out-going Chair Dinkins and thanked him for his contributions to the society. Chair Dinkins thanked out-going Treasurer George and Secretary Blanton for serving two terms in these positions and playing a critical role in the success of the society's first and subsequent stand-alone meetings. Former Chair Kuhajda noted that he would be cycling off the executive committee and thanked N. Burkhead and other committee members for their assistance throughout his tenure.

Technical Advisory Committee – Steve Powers, Patrick Rakes, Ginny Adams, Charlie Saylor, Jim Williams, Hank Bart, and Brett Albanese

Jim Williams informed the membership that he would be stepping down from this committee and Anna George volunteered to serve as his replacement. George noted that she would be focusing on completion of the Fishes In Need conservation reports for Tier 1 and Tier 2 fishes with the hopes of completing all by the following year.

Membership Committee – Hank Bart and Rebecca Blanton

Nothing to report.

Website Committee – Jake Schaefer

Nothing to report

Old Business

Keeping the membership informed of federal register actions.

Jim Williams noted that he would continue to monitor register actions and pass pertinent information on to the SFC executive committee.

Potential relationship with Patagonia.

Anna George noted that no relationship or agreement had been established with Patagonia.

New US Fish and Wildlife Position stationed at the Tennessee Aquarium.

Anna George said that the proposed plan to create a USFWS position had not been established and likely would not be established.

Status of Center for Biological Diversity Petition.

T. Curry from the CBD provided an update on the petition status. She requested SFC members provide comments, critical habitat, or other pertinent information to USFWS to assist with their review of petitioned species.

New Business

2012 SFC meeting venue

Chair Dinkins noted the 2012 meeting would be held in New Orleans, LA and that Hank Bart would serve as the local host. H. Bart noted the contributions of Royal Suttikus to SFC and southeastern fishes and suggested that the society honor these contributions at the 2012 meeting by holding a Suttikus-themed symposium and memorial.

Mel Warren asked for the subsequent meetings to not be held over the Veteran's Day holiday. Anna George noted that an effort could be made to avoid scheduling the meeting over this holiday, but due to conflicts with other events scheduled at meeting venues, that it could not always be avoided.

Policy on presentation cancellations

Chair Dinkins requested that the membership avoid last-minute cancellations of presentations. He asked the membership to entertain the idea

of imposing repercussions, such as preventing a speaker from submitting an abstract in the subsequent year, for non-justified, last-minute cancellations. Bruce Bauer moved to accept this policy, A. George seconded, and the motion carried unanimously.

Registration charges at future meetings

Chair Dinkins requested that the membership follow deadlines posted with respect to early registration and hotel reservations to the best of their ability. Treasurer George requested an increase in the late registration cost to alleviate the costs associated with accommodating the large number of late or walk-up registrants. N. Burkhead expressed concerns with this plan due to the fiscal year restrictions of government employees that often restricted their ability to pay registration fees before the early-registration deadline. Mollie Cashner suggested increasing walk-up registration fees only, as this seemed to be the largest concern with respect to creating unexpected expenses at the meeting. Pat Rakes suggested an RSVP option for members that planned to attend, but would not be able to pay prior to the early-registration deadline, to allow the society to account for those individuals in the overall meeting numbers, while allowing government employees more time to resolve their fiscal year budget issues. After additional discussion, Chair Dinkins asked for a motion to double the current walk-up registration fee. H. Bart moved, J. Williams seconded, and the motion carried unanimously.

Vendors

The attendance of vendors and potential fees to vendors at subsequent meetings was discussed. J. Williams moved that vendors be permitted, but charged a vendor fee (registration and table fee). The motion carried unanimously.

Horse "Park" on Citico Creek

Pat Rakes noted a proposal to establish a horse stable and 4-acre parking lot on Citico Creek adjacent to a known site of the Yellow-fin Madtom. He further noted that there was also a proposal in place to develop the parking lot and

other features at an alternative site. He said that work by the Southern Environmental Law Center indicated that the alternative site would have a minimum impact to Citico Creek overall. Noel Burkhead asked the executive committee to write a letter on behalf of SFC opposing the development at the Yellow-fin Madtom location. Chair Dinkins noted that he had written a letter previously. N. Burkhead and J. Williams proposed that the letter request complete removal of the planned development from the Citico Creek watershed given the other federally listed species there. Peggy Shute pointed out that the Biological Opinion notice associated with the planned development was posted on Christmas Eve, and no formal consultation of plan had been conducted. Dinkins called for a motion to write and submit a resolution and letter on behalf of SFC opposing construction of the horse area and parking lot in the Citico Creek watershed. H. Bart and C. Johnston moved, J. Williams, seconded the motion, which passed unanimously.

Student Awards

Herbert Boschung noted the good financial standing of SFC and requested that the society increase the student award amounts. Treasurer George indicated that it would be feasible under the current budget to raise award amounts. An alternative proposition to offer travel awards was also proposed. Ginny Adams suggested restructuring award categories to include awards for undergraduate, Master's, and Ph.D. level students. B. Kuhajda called for a motion to double the amount of money allotted to student awards and then hold an email vote to determine how to divide or use the extra allotted money. N. Burkhead moved, J. Williams seconded, and the motion passed unanimously.

The meeting was adjourned by Chair Dinkins at 5:34 p.m.

Respectfully submitted, Secretary Rebecca Blanton Johansen

**2011 Treasurer's Report
for the Southeastern Fishes Council
Prepared by Anna George**

2011

Starting Balance	\$30,822.49
1 January 2011	

EXPENSES

Meeting Room Deposit	\$300.00
T-Shirts	\$1322.43
Gifts	\$184.24
Paypal Fees	\$359.51

TOTAL EXPENSES	\$2166.18
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INCOME

Memberships	
110 Regular at \$30	\$3300.00
66 Students at \$15	\$990.00
7 Family at \$40	\$280.00

2010 Meeting Registration	\$10,760.00
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Donations	\$312.86
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TOTAL INCOME	\$15,642.86
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ENDING BALANCE	\$44,299.17
(as of November 5, 2011)	

Southeastern Fishes Council Proceedings

INFORMATION FOR CONTRIBUTORS

The primary purpose of the *Proceedings* is to publish peer-reviewed research papers and critical reviews of activities; regional reports and notes; and other pertinent information pertaining to the biology and conservation of southeastern fishes. The *Proceedings* is also an outlet for range extensions, distributions, and status papers, covering ecology and conservation ichthyology. Life history studies, faunal surveys, management issues, behavior, genetics and taxonomy of southeastern fishes are appropriate topics for papers in the *Proceedings*. Review papers or information on imperiled waters or fishes are particularly appropriate.

Manuscripts can be submitted electronically via email (send to: dave.neely@gmail.com) or mailed as hard copies to the address below. Mailed hard copies should be submitted in triplicate. A good guide for manuscript preparation is the Sixth Edition of the *CBE Style Manual* available from the Council of Biology Editors, One Illinois Center, Suite 200, 111 East Wacker Drive, Chicago, IL 60601-4298.

The entire manuscript including the Abstract (required for longer articles), Introduction, Methods, Results, Discussion, Acknowledgments, Literature Cited, Appendices, Tables, and Figure Legends must be double-spaced. The title, author's name and author's address (including fax number and email address for corresponding author) should be centered on the first page. Indicate a suggested running head of less than ten words at the bottom of the first page. An Abstract (if necessary) will be placed at the beginning of the text. Acknowledgments will be cited in the text immediately before the Literature Cited. All references cited in the paper will follow the standard format of using the last name of the author(s) followed by the year of publication of the paper. In the Literature Cited, the references will be alphabetical by the author's last name and chronological under a single authorship. Literature cited should be standardized and abbreviated, using the *World List of Aquatic Sciences And Fisheries Serial Titles* or guidelines in *CBE Manual for Authors, Editors, and Publishers, 6th edition* for journals not included in the World List.

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Regional reports, news notes and other short communications will also be edited and included when possible in the next number.

Only manuscripts from members of The Southeastern Fishes Council will be considered for publication. There is no charge for publishing in the *Proceedings*. All manuscripts and short communications should be sent to the editor:

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Proceedings is a publication of the Southeastern Fishes Council, Inc., and is published in Milledgeville, Georgia. Officers are Carol Johnston, Chair; Ginny Adams, Chair-Elect; Gerry Dinkins, Immediate Past Chair; Mollie Cashner, Secretary; Steve Rider, Treasurer; and Jake Schaefer, Webmaster. Managing Editor for the Proceedings is David Neely, Southeastern Fishes Council Proceedings, Tennessee Aquarium Conservation Institute, 1 Broad Street, Chattanooga, TN 37402, Phone: (415) 608-9227 Email: dave.neely@gmail.com; Associate Editor is Christopher E. Skelton, Department of Biological and Environmental Sciences, Georgia College & State University, Milledgeville, GA 31061, (478)-445-2440, chris.skelton@gcscu.edu. Proceedings is printed on recycled paper.

