

3-1-1998

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O'Connell, Martin T.; Ross, Stephen T.; Ewing, John A. III; and Slack, William T. (1998) "Distribution and Habitat Affinities of the Blackmouth Shiner (*Notropis melanostomus*) in Mississippi, Including Eight Newly Discovered Localities in the Upper Pascagoula River Drainage," *Southeastern Fishes Council Proceedings*: No. 36.

<https://doi.org/10.7290/sfcp363cri>

Available at: <https://trace.tennessee.edu/sfcproceedings/vol1/iss36/3>

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ABSTRACT

Lakes, ponds, and sloughs of the Upper Pascagoula River Drainage, Mississippi, were surveyed for the blackmouth shiner (*Notropis melanostomus*) from April to June, 1995. Because of its limited distribution, the species is federally listed as threatened. Blackmouth shiners were discovered in eight previously unknown sites. These newly discovered Mississippi populations of *N. melanostomus* are similar to Florida populations in that they are associated with clear, stained, acidic waters and abundant submerged vegetation. Unlike Florida populations, the Mississippi populations occupy isolated oxbow lakes and temporary floodplain pools that are only interconnected during floods of the Pascagoula River. The ephemeral nature of some of these habitats may explain why Mississippi populations of *N. melanostomus* appear to be less abundant than Florida populations. This knowledge of the specific habitat affinities of *N. melanostomus* should prove useful in management of the species.

INTRODUCTION

The blackmouth shiner, *Notropis melanostomus*, is currently known only from three separate drainages: 1) Blackwater River Drainage, Florida; 2) Yellow River Drainage, Florida; and 3) Pascagoula River Drainage, Mississippi (Suttkus and Bailey, 1990). In Mississippi, blackmouth shiners have been found in three areas within the Pascagoula River Drainage: 1) Doctor Lake in the Black Creek System; 2) the upper Pascagoula River downstream of state highway 26; and 3) an unnamed oxbow lake off of the lower Chickasawhay River (Ross, in prep.). Although local populations in Florida and Mississippi can be abundant, overall the blackmouth shiner is rare (Suttkus and Bailey, 1990).

Because of "its limited distribution and virtual dearth of information on the status of the extant populations" (Bortone, 1993), *N. melanostomus* is listed as "Threatened" by the American Fisheries Society (Williams et al. 1989). Most of the information about the blackmouth shiner comes from the relatively recent description of the species (Bortone, 1989) and subsequent descriptions of its biology (Suttkus and Bailey, 1990; Bortone, 1993). It is one of the smallest American cyprinids (the largest adult taken at Pond Creek, Florida was 34.6 mm SL) and has a relatively short (< two years) life span

(Suttkus and Bailey, 1990). Other distinct morphological characteristics include a large, oblique mouth and long, well-developed gill rakers (Suttkus and Bailey, 1990). These features suggest that the blackmouth shiner's closest relative is the Kiamichi shiner, *N. ortenburgeri* (Swift et al., 1986; Robison and Buchanan, 1988; Suttkus and Bailey, 1990). Although morphologically similar, the two species have profoundly different habitat preferences and distributions.

Collection records from Florida and Mississippi indicate that the blackmouth shiner may be restricted to oxbow lakes and backwater areas. Suttkus and Bailey (1990) noted that no blackmouth shiners were collected from main channel habitats. Although a 1989 survey (Ross et al. 1989) yielded two of the known localities of blackmouth shiners from backwater areas, this sampling focused primarily on main channel habitats of the Leaf, Chickasawhay, and Pascagoula rivers. Little sampling occurred in oxbow lakes and backwater areas, which are abundant in the Upper Pascagoula River Drainage. Because such areas could contain "other undiscovered disjunct populations [of blackmouth shiners]" (Suttkus and Bailey, 1990), we conducted a survey of oxbow lakes and backwater habitats in the vicinity of the three historic sites. Our objectives were to find undiscovered populations of blackmouth shiners and then describe their habitats.

METHODS

From United States Geological Survey topographic maps (1:24,000), we identified a study area in the Pascagoula River State Wildlife Management Area. The study area extended from Graham Lake near the town of Wade, Jackson County, upstream to the confluence of the Leaf and Chickasawhay rivers, near the town of Merrill, George County. Before sampling began, more potential sites were identified on two scouting trips (14 and 20 April 1995). Bortone (1993) suggested that oxbow lakes and backwater areas of streams were the preferred habitats of Florida blackmouth shiners. Because little is known about preferred blackmouth shiner habitats in Mississippi, our potential sites included habitats that were likely to contain blackmouth shiners (oxbow lakes, backwater areas) and areas less likely to contain blackmouth shiners (sloughs, ephemeral ponds, rivers). Sampling began on 23 May and ended 22 June 1995. A total of 52 collections of fishes, including measurement of habitat variables, was made in the study area.

Initially we used three methods for collecting fishes. In the first method, based on Bortone (1993), we used polarized sunglasses to scan shoreline areas from a 4.3 m (14 ft) aluminum boat powered by an electric trolling motor. When we located a school of fish that could potentially be blackmouth shiners, we attempted to collect the fish using fine-mesh (3.2 mm) dipnets. Fishes collected in this manner were preserved in 10% formalin for identification in the laboratory. No serious effort was given to collecting from schools of fishes that were determined not to be blackmouth shiners (e.g., larger, non-minnow-like fishes). For each site, we recorded the length of shoreline with appropriate habitats (as described by Bortone, 1993) that was scanned and sampled for fishes.

The second method involved seining appropriate habitats with either a 3 x 1.22 m straight seine (3.2 mm mesh size) attached to wooden brailes or a 6 x 1.22 m bag seine (3.2 mm mesh size) attached to ropes. The latter method was used in an appropriate habitat where it was left undisturbed for 15-20 min. After this period, the seine was retrieved by pulling on the ropes attached to its corners. This method allowed for quick retrieval of the seine in areas where the soft, muddy substratum precluded seining by wading. Once we determined that blackmouth shiners were collected more efficiently using the dipnetting method, we discontinued seining on 8 June (last seining collection: MT95-024).

We also placed out two hardware cloth minnow traps in appropriate habitats. Each trap was baited with catfood and was fished for 12-24 h. This method was discontinued after June 5 (last trapping attempt: MT95-016) because no fishes had been caught.

At each site, we measured turbidity, pH, temperature, and water depth. Water samples were placed on ice, returned to the laboratory, and measurements were taken within 48 h of collection. Turbidity (NTU) was determined with a turbidity

meter (HF instruments, Model DRT 1000) and pH was determined with a portable pH meter (Orion Research, Model SA 210). Water temperature was determined with a hand held thermometer. Type and relative proportion of different substrata were visually estimated. Five depth readings were taken in an area considered representative of the area sampled. When blackmouth shiners were collected, the five depth readings were taken at the place of collection. Additional observations included numbers and types of other fish species seen, descriptions of vegetation, and notes on blackmouth shiner behavior.

Using these data on environmental variables, a discriminant function (DISCRIM SPSS^x 2.1; SPSS, 1985) and a canonical variates analysis (CVA; CANOCO 3.12; ter Braak, 1991) were calculated. The objective of these exploratory analyses was to find linear combinations of the environmental variables that separated sites with and without *N. melanostomus* (James and McCulloch, 1990). The relative importance of each variable in identifying blackmouth shiner habitats was also determined. Because our sample size is large relative to the dimensionality of the data, any patterns exhibited are likely to be ecologically consequential (Williams, 1983).

All fishes that were preserved in the field in 10% formalin were later transferred to 45% isopropanol and identified in the laboratory. Morphometric, scale, and fin-ray characters were measured following Hubbs and Lagler (1958), using a dissecting microscope and calipers. Collected material is archived in the University of Southern Mississippi Museum of Ichthyology.

RESULTS

We collected blackmouth shiners (N=439) from eight previously undiscovered sites in the Pascagoula River System (Fig. 1, Appendix). Morphometric, scale, and fin-ray characters of these fish were similar to those recorded by Bortone (1993) for blackmouth shiners in Florida (Table 1). Two size classes were represented in the collected specimens (Fig. 2). We assumed that the larger size class of fish (SL > 25 mm)

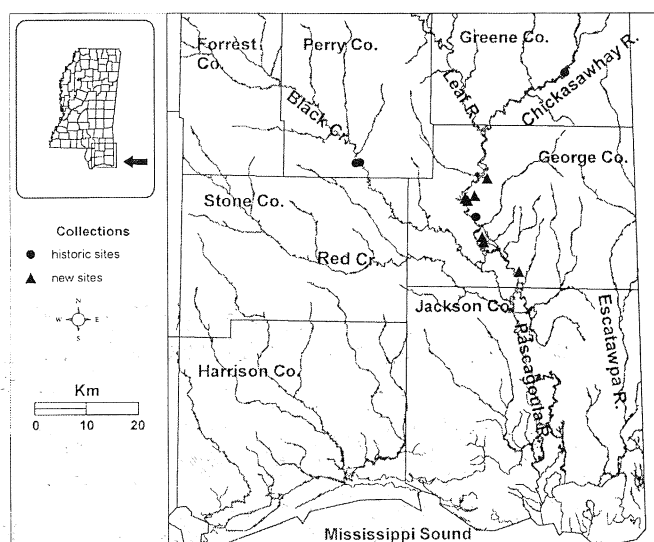


Figure 1. Distribution of the blackmouth shiner in Mississippi including historic sites and sites resulting from the present study.

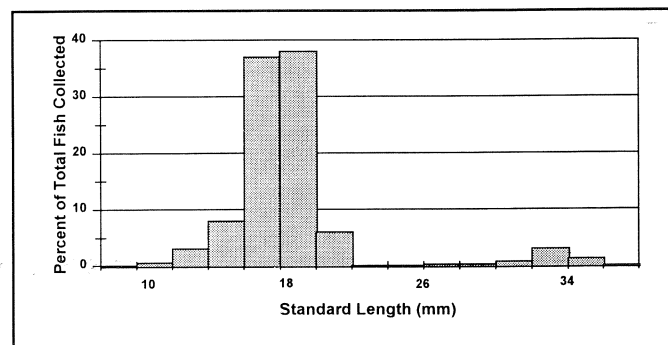


Figure 2. Length-frequency histogram of 439 blackmouth shiners (*Notropis melanostomus*) collected during the survey period of May 23 - June 22, 1995, in the Upper Pascagoula River System.

Table 1. Summary of some morphometric, scale, and fin-ray characters from 87 specimens of blackmouth shiners from the Pascagoula River System, Mississippi. The standard lengths are based on 439 specimens collected (does not include specimens collected for photograph material). Head length and body depth are recorded as a proportion of standard length. Scale count and pored scales are in reference to lateral scales.

Character	Mean	Min	Max	S.D.
Standard length (mm)	18.54	11.50	35.00	3.85
Head length	0.2420	0.2244	0.2727	0.01
Body depth	0.1653	0.1241	0.1981	0.02
Scale count	34.20	32	36	0.99
Dorsal rays	8.02	8	9	0.15
Anal rays	10.40	9	12	0.58
Left pectoral rays	10.90	9	12	0.70
Left pelvic rays	8.09	7	9	0.42
Pored scales	3.65	0	7	1.48

Table 2. Summary of five environmental variables measured at the eight sites where blackmouth shiners were collected in the Upper Pascagoula River System, Mississippi, between 23 May and 22 June 1995. Measurements were taken at the location and depth of where blackmouth shiners were collected.

Variable	Mean	Min	Max	S.D.
water temperature (C)	29.25	24.00	34.00	3.01
air temperature (C)	32.25	28.00	35.00	2.31
pH	6.14	5.51	6.79	0.42
turbidity (NTU)	4.01	2.20	6.00	1.42
capture depth (cm)	67.09	21.00	101.50	19.12

represented adults because 21 mm SL was proposed as the minimum adult size by Suttkus and Bailey (1990). Also, of these 25 larger fish collected, ten possessed pectoral fin tubercles, as described by Bortone (1993). Gonads of tuberculate and non-tuberculate larger fish were excised, treated with methylene blue, examined microscopically, and compared to determine sex. These examinations revealed that only male fish possessed tubercles. This suggests an adult male-female ratio of 1:1. Fish in the larger size class (adults) were found only at two of the eight sites (MT95-017 and MT95-031). The majority (414 of 439) of blackmouth shiners collected, and most of the blackmouth shiners observed, were in the smaller size class (juveniles).

At the eight sites where blackmouth shiners were collected, there was no water current and the water was warm, clear, shallow, and moderately acidic (Table 2). Compared with environmental parameters given by Bortone (1993) at sites

Table 3. Fish species collected at blackmouth shiner sites.

Species	Common name
<i>Aphredoderus sayanus</i>	pirate perch
<i>Esox niger</i>	chain pickerel
<i>Notemigonus crysoleucas</i>	golden shiner
<i>Pteronotropis welaka</i>	bluenose shiner
<i>Erimyzon tenuis</i>	sharpfin chubsucker
<i>Ameiurus nebulosus</i>	brown bullhead
<i>Fundulus notti</i>	southern starhead topminnow
<i>Fundulus chrysotus</i>	golden topminnow
<i>Gambusia holbrooki</i>	eastern mosquitofish
<i>Labidesthes sicculus</i>	brook silverside
<i>Micropterus salmoides</i>	largemouth bass
<i>Pomoxis nigromaculatus</i>	black crappie
<i>Lepomis macrochirus</i>	bluegill
<i>Lepomis marginatus</i>	dollar sunfish
<i>Lepomis microlophus</i>	redear sunfish
<i>Enneacanthus gloriosus</i>	bluespotted sunfish
<i>Elassoma zonatum</i>	banded pygmy sunfish
<i>Etheostoma fusiforme</i>	swamp darter

Table 4. Results of discriminant function analysis of environmental variables measured at sites with and without blackmouth shiners.

Predictor variable	Correlations of predictor variables with discriminant functions	Univariate F (3,28)
vegetation presence	0.90	45.12
detritus substratum	0.31	1.53
sand substratum	0.08	1.68

Canonical R = 0.83

Eigenvalue = 2.16

where blackmouth shiners were collected in Florida, these Mississippi collection sites were deeper and the water was less acidic. The aquatic vegetation associated with these sites included *Potamogeton*, *Nymphaea*, and *Najas*. There were 18 other fish species recorded from these sites (Table 3).

The analyses of the environmental data yielded differences between sites with and without blackmouth shiners. Using measurements of habitat parameters, a discriminant function was calculated for sites with and without blackmouth shiners ($\chi^2 = 32.81$, $p < 0.01$, canonical R = 0.83). Submerged vegetation, detritus (negative loading), and sand were the three habitat parameters significant in determining the presence or absence of blackmouth shiners, but the presence of submerged

vegetation was the single most critical variable (Table 4). The ordination diagram based on canonical variates analysis (CVA) of sites with and without blackmouth shiners, with respect to four habitat parameters (turbidity, presence of sand, detritus, or clay), suggests that water clarity (NTU) had the greatest contribution in determining differences between blackmouth shiner habitats and non-blackmouth shiner habitats (Fig. 3). Also, sites with blackmouth shiners were significantly less turbid than sites without blackmouth shiners (Wilcoxon two-sample test, $t_s = 3.72$, $p < 0.0001$).

In comparing collection sites with the orientation of shorelines, field observations suggested that blackmouth shiners were more common adjacent to shorelines that had a north-south orientation. To test this hypothesis, we compared the orientation of the four permanent lakes where blackmouth shiners were collected to the orientation of 14 other sampled permanent lakes where blackmouth shiners were not collected, using a Mardia-Watson-Wheeler test. Lake orientation (the axis of the longest dimension) was determined from USGS topographic maps (1:24,000). There was no significant difference between the orientation of the four permanent lakes where blackmouth shiners were collected and the orientation of fourteen permanent lakes where blackmouth shiners were not collected (Mardia-Watson-Wheeler test, $B = 6.38$, $p > 0.10$).

DISCUSSION

Our addition of eight blackmouth shiner sites to the known distribution of this species confirms suspicions that other undiscovered disjunct populations exist (Suttkus and Bailey, 1990; Bortone, 1993). We also suspect that further surveys of appropriate habitats in the Pascagoula River Drainage (Leaf River, Chickasawhay River, Black Creek, Red Creek, and others) will yield additional populations of blackmouth shiners. Only two of these blackmouth shiner sites (Hudson Lake and Lower Rines Lake) appeared to have anything close to the "thriving" Florida populations with "schools of several thousand individuals" described by Bortone (1993). The biggest shoals we observed contained hundreds of individuals and these were always juveniles. At the two sites where only adult blackmouth shiners were collected, the shoals never numbered more than twenty individuals. These lower numbers may suggest that Mississippi populations of blackmouth shiners are less viable than Florida populations. The Mississippi populations may also represent more of a patchy distribution than the Florida populations.

The two size classes of blackmouth shiners support Bortone's (1993) contention that blackmouth shiners live no longer than a year to a year and a half, as predicted by Suttkus and Bailey (1990). Adult and juvenile blackmouth shiners were only collected together at one site (MT95-017: 14 adults and two juveniles collected). Blackmouth shiner collections from the other sites were exclusively adults or juveniles. This may be due to the isolation of the populations sampled. Unlike the Florida populations of blackmouth shiners in the Lower

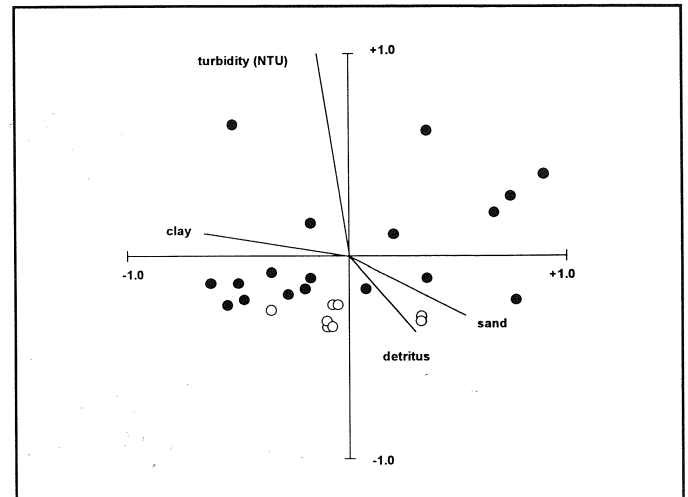


Figure 3. Ordination diagram based on canonical variates analysis (CVA) of sites where blackmouth shiners were found (open circles). The length and direction of the turbidity (NTU) vector suggests that water clarity had the greatest contribution in separating blackmouth shiner habitats from non-blackmouth shiner habitats.

Blackwater River Drainage, there are no permanent water connections between any of the sites where we collected blackmouth shiners. It is possible that each of the populations we sampled was the result of a temporally and spatially unique spawning event. Seasonal flooding may provide the temporary connection between these ponds and pools that allows blackmouth shiners to colonize different areas at different times.

Mississippi populations of blackmouth shiners exhibit a marked affinity toward clear water and submerged vegetation. The Mississippi collection sites were deeper and the water was less acidic than the Florida sites. It is not known if this represents a significant difference in habitats between the Mississippi and Florida populations of blackmouth shiners. This difference may be due to the nature of the Mississippi habitats (oxbow lakes, floodplain ponds) versus the Florida habitats (mostly backwater areas).

Our observations suggest that blackmouth shiners may only occur in lakes and ponds whose geography promotes the growth of submerged aquatic vegetation, low turbidity, or both. The orientation of a lake affects the presence of vegetation because plant colonization is governed primarily by turbulence, substratum, and light intensity and quality (Sculthorpe, 1971). We suggest that the orientation of the shoreline affects whether submerged aquatic vegetation is present, possibly because of protection from east to west winds or increased exposure to light, or both. Because blackmouth shiners are highly associated with submerged vegetation, shoreline orientation may determine if blackmouth shiners will be present in a permanent lake. Although our comparison of lake orientations showed no differences between lakes with and without blackmouth shiners, this could have been due to small sample size.

Every aspect of blackmouth shiner behavior described by Bortone (1993) was observed for Mississippi populations of the species. The blackmouth shiners formed tight schools or shoals that were globular or irregular in shape. These shoals were never less than 20 cm from the water surface and were always associated with submerged vegetation. Blackmouth shiners were easily distinguished from other fishes due to their distinct shoaling. For example, although brook silversides (*Labidesthes sicculus*) were often associated with blackmouth shiners (as with the Florida populations), their schools could be distinguished from *N. melanostomus* schools due to their higher position in the water column, faster swimming, and more orderly schooling.

An important point concerning future management of this species in Mississippi is the ephemeral condition of blackmouth shiner habitats (Bortone, 1993). Four of the eight sites in this study appeared to be temporary floodplain pools or ponds. Over the study period, the water level in these ponds and pools declined, and these habitats could be eliminated during long dry periods. We sampled a historical site in a backwater area of the Pascagoula River and found that the area was completely dry. Also, Suttkus and Bailey (1990) could not find any blackmouth shiners at another historical site (Doctor Lake) during subsequent collection trips. We have found this area to be essentially dried up on at least two occasions since.

The eight sites discovered during this survey were located in the Pascagoula River Wildlife Management Area. As long as this area is maintained as a wildlife area, these blackmouth shiner habitats are not likely to be destroyed by anthropogenic disturbances.

ACKNOWLEDGMENTS

We thank B.W. Albanese and A.M. O'Connell for help with field work.

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APPENDIX

Site	Field Number	Date	Water Body	Location	Gear	Length of Sampled Reach (m)
8	MT95-008	29 May 95	unnamed pond	at corner of road to Dace Lake and road to Hudson Lake; T2S, R8W, Sec 11 UTMX: 332800 UTMY: 3417800	3 m seine	10
24	MT95-024	7 Jun 95	unnamed pond	under rt 26 bridge, first pond west of Big Slough; T2S, R8W, Sec 15 UTMX: 330900 UTMY: 3417300	dipnet	100
31	MT95-031	13 Jun 95	unnamed pond	northernmost of 2 lakes south of Boneyard Lake; T3S, R7W, Sec 23 UTMX: 341300 UTMY: 3404200	dipnet	400
33	MT95-033	13 Jun 95	unnamed pond	west of road to Cochrans Dead Lake; T1S, R7W, Sec 31 UTMX: 335200 UTMY: 3420900	dipnet	300
38	MT95-038	19 Jun 95	unnamed pond	under rt 26 bridge, third pool west of Big Slough; T2S, R8W, Sec 15 UTMX: 331000 UTMY: 3417400	dipnet	170
39	MT95-039	19 Jun 95	Hutson (Hudson) Lake	south of rt 26, west of Big Slough; T2S, R3W, Sec 24 UTMX: 331500 UTMY: 3416900	dipnet	300
48	MT95-048	22 Jun 95	Lower Rhymes	second large lake south of Bilbo Basin; T3S, R7W, Sec 6 UTMX: 334500 UTMY: 3409500	dipnet	150
49	MT95-049	22 Jun 95	Upper Rhymes (Rines) Lake	first large lake south of Bilbo Basin; T3S, R8W, Sec 1 UTMX: 334200 UTMY: 3410400	dipnet	400