Bulletin Alabama Museum of Natural History

april 6, 1981

number six

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Royal D. Suttkus and Robert C. Cashner

Karyotypes in Populations of the Cyprinodontid Fishes of the *Fundulus notatus* Species-complex: A Geographic Analysis

W. Mike Howell and Ann Black

An Isozymic Analysis of Several Southeastern Populations of the Cyprinodontid Fishes of the *Fundulus notatus* Species-complex

Fred Tatum, Ronald Lindahl and Herbert Boschung

BULLETIN ALABAMA MUSEUM NATURAL HISTORY is published by the Alabama Museum of Natural History, The University of Alabama. The BULLETIN is devoted primarily to the subjects of Anthropology, Archaelology, Botany, Geology and Zoology of the Southeast. The BULLETIN appears irregularly in consecutively numbered issues. Manuscripts are evaluated by the editor and an editorial committee selected for each paper.

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Price this Number: $3.50

A New Species of Cyprinodontid Fish, Genus *Fundulus (Zygonectes)*, from Lake Pontchartrain Tributaries in Louisiana and Mississippi[1]

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ABSTRACT: Suttkus, Royal D. and Robert C. Cashner, 1981. A new species of cyprinodontid fish, genus *Fundulus (Zygonectes)* from Lake Pontchartrain tributaries in Louisiana and Mississippi. Bulletin Alabama Museum of Natural History, Number 6:1-17, 10 tables, 6 figs. The broadstripe topminnow, *Fundulus euryzonus* n. sp., is endemic to the Tangipahoa and Amite rivers in Louisiana and Mississippi, where it is sympatric with its close relatives, *F. notatus* and *F. olivaceus*. The three species are distinguished from other species of *Fundulus*, including other nominal members of subgenus *Zygonectes*, by a dark lateral stripe from the snout tip to the caudal base, and by a pair of small metacentric chromosomes. The new species differs from *F. notatus* and *F. olivaceus* in: fewer dorsal rays, fewer gill rakers, a wider lateral stripe and other details of pigmentation. *Fundulus euryzonus* and *F. olivaceus* are syntopic throughout the upper middle regions of the Tangipahoa and Amite rivers, with *F. euryzonus* more common in smaller tributaries and *F. olivaceus* more abundant in the main channel. Where syntopic, *F. euryzonus* prefers more sheltered habitat and *F. olivaceus* is in more open areas.

Introduction

The new species of topminnow described below is relatively common in the Tangipahoa River and its tributaries and in the upper part of the Amite River system. Both these rivers drain into Lake Pontchartrain as do several other similar small rivers. This new topminnow seems to be the only fish endemic to the Lake Pontchartrain drainage.

The new form was first collected by the late Dr. Fred R. Cagle in 1948. The two small specimens collected by him and material collected by the senior author in the early 1950s was thought to represent a variant of the blackstripe topminnow. However, subsequent collections and observations in the early 1970s revealed the blackstripe topminnow, *Fundulus notatus*, in the lower part of the Amite River, the new form in the upper part and the blackspotted topminnow, *Fundulus olivaceus*, throughout the Amite River system. A recent collection revealed all three species at the same site, evidence that the nominal variant is a third species of dark-striped topminnow indigenous to the Lake Pontchartrain drainage.

[1]Contribution No. 25, Tulane University Museum of Natural History.

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Bull. Alabama Mus. Nat. Hist. 6:1-17.

In this paper, we describe the new form and compare it with the other two currently recognized dark-striped topminnows. Prior to 1950 there was confusion and difference of opinion with regard to the status of *F. notatus* and *F. olivaceus*. Moore and Paden (1950) presented tabular comparative data that was compiled by the late Dr. Carl L. Hubbs, and thereafter most authors recognized *F. notatus* and *F. olivaceus* as valid species. Brown (1956) presented a historical review of the pertinent literature covering the period from 1895 to 1954. Miller (1955) included both *F. notatus* and *F. olivaceus* in his list of the American cyprinodontid fishes of the genus *Fundulus*.

Acknowledgements

Numerous past students and curatorial assistants helped collect the study material for which we are grateful. We feel obliged to mention a few individuals who made special trips for *Fundulus* material or who made specific local faunal studies for us. We thank the following individuals for their efforts: Henry Bart, Eugene and Esther Beckham, Gantt Boswell, Erin M. Burks, Linda S. Dunn, Wayne Forman, Ralph W. Holzenthal, Greg Laiche and Carolyn A. Miller. We thank Ralph W. Holzenthal for the halftone illustrations and we extend our deep appreciation to Jeanne Suttkus for the photographs. We are grateful to W. Mike Howell and Ann Black for their collecting aid and also for sharing their prepublication information on karyotypes.

Materials and Methods

The description is based on 1121 specimens taken from Tangipahoa and Amite rivers of Louisiana and Mississippi. The type material consists of 340 specimens, 17.5 to 68.7 mm in standard length, which was seined from two sites along East Fork Big Creek. The rest of the material of the new form include 781 specimens (16.4 – 68.2 mm SL) from the Tangipahoa and Amite river systems. In the enumeration of the type material below, the number of specimens and range of standard length in millimeters are given in parentheses; for the rest of the listed material only the number of specimens is given in parentheses. In addition to standard abbreviations for compass directions, the following are used: Hwy = Highway, km = kilometer or kilometers, jct = junction, trib = tributary (to), ANSP = Academy of Natural Sciences, Philadelphia; INHS = Illinois Natural History Survey; KU = University of Kansas; MCZ = Museum of Comparative Zoology; TU = Tulane University Museum of Natural History; UAIC = University of Alabama Ichthyological Collection; UF = Florida State Museum; UMMZ = University of Michigan Museum of Zoology; UNO = University of New Orleans; USNM = United States National Museum of Natural History.

Most counts and measurements were made following Hubbs and Lagler (1947); however, dorsal and anal fin ray and lateral line scale counts were made following Brown (1957).

Fundulus euryzonus, new species
Broadstripe Topminnow
Figures 1,2,3 and 4

Holotype. – TU 116631, an adult male, 65.4 mm SL from East Fork Big Creek, trib Tangipahoa River 11.2 km E of Arcola, Tangipahoa Parish, Louisiana, on 4 March 1980, RDS 7283, by Royal D. Suttkus, Robert and James Reynolds.

Paratypes. – Taken with the holotype were 44 paratopotypes, TU 116632 (44, 21.3–65.3). Other paratopotypes include TU 106601 (58, 19.8–63.7), from the same locality, 27 March 1978, RDS 6673, R.D. Suttkus, W. Mike Howell, Ann Black, and Eugene C. Beckham. Also included as paratypes are specimens taken from East Fork Big Creek 7.5 km E of Arcola, Hwy 1054, Tangipahoa Parish, Louisiana. This site is only 3 air km downstream from the aforementioned site on the same creek. Paratypes from this latter site include TU 83182 (40, 23.0–68.7), ANSP 144091 (10, 26.2–53.6) INHS 87086 (10, 25.9–59.6), KU 18414 (10, 26.3–51.7), UAIC 5969.01 (10, 26.2–58.5), UF 29999 (10, 25.4–55.4), UMMZ 205436 (10, 29.5–56.3), UNO 2953 (10, 30.7–57.1), and USNM 221477 (10, 27.4–58.0) all taken on 10 March 1972, RDS 5180, R.D. Suttkus et al.; TU 116357 (21, 21.8–54.2), 4 October 1974, RDS 5763, R.D. Suttkus and Rebecca Odom; TU 106576 (22, 22.4–51.8) 27 March 1978, RDS 6672, R.D. Suttkus, W.M. Howell, Ann Black, and E. C. Beckham; TU 116504 (22, 20.5–55.9) 24 February 1980, RDS 7273, R.D. Suttkus and R.C. Cashner; TU 116597 (42, 17.5–60.1) and MCZ 56589 (10, 29.6–51.3), 4 March 1980, RDS 7281, R.D. Suttkus, R. Reynolds, and J. Reynolds.

Other material from Tangipahoa River system. Tangipahoa Parish, Louisiana: TU 116365 (2), Tangipahoa River 1.6 km E of Amite, 9 June 1948; TU 116366 (1), Beaver Creek, 6.2 km S of Kentwood, US Hwy 51, 11 March 1951; TU 116358 (10), Conners Creek 3.2 km off Franklinton Hwy, near Amite, 26 May 1955; TU 116359 (3), Tangipahoa River, 1.3 km E of jct Hwy 10 and US Hwy 51 at Hwy 10 bridge, 22 April 1967; TU 116356 (3), 4 October 1974; TU 116364 (21), trib Tangipahoa River 1.6 km E of Kentwood, Hwy 38, 28 January 1972; TU 79994 (3), Chappepeela Creek, at Hughes Cemetery, 4.8 km SE of Holton, 8 October 1972; TU 83048 (3) 18 January 1973; TU 83066 (8), 15 April 1973; TU 84125 (1) 19 May 1973; TU 84148 (10), 24 June 1973; TU 84165 (1), 19 July 1973; TU 84184 (3), 28 August 1973; TU 116360 (1), Chappepeela Creek, 10 December 1972; TU 116361 (1), Beaver Creek, 4.8 km S of Kentwood exit off Interstate Hwy 55, 16 February 1973; TU 116362 (1), Chappepeela Creek, 3.2 km S of Husser, Hwy 445, 26 February 1973; TU 83027 (1), 15 April 1973; TU 84132 (10), 19 May 1973; TU 116363 (1), 19 July 1973; TU 84203 (9), 23 September 1973; TU 84222 (7), Chappepeela Creek, near entrance to Zemurray Gardens, Hwy 40, 23 September 1973; TU 109245 (4), 27 September 1978; TU

117004 (38), 22 April 1980; TU 101773 (1), Tangipahoa River, about 150 m downstream from Hwy 10, 9 April 1977; TU 101798 (1), Tangipahoa River about 4 km downstream from Hwy 440, 8 April 1977; TU 101799 (1), Tangipahoa River about 12.8 km downstream from Hwy 440, 8 April 1977; TU 109817 (2), Tangipahoa River 12.8 km E of Hammond, US Hwy 190, 22 October 1978; TU 109846 (28), Chappepeela Creek, 5.9 km N of Robert just W off Hwy 445, 22 October 1978; TU 109873 (9), Chappepeela Creek, 18.7 km N of Robert, Hwy 445, 22 October 1978; TU 112798 (2), Chappepeela Creek, 9.6 km N of Robert, 31 March 1979; TU 112816 (12), Spring Creek, 5.1 km E of Loranger, Hwy 1062, 6 April 1979; TU 112838 (7), Chappepeela Creek, Hwy 445, 6 April 1979.

Pike County, Mississippi: TU 14339 (2), 3.8 km S of Magnolia, US Hwy 51, 13 June 1955; TU 67105 (35), Tangipahoa River, 1.1 km S of Magnolia and Gillsburg, Hwy 568 exit from Interstate Hwy 55, 29 December 1969; UNO 508 (13), Tangipahoa River, along Muddy Springs Road, 3.5 km W of Interstate Hwy 55, Exit 4, 14 October 1978; TU 116680 (26), 15 March 1980; TU 117147 (18), 10 May 1980.

Other material from Amite River system. St. Helena Parish, Louisiana: TU 75695 (37), Darlings Creek, trib Amite River, 1 km E of Chipola, Hwy 38, 28 January 1972; TU 106973 (51), 21 April 1978; UNO 554 (7), 14 October 1978; UNO 534 (36), Amite River, 6.6 km W of Chipola, Hwy 432, 14 October 1978; UNO 599 (1), Amite River, 1.1 km W of Hwy 448, 8.3 km S of Darlington, 11 November 1978; UNO 628 (1), Amite River, 4.6 km W of Hwy 38 jct in Coleman Town, Hwy 10, 11 November 1978; UNO 648 (17), Darlings Creek, 2.6 km W of jct Hwy 44 at Darlington, Hwy 10, 11 November 1978; UNO 2224 (4), Darlings Creek, at Church Road, 0.5 km N of Hwy 448, Sec 40, T2S, R4E, 24 March 1979; UNO 2841 (1), Amite River, 0.8 km N of Hwy 10, 8 April 1979; UNO 2854 (1), 4 km N of Hwy 10, 8 April 1979; UNO 2833 (5), Amite River, 6 August 1979; UNO 2838 (13), Amite River at confluence of East Fork and West Fork, 6 August 1979; UNO 2851 (5), Amite River, 2.4 km S of East Fork and West Fork confluence, 6 August 1979.

East Feliciana Parish, Louisiana: TU 75523 (3), Amite River, 17.4 km E of Clinton, Hwy 10, 28 January 1972; TU 116355 (1), 17 March 1973; UNO 2359 (6), 16 February 1979; UNO 2845 (3), 23 June 1979; UNO 2860 (20), Amite River at confluence of East Fork and West Fork, 5 August 1979.

Amite County, Mississippi: TU 75622 (26), East Fork Amite River, 11.7 km E of Liberty, Hwy 24, 29 January 1972; UNO 433 (7), 14 October 1978; TU 76057 (96), West Fork Amite River, 5.1 km SW of Liberty, Hwy 48, 29 January 1972; UNO 1569 (1), 17 September 1978; UNO 2836 (19), 24 April 1979; UNO 2848 (1), 23 May 1979; UNO 812 (8), East Fork Amite River, 12.2 km W of Gillsburg on dirt road, 6 January 1979; UNO 844 (5), Beaver Creek, 7.2 km W of Woodland, Hwy 569, 6 January 1979; UNO 969 (14), West Fork Amite River, W. Gillsburg on dirt road, 6 January 1979; UNO 988 (3), East Fork Amite River, 11.2 km SE of Liberty, Hwy 584, 14 October 1978; UNO 2121 (7), East Fork Amite River, 15.2 km S of Liberty, Liberty Road, 25 March 1979; UNO 2832 (15), East Fork Amite River, Hwy 570, 8 September 1979; UNO 2839 (15), West Fork Amite River, 23 May 1979; UNO 2850 (1), Wagoner Creek at Hwy 48, 1 km E of Hwy 569 jct, 23 May 1979; UNO 2844 (10), Amite River at dirt road 14.6 km S of Liberty, 23 June 1979; UNO 2855 (17), East Fork Amite River, Sec 27; T1N, R4E, 5 August 1979; UNO 2857 (4), East Fork Amite River, 5 August 1979.

Lincoln County, Mississippi: UNO 2333 (2), East Fork Amite River, near Auburn, US Hwy 98, 14 June 1979; TU 117170 (19), 10 May 1980.

Comparative material of *Fundulus notatus* and *Fundulus olivaceus* is listed under Material Examined.

Diagnosis.—A moderate-sized species of *Fundulus* closely allied to *Fundulus olivaceus* and *Fundulus notatus* that differs from these two species by its broader lateral stripe which is purplish brown in life rather than steel bluish. In preservative the lateral stripe is brown in the new form and black in the other two species. The lateral stripe in the broadstripe topminnow extends ventrally much further on the prepectoral region and on the posterior part of the body than in other two species. There are no vertical extensions (cross bars) on the lateral stripe of the male broadstripe topminnow as in the other two. The spots or blotches on the body are somewhat diffuse as in *Fundulus notatus*. *Fundulus euryzonus* has fewer dorsal fin rays, modally 8, whereas *F. notatus* has modally 9 and *F. olivaceus* modally 10 in the Lake Pontchartrain area. Also the gill-raker count is lower for the new form, averaging 7.58 compared to 9.14 and 9.03 for *F. notatus* and *F. olivaceus*, respectively.

Description and Comparisons.—Proportional measurements of 20 males and 20 females were determined with a dial calipers. Ten males and ten females were from the Tangipahoa River and ten each from the Amite River (Table 1). Fin-ray, scale, gill-raker and vertebral counts, and pigmentation are given in Tables 2–10. The genital pouch is moderately developed in the female.

Fundulus euryzonus has a moderately deep body (Fig. 1), intermediate between the deeper body of male *F. olivaceus* and the more slender male *F. notatus* (Table 1). The caudal peduncle depth is similar in all three species; the males have a deeper peduncle than conspecific females. *Fundulus euryzonus* and *F. olivaceus* are similar in head length, both having proportionally longer heads than *F. notatus*. There are some interspecific and sexual dimorphic differences with regards to position of fins. In females, dorsal and pelvic fins are inserted farther posterior than in males of all three species. The dorsal fin origin is most posterior in *F. euryzonus*, intermediate in *F. notatus* and closest to the

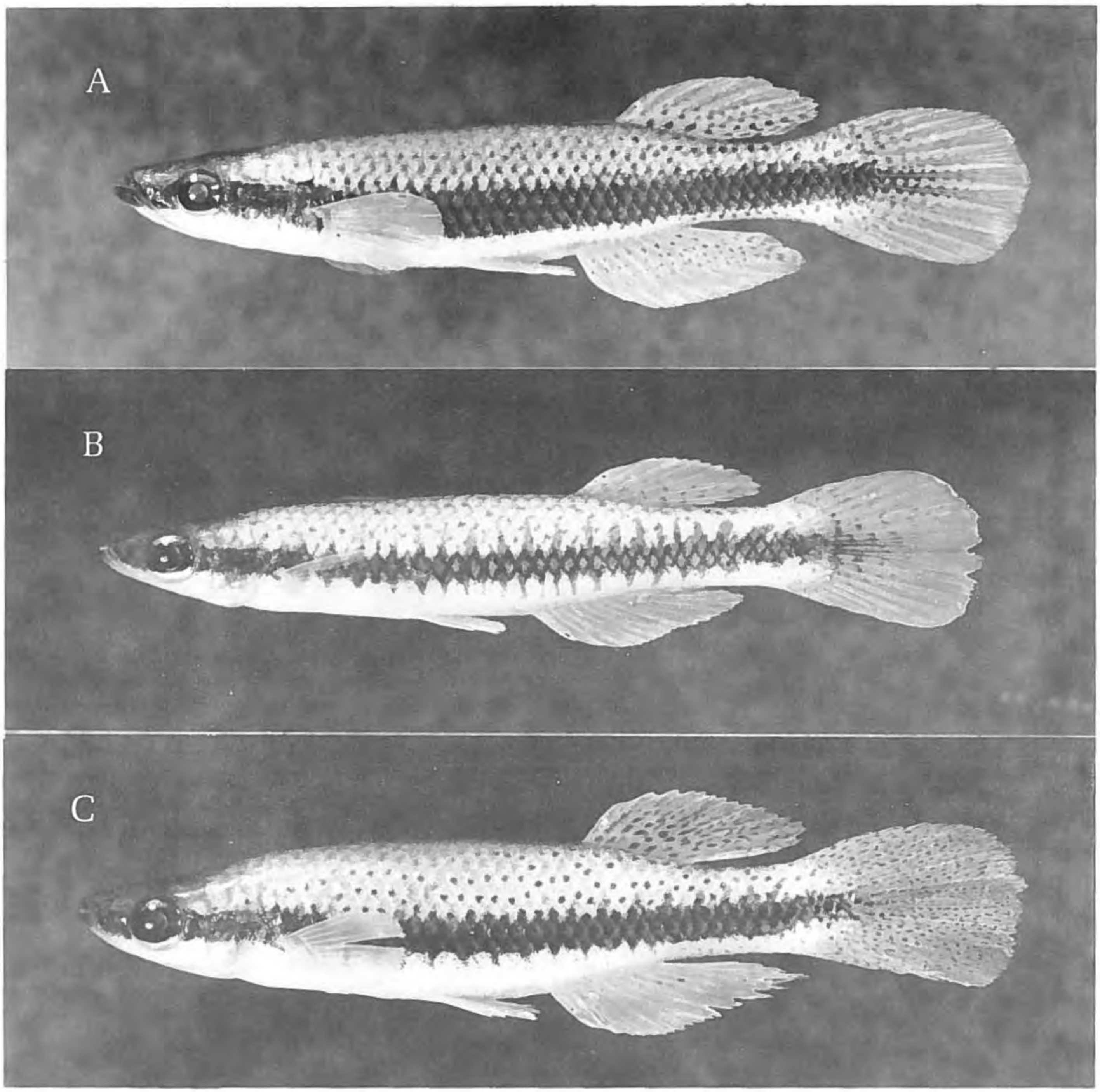

Fig. 1. (A) *Fundulus euryzonus* holotype (TU 116631) male, 65.4 mm SL, East Fork Big Creek, 11.2 km E of Arcola, Tangipahoa Parish, Louisiana (B) *Fundulus notatus* (TU 116479) male, 50.4 mm SL, Amite River, 4.3 km W of Denham Springs at US Hwy 190, Livingston Parish, Louisiana. (C) *Fundulus olivaceus* (TU 116598) male, 63.0 mm SL, East Fork Big Creek, 7.5 km E of Arcola, Hwy 1054, Tangipahoa Parish, Louisiana.

snout tip in *F. olivaceus*. The pelvic fin insertion is similar in both *F. euryzonus* and *F. olivaceus* males and is farther posterior than in males of *F. notatus* (Table 1). Males of all three dark-striped topminnows have dorsal and anal fins greatly elevated in comparison to females. Male *F. euryzonus* have less elevated dorsal fins than males of either *F. notatus* or *F. olivaceus*. The anal fin of the male *F. euryzonus* is intermediate in elevation. Male *F. notatus* have the most elevated anal fins of the three species. In males, the pectoral fin length is similar in *F. euryzonus* and *F. olivaceus* and longer than that of male *F. notatus*. *Fundulus euryzonus* males have the longest pelvic fins, *F. olivaceus* males the next longest, and *F. notatus* males the shortest (Table 1). The caudal-fin length, snout length and horizontal-orbit diameter exhibit only slight differences (Table 1). In general *F. euryzonus* morphometrics are more like *F. olivaceus* than *F. notatus*.

The three dark-striped topminnows have similar general pigmentation, that is, a dark lateral stripe, few to numerous small spots on body and median fins and little pigmentation on ventral region below lateral stripe. In his comparison of *F. notatus* and *F. olivaceus*, Thomerson (1966) characterized the lateral stripe as smooth, rough, or with few, several or many vertical extensions. *Fundulus euryzonus* has a broad lateral stripe that is usually smooth on its lower margin (in most females and some males) or rough. The upper margin of the lateral stripe is usually rough in both sexes although a few specimens have a smooth upper margin. The vertical extensions typically present on *F. notatus* and *F. olivaceus* males are lacking on males of *F. euryzonus* (Fig. 1, Table 10).

The dark lateral stripe extends from the anterior tip of the lower lip posteriorly across the lower two-thirds of the orbit through the upper half of the pectoral fin base to the base of the middle caudal fin rays (Fig. 2). There is a slight ventral curvature of the stripe at the mid-body region. Above the dark brown lateral stripe the sides, upper portion of the eye, the back and the top of the head is olive-brown. There is very little pigment ventral to the lateral stripe. Both males and females have small visible patches of melanophores posterior to the rictus (Fig. 3, Table 10). Both sexes have pigment around the vent, along the base of the anal fin and on the ventral surface of the caudal peduncle. More melanophores are scattered along the lateral surface of the caudal peduncle, below the lateral stripe, in males than in females.

The pelvic fins of both sexes are devoid of pigment as are the pectoral fins except for the basal portion of the upper fin rays that are covered by the lateral stripe (Figs. 1 and 2, Table 10). The basal two-thirds of the median fins of the male is densely spotted with moderate sized diffuse spots. The distal third of the dorsal and the caudal is dusky with a dense pattern of fine melanophores. The dusky area on the anal fin of the male is limited to a narrow band that is distal to the spotted basal portion. The rest of the fin margin distal

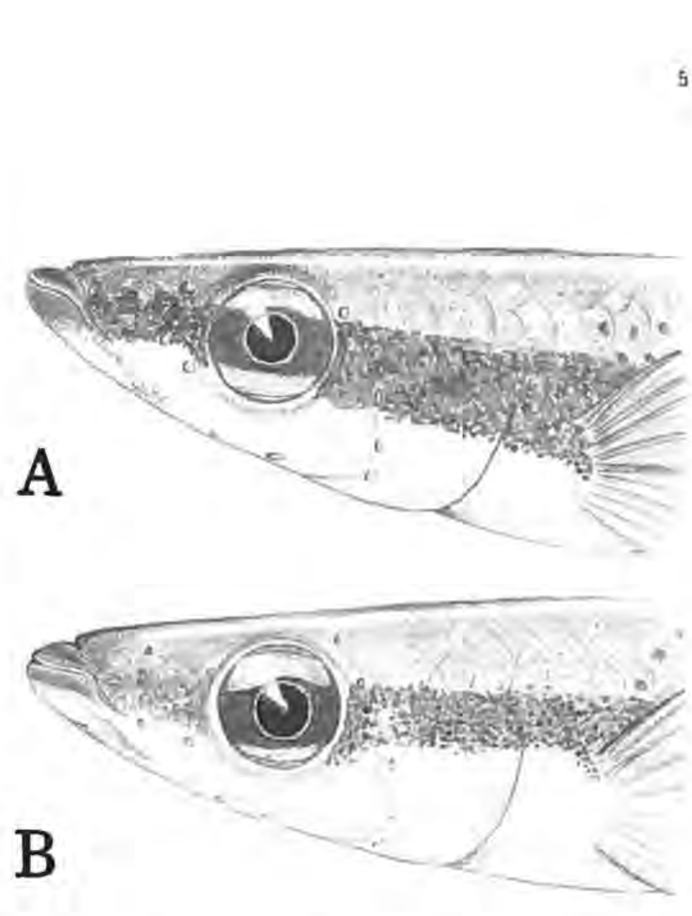

Fig. 2. (A) Lateral view of head pigmentation of *Fundulus euryzonus* (UNO 433) female, 56.9 mm SL, East Fork Amite River, 11.7 km E of Liberty, Hwy 24, Amite County, Mississippi. (B) *Fundulus olivaceus* (UNO 845) female, 58.0 mm SL, Beaver Creek, Hwy 569, 7.3 km N of Hwy 432 jct, Amite County, Mississippi.

to the dusky band is clear or devoid of pigment. Median fins in females have considerable less pigment, both in the form of spots and duskiness. Many females have very little duskiness on the membranes of the dorsal and the caudal fins. Spots and the minute specks of pigment (dusky area) are fewer and more widely scattered in the anal fin of the females so that to the unaided eye some females appear to lack pigment in the anal fin. A tabular comparison of the pigmentation in the three dark striped topminnows is presented in Table 10.

Color in life was observed and recorded for specimens collected from the Tangipahoa River system on 22 April and 10 May 1980. The lateral stripe on the body of *F. euryzonus* was purplish-brown with green flecks. The dark brown diffuse spots on the dorsal area above the lateral stripe contrasted with the lighter olive-brown background color. The upper lip was pinkish-gold in both the male and the female, but the lateral stripe on the snout was more intensely colored in the male. This snout stripe was deep pinkish-gold along its upper half, then golden below with a greenish ventral margin. The upper portion of the iris was bright-golden in females and golden brown in males. The lower two-thirds of the iris was a rich dark brown in both sexes. There was a fine golden edging or ring around the pupil and the extreme

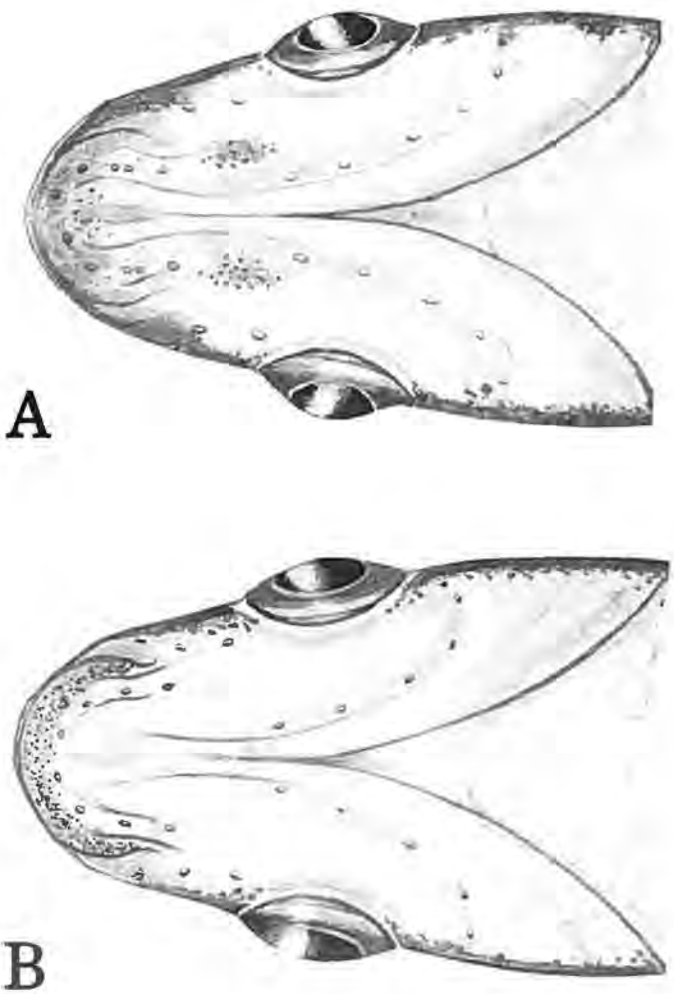

Fig. 3. (A) Ventral view of head pigmentation of *Fundulus euryzonus* (UNO 433) female, 56.9 mm SL, East Fork Amite River, 11.7 km E of Liberty, Hwy 24, Amite County, Mississippi. (B) *Fundulus olivaceus* (UNO 845) female, 58.0 mm SL, Beaver Creek, Hwy 569, 7.3 km N of Hwy 432 jct, Amite County, Mississippi.

outer, ventral margin of the iris was white with a pale-blue iridescence. The chin band part of the lateral stripe was dark brown in both sexes. The gular region posterior to the chin band was yellowish-olive. The opercular area of the lateral stripe was purplish-brown with an overlay of iridescent greenish-blue. There was a yellow trim on the ventral two-thirds of the opercular membrane. All fins of the large males had yellow to deep-orange color. The entire dorsal fin was amber or deep-orange in some males but in others the dorsal fin was less intensely colored on distal margin. The basal spotted portion of the anal fin was amber or deep-orange whereas distal to the spots the fin was bright-orange. The basal portion of the caudal fin was amber or deep-orange and the lower edge including the procurrent rays was bright-orange. Also, a bright-orange crescent covered the distal portion of the central caudal rays. This orange crescent was bordered posteriorly by a thin grey edging on the tips of the rays. The pelvic fins were uniform yellowish but the pectoral fins were pale-yellow at the base with a wide bright-yellow distal border. There was less amber and deep-orange in the fins of the females.

Observations were made on color of live *F. olivaceus* from the Tangipahoa River system on 22 April and 10 May 1980. Color notes on *F. notatus* specimens from the Amite River system were made on 22 April 1980. Both *F. notatus* and *F. olivaceus* had a steel-bluish-black lateral stripe with numerous bluish-green flecks. The dorsal area above the lateral stripe was yellowish-olive. *Fundulus olivaceus* was darker above than *F. notatus*. The upper lip of the *F. olivaceus* males collected on 10 May was pinkish as well as the anterior end of the snout stripe. The upper border of the snout stripe from the pink area posterior to the orbit was light blue. The rest of the snout stripe was greenish anteriorly and blue posteriorly. The iris was similar in coloration to that described for *F. euryzonus*, that is, the upper portion was golden, the lower part was brown and there was a golden edge or rim around the pupil. The extreme lower part of the iris was white with light iridescent-blue. There was a yellow trim on the ventral portion of the opercular membrane. The pectoral and pelvic fins were bright-yellow, but the median fins were amber proximally and yellow distally.

Our recorded observations on color in *F. notatus* was limited to specimens collected from the Amite River on 22 April 1980. The lateral stripe was steel-bluish-black as mentioned above; thus, both *F. notatus* and *F. olivaceus* differed from *F. euryzonus* because the latter had a purplish-brown lateral stripe. The upper lip in *F. notatus* was bright iridescent-blue. This iridescent-blue of the lip was continuous with the bright iridescent-blue upper border of the snout stripe. There was a small area of greenish-blue iridescence on the upper border of the stripe immediately posterior to the orbit and a more extensive greenish-blue area along lower border of postorbital stripe. The lower lip, chin and anterior gular region were yellowish-olive. The cheek and lower opercular area was tinted with a light blue iridescence. The median fins were yellowish-olive. We failed to record coloration of paired fins. The females had the bluish upper lip and iridescent-blue elsewhere on the head as described for males, but in each area the blue pigmentation was less extensive.

VARIATION.—Meristic data for all three species of dark-striped topminnows from the Lake Pontchartrain, Thompson Creek, and Pearl River drainages are presented in Tables 2–9.

Specimens of *F. euryzonus* from the Amite and Tangipahoa rivers show little variation and no significant differences in meristic characters. The means for dorsal, anal, pectoral fin rays, predorsal scales and gill rakers are virtually identical. Specimens from the Amite River do show slightly higher values for caudal rays ($\overline{X} = 13.7$) and lateral scales ($\overline{X} = 34.8$) compared to those from the Tangipahoa River ($\overline{X} = 13.2$ and 34.5 respectively).

ETYMOLOGY.—The name *euryzonus*, is derived from *eurys*, broad and *zon*, zone, and refers to the broad lateral stripe.

Relationships

The new form, *Fundulus euryzonus*, herein described, is closely allied to *F. notatus* and *F. olivaceus*. Jordan and Evermann (1896) grouped the species of *Fundulus* under five subgenera. They placed *F. notatus* and *Poecilia olivacea* Storer as synonym of *F. notatus* in the subgenus *Zygonectes* along with the following currently recognized species, *jenkinsi, pulvereus, sciadicus, luciae, chrysotus, cingulatus*, and the *notti*-group. Brown (1957) divided the subgenus *Zygonectes* into two sections and placed the dark-striped topminnows *F. notatus* and *F. olivaceus* with the *notti*-group. More recently, Hubbs and Burnside (1972), based on karyology and developmental sequences, proposed that *Zygonectes* be restricted to *notatus* and *olivaceus* and be elevated to full generic status. *Fundulus euryzonus* shares with *F. notatus* and *F. olivaceus* the unique pronounced dark lateral stripe and pair of small metacentric chromosomes (Setzer, 1970; Chen, 1971; Black and Howell, 1978 and Howell and Black, 1981). We retain *Fundulus* here, even though we recognize *F. notatus, F. olivaceus* and *F. euryzonus* as distinct from other nominal *Zygonectes*.

Ecology and Biology

Several authors (Braasch and Smith, 1965; Thomerson, 1966; Thomerson, 1967; and Thomerson and Wooldridge, 1970) reported on various aspects of ecology and biology of more northern populations of *Fundulus notatus* and *F. olivaceus*.

Fundulus euryzonus is a common inhabitant of the major tributaries of the Tangipahoa and upper Amite river systems as well as the upper sections of both of these rivers (Fig. 4). None was taken in the small headwater tributaries, particularly those of intermittent flow. There seemed to be a direct correlation between the increase in size of river channel with decreasing coverage by overhanging vegetation and the decrease in density of *Fundulus euryzonus*. *Fundulus olivaceus* occurred in about equal numbers in most tributaries and the upper parts of the two rivers; however, in the downstream sections of the rivers where the channels are wide without overhanging banks and vegetation, *F. olivaceus* greatly outnumbered *F. euryzonus*. *Fundulus euryzonus* most frequently occurred along overhanging banks, overhanging shrubs or trees that were partially submerged, around stumps, snags, trunks of living trees such as bald cypress, tupelo, swamp red maple, etc., that

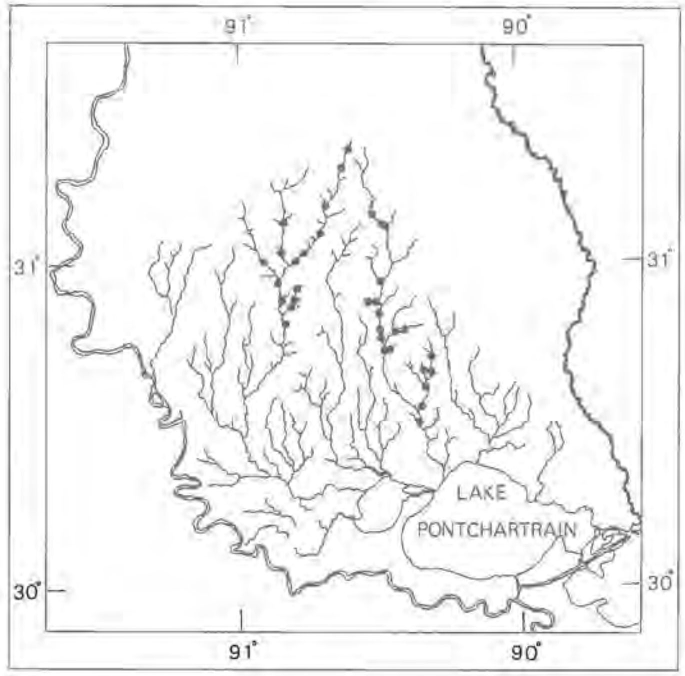

Fig. 4. Distribution of collection sites of *Fundulus euryzonus*. Star symbol indicates type-locality.

were standing in the water close to the bank. In these same sections of stream, *F. olivaceus* frequented the more open area along sand bars, particularly where the shoreline was irregular and where there were shallow coves.

Big Creek at the type locality varies from about five to ten meters in width with alternating pools and riffles. During the two collections, the deeper pools were slightly over two meters in depth. The substrate was mostly sand with a silt or detritus covering layer in slack areas. The riffles had a mixture of gravel and sand, and in a few places the hard clay of the bank extended out to form limited areas of the stream bed.

East Fork Big Creek is a relatively rich stream for its size. The following 23 species were taken in the same collection with the holotype: *Ichthyomyzon gagei, Esox americanus, Erimyzon oblongus, Hypentelium nigricans, Notemigonus crysoleucas, Notropis roseipinnis, N. texanus, N. venustus, Noturus leptacanthus, N. miurus, Fundulus olivaceus, Gambusia affinis, Ambloplites ariommus, Chaenobryttus gulosus, Lepomis cyanellus, L. macrochirus, L. marginatus, L. megalotis, Etheostoma proeliare, E. stigmaeum, E. swaini, E. zonale* and *Percina nigrofasciata*. The other collection taken from the type locality on 27 March 1978 included six additional species: *Esox niger, Notropis chrysocephalus, N. emiliae, Labidesthes sicculus, Elassoma zonatum*, and *Lepomis punctatus*, making a total of 29 associates of *F. euryzonus* at this site. Four collections were obtained from the other site on East Fork Big Creek approximately three kilometers downstream from the type locality. All but three *(Erimyzon oblongus, Hypentelium nigricans, Noturus miurus)* of the species taken at the type locality were taken at the lower site also. In addition, the following species were collected at the lower site with *F. euryzonus: Erimyzon tenuis, Hybopsis winchelli, Notropis volucellus, Aphredoderus sayanus, Centrarchus macropterus, Micropterus punctulatus, M. salmoides, Etheostoma chlorosomum,* and *Percina sciera.* Thus, a total of 35 associates of *F. euryzonus* were taken at the lower site.

Fundulus euryzonus is intermediate in size in comparison to *F. notatus* and *F. olivaceus* from Lake Pontchartrain tributaries. Twenty-one samples of *F. euryzonus* totaling 760 specimens were analyzed for largest male and female in each sample. The samples ranged from 7 to 120 specimens with an average of 36.2 individuals per sample. The largest male was 69.4 mm SL and the smallest 49.9 mm SL. The largest female was 65.2 mm SL and the smallest 47.6 mm SL for the respective samples. The average size for the (21) males is 61.6 mm SL versus 56.5 mm SL for the (21) females.

Seven samples totally 160 specimens of *F. notatus* were analyzed for largest males and females. The samples ranged in size from seven to 59 specimens with an average of 22.8 specimens per sample. The seven largest males range from 47.0 to 60.3 mm SL and the seven largest females in respective collections range from 42.1 to 58.6 mm SL. The average size for the (7) males is 55.8 mm SL and 51.8 mm SL for the (7) females.

Fifty-eight samples totaling 2357 specimens of *F. olivaceus* were analyzed for largest male and female in sample. The samples ranged in size from seven to 121 specimens with an average of 40.6 specimens per sample. The 58 males range in size from 40.3 to 74.8 mm SL and the 58 females in the respective samples range from 40.6 to 72.3 mm SL. The males average 63.7 mm SL and the females average 57.3 mm SL. The ten largest males are 74.8, 70.7, 70.4, 70.0, 70.0, 69.9, 69.6, 69.0, 68.9 and 68.8 mm SL where as the ten largest females in the respective samples are 72.3, 66.5, 65.9, 65.5, 65.4, 65.2, 64.9, 64.9, 63.6 and 63.5 mm SL. A review of the foregoing figures shows that males average larger size than the females in each of the three species. Braasch and Smith (1965) reported the maximum size of 750 specimens of *F. olivaceus* as 85 mm in total length and of 1200 specimens of *F. notatus* as 74 mm in total length. These data are comparable with our standard length data.

Range

The broadstripe topminnow, *Fundulus euryzonus,* is known only from the Tangipahoa and Amite rivers in Louisiana and Mississippi. Both rivers are tributaries to the Lake Pontchartrain estuary. *Fundulus euryzonus* occurs throughout most of the Tangipahoa River system. Some parts of the system were not sampled by us; therefore, our map (Fig. 4) may reflect collecting gaps and not distributional gaps. In the Amite River system, to the west, the species is restricted to the upper and middle reaches.

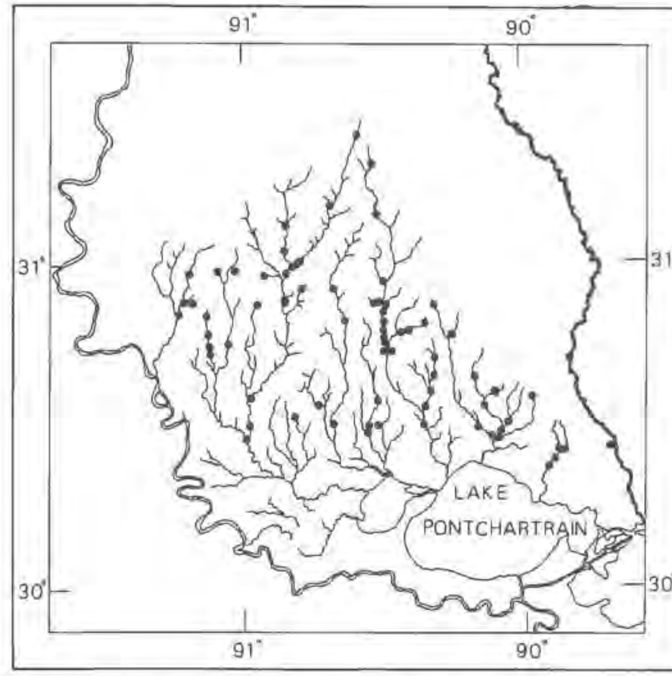

Fig. 5. Distribution of collection sites of *Fundulus olivaceus.*

All three dark-striped topminnows occur in the Amite River and have been taken syntopically at one site. *Fundulus olivaceus* was taken with *F. euryzonus* at nearly all sites in both the Amite and Tangipahoa rivers (Figs. 4 and 5).

The blackspotted topminnow, *F. olivaceus,* occurs in all the Lake Pontchartrain tributaries, and is the only dark-striped topminnow recorded from the easternmost Lake Pontchartrain tributaries, the Tchefuncte River and Bayou Lacombe.

Fundulus olivaceus and *F. notatus* occur sympatrically in Thompson Creek, the Pearl River and in the Natalbany River branch of the Tickfaw River system (Figs. 5 and 6).

Fundulus notatus has been collected only from the middle and lower sections of the Amite River, and the lower section of the Natalbany River of the Lake Pontchartrain tributaries.

Materials Examined

The following material was examined and used for counts, measurements, description of pigmentation, and for the distributional maps. Materials are listed by drainage from east to west. Standard abbreviations and compass points are used. All *F. notatus* specimens are from the state of Louisiana, but *F. olivaceus* specimens are from both Louisiana and Mississippi. A total of 292 *F. notatus* and 3661 *F. olivaceus* specimens was used.

Fundulus notatus

Pearl River. WASHINGTON PARISH TU 37118 (54), Pearl River flood pools 11.2 km E of Varnado, 24 September 1955; TU 53865 (6), Pearl River

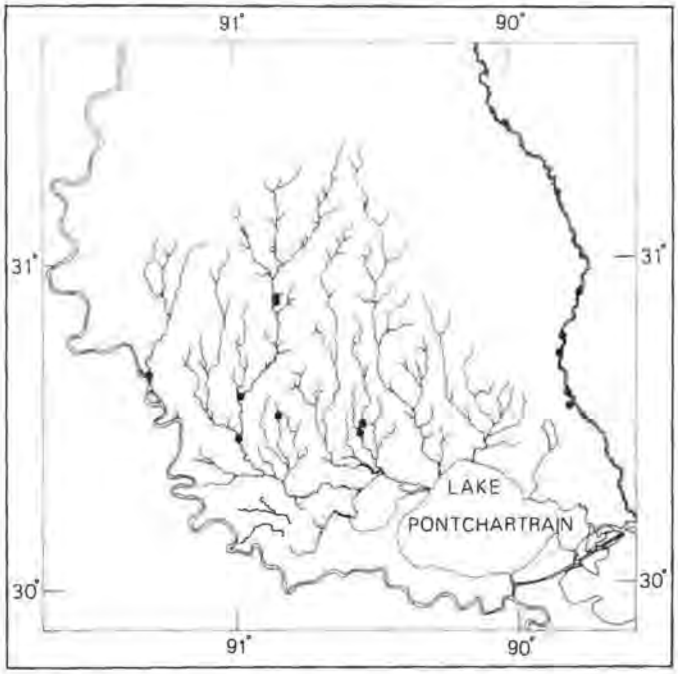

Fig. 6. Distribution of collection sites of *Fundulus notatus*.

mouth of Coburn and Bogalusa Creeks, 2.4 km E of Bogalusa, 23 October 1968; TU 100395 (38), swamp at Pearl River Canal at Pools Bluff Sill, 6.4 km S of Bogalusa, 10 December 1976. ST. TAMMANY PARISH: TU 86587 (14), Pearl River 28 km downriver from Pools Bluff Sill, 17 October 1973; TU 110103 (4), West Pearl River 3.2 km below mouth of Wilson Slough, 27 October 1978.

Natalbany River. TANGIPAHOA PARISH: TU 86446 (4), Natalbany River 10.7 km W of Hammond at Interstate 12 bridge, 11 February 1974; TU 106923 (7), 21 April 1978.

Amite River. EAST BATON ROUGE PARISH: TU 35697 (19), Amite River 11.2 km E of Baton Rouge at US Hwy 190 bridge, 25 October 1964; UNO 823 (9), 11 November 1978; UNO 2157 (10), Amite River at confluence of Sandy Creek, 10 April 1979. LIVINGSTON PARISH: UNO 2253 (2), Middle Colyell Creek at Hwy 1025, 16.6 km E of Hwy 447, 28 February 1979; TU 106947 (59), Amite River 4.3 km W of Denham Springs at US Hwy 190 bridge, along east bank, 21 April 1978; TU 116479 (37), 24 February 1980; TU 117022 (19), 22 April 1980. EAST FELICIANA PARISH: UNO 2846 (1), Amite River at Hwy 10 bridge, 4.6 km W of Coleman Town, 23 June 1979. ST. HELENA PARISH: UNO 2842 (1), Amite River about 0.8 km upriver from Hwy 10 bridge, 8 April 1979.

Thompson Creek. EAST BATON ROUGE PARISH: TU 113403 (8), mouth of Thompson Creek at confluence with Mississippi River, River Mile 256, 28 June 1979.

Fundulus olivaceus

Pearl River. **Louisiana:** WASHINGTON PARISH: TU 40390 (45), Pearl River just below Pools Bluff Sill, 6.4 km S of Bogalusa, 2 April 1966; ST. TAMMANY PARISH: TU 48195 (30), Holmes Bayou 3.2 km downstream from its upper end, 6 October 1967.

Bayou Lacombe. **Louisiana:** ST. TAMMANY PARISH: TU 373 (40), Bayou Lacombe N of Lacombe near St. Tammany, 7 January 1951; TU 46097 (15), Bayou Lacombe 17.6 km E of Abita Springs, Hwy 36, 7 April 1967; TU 47687 (2), Bayou Lacombe near Hwy 434, 8 km NW of Lacombe, 16 August 1967; TU 47700 (6), 17 August 1967.

Tchefuncte River. **Louisiana:** ST. TAMMANY PARISH: TU 967 (33), Tchefuncte River at Covington, 9 June 1948; TU 239 (1), Bogue Falaya River at Covington, 10 December 1950; TU 7685 (2), 8 November 1952; TU 9811 (14), 4 December 1954; TU 14512 (18), 14 August 1955; TU 23808 (11), 5 November 1960; TU 45040 (36), 8 April 1967; TU 46120 (9), 26 April 1967; TU 74825 (13), 17 December 1970; TU 74860 (21), 9 January 1971; TU 74929 (14), 6 February 1971; TU 74964 (9), 6 March 1971; TU 75003 (18), 4 April 1971; TU 75109 (17), 9 May 1971; TU 75144 (10), 3 July 1971; TU 75167 (4), 5 June 1971; TU 79919 (37), 9 August 1971; TU 80562 (12), 13 September 1971; TU 80678 (2), 6 September 1971; TU 81090 (11), 16 October 1971; TU 81130 (13), 10 November 1971; TU 92659 (19), 8 April 1970; TU 5901 (52), Tchefuncte River 8.2 km W of Covington, US Hwy 190, 17 June 1952; TU 7987 (9), trib. to Simalusa Creek at Blond, 8 km SE of Folsom, 26 September 1953; TU 36023 (20), Bogue Falaya River at Delta Primate Research Center, 7 June 1963; TU 36104 (2), 2 August 1963; TU 36030 (4), Tchefuncte River, 27 April 1963; TU 74846 (20), Bogue Falaya River 9 km SSE of Folsom, 17 December 1970; TU 74916 (41), 9 January 1971; TU 74949 (88), 6 February 1971; TU 74986 (15), 6 March 1971; TU 75027 (68), 4 April 1971; TU 75043 (38), 9 May 1971; TU 75128 (5), 3 July 1971; TU 75191 (39), 5 June 1971; TU 79944 (6), 9 August 1971; TU 80585 (9), 16 October 1971; TU 81043 (6), 13 September 1971; TU 74883 (27), Bogue Falaya River 3.4 km NNE of Folsom, 5 June 1971; TU 74900 (117), 13 March 1971; TU 75059 (28), 4 April 1971; TU 75075 (43), 10 May 1971; TU 75093 (45), 3 July 1971; TU 80871 (15), 22 January 1972; TU 80878 (16), 19 February 1972; TU 80949 (13), 13 September 1971; TU 80975 (13), 16 December 1971; TU 81012 (19), 10 November 1971, TU 81028 (35), 8 August 1971; TU 81073 (22), 16 October 1971; TU 80891 (2), East Fork Little Bogue Falaya River at Hwy 1082, 5.3 km SW of Waldheim, 18 September 1971; TU 80994 (4), Abita Creek at Hwy 21, 4.6 km NE of Waldheim, 18 September 1971; TU 81130 (13), Bogue Falaya River 1 km NE of Covington, 10 November 1971. WASHINGTON PARISH: TU 80915 (8), Tchefuncte River at Hwy 440, 10.2 km WNW of Richardson, 28 May 1972; TU 81059 (14), Gorman Creek at Hwy 450, 5 km SSE of Stoney Point, 28 May 1972.

Tangipahoa River. **Louisiana:** TANGIPAHOA PARISH: TU 1034 (9), flood pool of Tangipahoa River, 1.6 km E of Amite, 7 June 1948; TU 1301 (2), Tangipahoa River 1.6 km E of Amite, 9 June 1948; TU 3747 (1), Beaver Creek 6.2 km S of Kentwood, US Hwy 51, 11 March 1951; TU 9878 (9), Tangipahoa River 3.2 km off Franklinton Hwy, 26 May 1955; TU 15843 (8), Tangipahoa River 2.4 km E of Amite, LA Hwy 16, 12 May 1957; TU 45333 (7), Tangipahoa River 3.2 km E of Amite off Hwy 16, 8 April 1967; TU 45547 (37), Tangipahoa River 1.3 km E of Arcola, LA Hwy 10, 22 April 1967; TU 90128 (70), 4 October 1974; TU 75596 (3), trib. Tangipahoa River 1.6 km E of Kentwood, Hwy 38, 28 January 1972; TU 79995 (1), Chappepeela Creek at Hughes Cemetery, 4.8 km SE of Holton, 8 October 1972; TU 83050 (15), 18 January 1973; TU 83067 (1), 15 April 1973; TU 84149 (82), 24 June 1973; TU 84166 (2), 19 July 1973; TU 80020 (9), Chappepeela Creek at Hwy 445, 3.2 km SE of Husser, 19 November 1972; TU 83010 (6), 26 February 1973; TU 83029 (4), 15 April 1973; TU 84134 (17), 19 May 1973; TU 84174 (10), 19 July 1973; TU 84190 (4), 28 August 1973; TU 84205 (7), 23 September 1973; TU 80024 (1), Chappepeela Creek, 10 December 1972; TU 81626 (3), Beaver Creek 4.8 km S of Kentwood, Interstate 55, 16 February 1973; TU 83183 (59), East Fork Big Creek 7.5 km E of Arcola, Hwy 1054, 10 March 1972; TU 90155 (22), 4 October 1974; TU 106577 (42), 27 March 1978; TU 116505 (39), 24 February 1980; TU 116598 (24), 4 March 1980; TU 84223 (33), Chappepeela Creek at Hwy 40 near entrance to Zemurray Park, 23 September 1973; TU 109247 (12), 27 September 1978; TU 117006 (112), 22 April 1980; TU 101732 (16), Tangipahoa River at Hwy 440, just E of Tangipahoa, 8 April 1977; TU 101744 (9), Tangipahoa River about 4 km downstream from Hwy 440, 8 April 1977; TU 101753 (1), Tangipahoa River about 8 km downstream from Hwy 440, 8 April 1977; TU 101763 (20), Tangipahoa River about 13 km downstream from Hwy 440, 8 April 1977; TU 101784 (6), Tangipahoa River at mouth of a creek about 3 km SE of Arcola, 9 April 1977; TU 101794 (25), Tangipahoa River about 300 meters below confluence of Big Creek, about 3 km NE of Amite, 9 April 1977; TU 106602 (57), East Fork Big Creek, 11 km E of Arcola, 27 March 1978; TU 116633 (24) 4 March

1980; TU 109818 (105), Tangipahoa River 12.8 km E of Hammond, US Hwy 190, 22 October 1978; TU 109847 (51), Chappepeela Creek 5.9 km N of Robert just W off Hwy 445, 22 October 1978; TU 109875 (121), Chappepeela Creek 18.7 km N of Robert at Hwy 445 bridge, 22 October 1978; TU 116617 (1), Hamilton Creek 1.1 km E of Wilmer, Hwy 10, 4 March 1980. **Mississippi,** PIKE COUNTY: TU 116682 (94), Tangipahoa River along Muddy Springs Road, 3.5 km W of Interstate Hwy 55 at Exit 4, 15 March 1980; TU 117148 (9), 10 May 1980; TU 116983 (25), Tangipahoa River 7.2 km W of Interstate Hwy 55, at US Hwy 98 bridge, 11 April 1980.

Natalbany River. **Louisiana:** TANGIPAHOA PARISH: TU 3936 (4), Natalbany River 1.1 km W of Baptist, US Hwy 51, 17 February 1951; TU 4439 (2), trib. Natalbany River 5 km W of Hammond, 17 February 1951; TU 4878 (3), Natalbany River 3.4 km W of Tickfaw, Hwy 1080, 17 June 1952; TU 9870 (8), 26 May 1955; TU 81944 (39), Natalbany River 3.2 km W of Tickfaw, Hwy 442, 25 February 1972; TU 86447 (85), Natalbany River 6.7 km W of Hammond, Interstate Hwy 12, 11 February 1974; TU 106924 (82), 21 April 1978.

Tickfaw River. **Louisiana:** ST. HELENA PARISH: TU 926 (3), Tickfaw River at Liverpool, 13 August 1948; TU 45312 (8), Tickfaw River 4.8 km E of Greensburg, Hwy 10, 8 April 1967; TU 75869 (48), Tickfaw River 1.3 km E of Easleyville, 28 January 1972. LIVINGSTON PARISH: TU 3790 (7), Tickfaw River 0.8 km W of Holden, US Hwy 190, 17 February 1951; TU 4929 (4), 17 June 1952, TU 83115 (14), West Hog Branch 21.1 km W of Tickfaw, Hwy 442, 25 February 1972.

Amite River. **Louisiana:** LIVINGSTON PARISH: TU 29043 (1), Amite River about 3.2 km NW of Denham Springs, 17 March 1963; TU 106948 (30), Amite River at US Hwy 190 bridge, 4.3 km W of Denham Springs, 21 April 1978; TU 116480 (1), 24 February 1980; TU 117023 (2), 22 April 1980; UNO 2081 (53), Hornsby Creek, trib. Colyell Creek at Hwy 1025, 1.1 km E of Hwy 449, 18 September 1979. EAST BATON ROUGE PARISH: TU 35691 (22), Amite River at US Hwy 190, 11.2 km E of Baton Rouge, 25 October 1964; TU 39940 (10), 26 September 1964. ST. HELENA PARISH: TU 106874 (1), Darlings Creek, 1.0 km E of Chipola, Hwy 38, 21 April 1978; UNO 2834 (1), Amite River 6 August 1979; UNO 2837 (16), Amite River at confluence of East and West forks, 6 August 1979; UNO 2843 (6), Amite River 0.8 km N of Hwy 10 bridge, 8 April 1979; UNO 2852 (37), Amite River about 2.4 km S of confluence of East and West forks, 6 August 1979. EAST FELICIANA PARISH: TU 4656 (39), Redwood Creek, 4.3 km N of Ethel, 18 July 1952; TU 4667 (11), Redwood Creek, 2.7 km NE of Ethel, 18 July 1952; TU 4707 (7), Woodland Creek, 2.1 km S of Felps, Hwy 36, 19 July 1952; TU 6159 (6), Richland Creek, 11.2 km NE of Norwood, Hwy 127, 19 July 1952; TU 7622 (25), Redwood Creek 7 km N of Ethel, Hwy 91, 1 May 1953; TU 7877 (4), Little Comite Creek, 3.7 km N of Norwood, Hwy 91, 1 May 1953; TU 14054 (13), Redwood Creek at Hwy 10, Mc Manus, 29 June 1956; TU 32329 (8), Redwood Creek, 13.9 km S of Wilson, Hwy 19, 9 May 1964; TU 75529 (5), Amite River, 17.4 km E of Clinton, Hwy 10, 28 January 1972; TU 81538 (17), 17 March 1973; UNO 2847 (4), 23 June 1979; TU 81513 (10), Comite River 14.2 km S of Clinton, Hwy 67, 17 March 1973; TU 86420 (21), 11 February 1972; TU 81556 (6), Sandy Creek at Hwy 10, 6.9 km E of Clinton, 17 March 1973; UNO 2835 (9), 23 June 1979; UNO 2853 (88), Redwood Creek at Hwy 10, 23 June 1979; UNO 2861 (5), Amite River at confluence of East and West Forks, 5 August 1979. **Mississippi:** AMITE COUNTY: TU 75623 (10), East Fork Amite River, 11.7 km E of Liberty, at Hwy 24, 29 January 1972; TU 117258 (1), West Fork Amite River 5.1 km SW of Liberty, Hwy 48, 29 January 1972; UNO 2849 (5), 23 May 1979; UNO 2840 (8), West Fork Amite River on unmarked gravel road, 23 May 1979; UNO 845 (8), Beaver Creek at Hwy 569, 7.3 km N of Hwy 432 jct, 6 January 1976; UNO 2856 (5), East Fork Amite River, T1N, R4E, Sec 27, 5 August 1979; UNO 2858 (14), East Fork Amite River, 5 August 1979; UNO 2859 (8), East Fork Amite River at Louisiana-Mississippi line, 5 August 1979. LINCOLN COUNTY: TU 76074 (5), east branch of East Fork Amite River, 4.2 km W of Arlington, 12 February 1972; TU 117171 (63), Amite River at US Hwy 98 bridge near Auburn, 10 May 1980.

Thompson Creek. **Louisiana:** WEST FELICIANA PARISH: TU 4615 (14), Thompson Creek 1.6 km W of Jackson, Hwy 10, 18 July 1952; TU 65769 (55), 23 October 1970; TU 66678 (29), 11 December 1970; TU 68997 (15), 12 February 1971. EAST FELICIANA PARISH: TU 5231 (20), Corney Branch of Beaver Creek, 7.0 km SW of Wilson, Hwy 311, 19 July 1953; TU 7898 (39), Thompson Creek 7.5 km W of Norwood, 1 May 1953; TU 30884 (43), Beaver Dam Creek, 11.5 km SW of Wilson, Hwy 952, 1 March 1964; TU 30905 (21), Thompson Creek at western limits of Jackson, Hwy 10, 1 March 1964.

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Table 1. Measurements of *Fundulus* in thousandths of standard length. For each character is given the range of variation (in parentheses) the mean.

Species		*F. euryzonus*		*F. notatus*		*F. olivaceus*	
Stream System							
Tangipahoa River	TU 116631	TU 75596 (2) TU 83182 (5) TU 109846 (3)	TU 83182 (5) TU 109846 (5)			TU 106577 (5) TU 106602 (5)	TU 106577 (5) TU 106602 (5)
Amite River		TU 75695 (5) TU 76057 (5)	TU 75695 (5) TU 76057 (5)	TU 106947 (10)	TU 106947 (10)	TU 4667(3) TU 7622 (2) TU 35691 (5)	TU 4667 (2) TU 7622 (3) TU 35691 (5)
			Number and Sex				
	Holotype ♂	20 ♂♂	20 ♀♀	10 ♂♂	10 ♀♀	20 ♂♂	20 ♀♀
Standard length	65.4	53.2–69.2	49.0–61.6	46.5–60.3	47.3–57.0	47.2–68.4	42.1–65.3
Dorsal orgin to snout tip	702	686–730 (705)	701–729 (716)	686–705 (694)	700–718 (708)	674–710 (691)	696–715 (705)
Dorsal origin to caudal base	321	289–341 (311)	281–312 (296)	309–330 (319)	286–310 (299)	308–339 (323)	297–329 (308)
Pelvic insertion to snout tip	482	459–493 (475)	463–501 (483)	454–480 (461)	463–483 (473)	448–493 (475)	447–497 (477)
Head length	275	268–283 (277)	267–294 (276)	254–267 (263)	256–275 (263)	266–285 (276)	248–284 (272)
Body depth	196	192–226 (203)	169–199 (183)	187–199 (194)	184–207 (197)	174–237 (210)	175–220 (200)
Caudal peduncle depth	112	118–136 (124)	107–117 (112)	118–128 (124)	114–123 (117)	111–132 (124)	106–126 (114)
Snout length	105	97–116 (103)	91–105 (97)	91–98 (93)	84–95 (89)	91–111 (100)	84–106 (96)
Orbit length	70	73–79 (75)	76–87 (81)	70–76 (73)	72–84 (78)	65–81 (74)	69–88 (78)
Dorsal fin, depressed fin	286	240–299 (277)	205–232 (217)	282–320 (300)	187–213 (203)	243–333 (300)	201–237 (218)
Anal fin, depressed length	326	323–369 (345)	229–259 (241)	322–370 (349)	224–244 (235)	292–361 (331)	209–256 (235)
Pectoral fin length	186	173–193 (183)	164–192 (177)	161–172 (166)	155–180 (166)	167–198 (182)	156–190 (178)
Pelvic fin length	171	148–197 (168)	127–148 (138)	134–161 (147)	121–139 (129)	135–185 (156)	125–144 (135)
Caudal fin depth	272	260–286 (272)	248–289 (264)	251–282 (268)	254–298 (273)	262–298 (275)	234–291 (267)

Table 2. Frequency distribution of dorsal fin rays in three species of *Fundulus*.

Species and Drainage	7	8	9	10	11	12	N	$\bar{x}$	SD	SE
F. euryzonus										
Tangipahoa River	1	38	11				50	8.20	0.45	0.06
Amite River	3	35	12				50	8.18	0.52	0.07
Total	4	73	23				100	8.19	0.49	0.05
F. notatus										
Pearl River			18	7			25	9.28	0.46	0.09
Natalbany River			10	1			11	9.09	0.30	0.09
Amite River		9	58	3			70	8.91	0.41	0.05
Thompson Creek			5	3			8	9.38	0.52	0.18
Total		9	91	14			114	9.04	0.45	0.04
F. olivaceus										
Pearl River			7	15	3		25	9.84	0.63	0.13
Bayou Lacombe			2	19	4		25	10.08	0.49	0.10
Tchefuncte River			6	17	2		25	9.84	0.55	0.11
Tangipahoa River			9	46	5		60	9.93	0.48	0.06
Natalbany River			8	16	1		25	9.72	0.54	0.11
Tickfaw River			7	15	2	1	25	9.88	0.73	0.15
Amite River			24	63	4		91	9.78	0.51	0.05
Thompson Creek			12	12	1		25	9.56	0.58	0.12
Total			75	203	22	1	301	9.83	0.56	0.03

Table 3. Frequency distribution of anal fin rays in three species of *Fundulus*.

Species and Drainage	11	12	13	14	N	$\bar{x}$	SD	SE
F. euryzonus								
Tangipahoa River	28	20	2		50	11.48	0.58	0.08
Amite River	18	30	2		50	11.68	0.55	0.08
Total	46	50	4		100	11.58	0.57	0.06
F. notatus								
Pearl River		17	7	1	25	12.36	0.57	0.11
Natalbany River		10	1		11	12.09	0.30	0.09
Amite River	5	59	6		70	12.01	0.40	0.05
Thompson Creek	1	5	2		8	12.13	0.64	0.23
Total	6	91	16	1	114	12.10	0.47	0.04
F. olivaceus								
Pearl River	2	19	4		25	12.08	0.49	0.10
Bayou Lacombe	1	14	10		25	12.36	0.57	0.11
Tchefuncte River	3	12	10		25	12.28	0.68	0.14
Tangipahoa River	7	43	10		60	12.05	0.53	0.07
Natalbany River	2	16	7		25	12.20	0.58	0.12
Tickfaw River	4	12	8	1	25	12.24	0.78	0.16
Amite River	4	67	19	1	91	12.19	0.52	0.05
Thompson Creek	1	11	13		25	12.48	0.59	0.12
Total	24	194	81	2	301	12.20	0.58	0.03

Table 4. Frequency distribution of caudal rays in three species of *Fundulus*.

Species and Drainage	12	13	14	15	16	17	N	$\bar{x}$	SD	SE
F. euryzonus										
Tangipahoa River	7	30	11	2			50	13.16	0.71	0.10
Amite River	2	17	23	8			50	13.74	0.78	0.11
Total	9	47	34	10			100	13.45	0.80	0.08
F. notatus										
Pearl River	1	20	4				25	13.12	0.44	0.09
Natalbany River	1	10					11	12.91	0.30	0.09
Amite River	16	28	23	3			70	13.19	0.84	0.10
Thompson Creek			1	6	1		8	15.00	0.54	0.19
Total	18	58	28	9	1		114	13.27	0.86	0.08
F. olivaceus										
Pearl River	2	4	11	7		1	25	14.08	1.08	0.22
Bayou Lacombe	1	7	15	2			25	13.72	0.68	0.14
Tchefuncte River		3	11	11			25	14.32	0.69	0.14
Tangipahoa River		3	33	24			60	14.35	0.58	0.08
Natalbany River		3	15	7			25	14.16	0.63	0.13
Tickfaw River	5	7	7	6			25	13.56	1.08	0.22
Amite River	3	8	30	46	4		91	14.44	0.85	0.09
Thompson Creek		1	7	9	6	2	25	15.04	1.02	0.20
Total	11	36	129	112	10	3	301	14.28	0.89	0.05

Table 5. Frequency distribution of left pectoral fin rays in three species of *Fundulus*.

Species and Drainage	12	13	14	15	16	N	$\bar{x}$	SD	SE
F. euryzonus									
Tangipahoa River			28	22		50	14.44	0.50	0.07
Amite River		1	19	29	1	50	14.60	0.57	0.08
Total		1	47	51	1	100	14.52	0.54	0.11
F. notatus									
Pearl River	1	20	4			25	13.12	0.44	0.09
Natalbany River	2	7	2			11	13.00	0.63	0.19
Amite River	1	40	28	1		70	13.41	0.55	0.07
Thompson Creek			5	3		8	14.38	0.52	0.18
Total	4	67	39	4		114	13.38	0.62	0.12
F. olivaceus									
Pearl River		5	16	4		25	13.96	0.61	0.12
Bayou Lacombe			14	11		25	14.44	0.51	0.10
Tchefuncte River		2	11	11	1	25	14.44	0.71	0.14
Tangipahoa River			31	29		60	14.48	0.50	0.07
Natalbany River		1	12	11	1	25	14.48	0.65	0.13
Tickfaw River		1	8	16		25	14.60	0.58	0.12
Amite River			40	50	1	91	14.57	0.52	0.06
Thompson Creek		1	12	12		25	14.44	0.58	0.12
Total		10	144	144	3	301	14.47	0.58	0.07

Table 6. Frequency distribution of lateral scales in three species of *Fundulus*.

Species and Drainage	33	34	35	36	37	N	$\bar{x}$	SD	SE
F. euryzonus									
Tangipahoa River		26	23	1		50	34.50	0.54	0.08
Amite River		14	33	3		50	34.78	0.55	0.08
Total		40	56	4		100	34.64	0.56	0.06
F. notatus									
Pearl River		8	16	1		25	34.72	0.54	0.11
Natalbany River		1	8	2		11	35.09	0.54	0.16
Amite River	1	15	54			70	34.76	0.46	0.06
Thompson Creek			5	2	1	8	35.50	0.76	0.27
Total	1	24	83	5	1	114	34.83	0.55	0.05
F. olivaceus									
Pearl River		4	20	1		25	34.88	0.44	0.09
Bayou Lacombe		7	15	3		25	34.84	0.63	0.13
Tchefuncte River		3	22			25	34.88	0.33	0.07
Tangipahoa River		12	46	2		60	34.83	0.46	0.06
Natalbany River		5	19	1		25	34.84	0.47	0.10
Tickfaw River		5	20			25	34.80	0.41	0.08
Amite River		18	54	12		84	34.93	0.60	0.07
Thompson Creek		3	19	3		25	35.00	0.50	0.10
Total		57	219	22		294	34.88	0.50	0.03

Table 7. Frequency distribution of predorsal scales in three species of *Fundulus*.

Species and Drainage	19	20	21	22	23	24	25	26	27	N	x̄	SD	SE
F. euryzonus													
Tangipahoa River		14	27	9						50	20.90	0.68	0.10
Amite River	2	14	24	9		1				50	20.88	0.90	0.13
Total	2	28	51	18		1				100	20.89	0.79	0.08
F. notatus													
Pearl River		5	11	4	2	3				25	21.48	1.26	0.25
Natalbany River		1	2	6	2					11	21.82	0.87	0.26
Amite River		5	29	23	11	2				70	21.66	0.93	0.11
Thompson Creek			1	3	2	2				8	22.62	1.06	0.38
Total		11	43	36	17	7				114	21.70	1.04	0.10
F. olivaceus													
Pearl River		12	9	4						25	20.68	0.75	0.15
Bayou Lacombe	5	7	10	1	2					25	20.52	1.12	0.23
Tchefuncte River	1	12	9	3						25	20.56	0.77	0.15
Tangipahoa River	11	21	21	6	1					60	20.42	0.96	0.12
Natalbany River	1	9	9	5	1					25	20.84	0.94	0.19
Tickfaw River	4	11	8	2						25	20.32	0.85	0.17
Amite River	6	30	27	7	2					72	20.57	0.89	0.10
Thompson Creek		6	8	6	2	2			1	25	21.64	1.63	0.33
Total	28	108	101	34	8	2			1	282	20.64	0.97	0.06

Table 8. Frequency distribution of gill rakers in three species of *Fundulus*.

Species and Drainage	6	7	8	9	10	11	12	13	N	x̄	SD	SE
F. euryzonus												
Tangipahoa River	1	22	25	2					50	7.56	0.61	0.09
Amite River		29	26	5					60	7.60	0.64	0.08
Total	1	51	51	7					110	7.58	0.63	0.06
F. notatus												
Pearl River			2	8	11	2	2		25	9.76	1.01	0.20
Natalbany River			3	5	2	1			11	9.09	0.94	0.29
Amite River		2	14	27	6	2			51	8.84	0.83	0.12
Total		2	19	40	19	5	2		87	9.14	0.98	0.11
F. olivaceus												
Pearl River			7	6	9	2		1	25	9.40	1.23	0.25
Bayou Lacombe		1	11	8	4	1			25	8.72	0.94	0.19
Tchefuncte River			5	11	6	1	2		25	9.36	1.11	0.22
Tangipahoa River		2	21	18	8		1		50	8.72	0.93	0.13
Natalbany River		1	3	7	9	3	2		25	9.64	1.22	0.24
Tickfaw River			12	10	2	1			25	8.68	0.80	0.16
Amite River		5	26	24	12	6	1		74	8.88	1.11	0.13
Thompson Creek			4	12	6	3			25	9.32	0.90	0.18
Total		9	89	96	56	17	6	1	274	9.03	1.08	0.06

Table 9. Total number of vertebrae in three species of *Fundulus*.

Species and Drainage	33	34	35	36	N	$\bar{x}$
F. euryzonus						
Tangipahoa River		8	41	1	50	34.86
Amite River		11	34	4	49	34.86
Total		19	75	5	99	34.86
F. notatus						
Pearl River	1	23	74	1	99	34.76
Natalbany River			9	1	10	35.10
Amite River		4	24		28	34.86
Total	1	27	107	2	137	34.80
F. olivaceus						
Pearl River		14	55	4	73	34.86
Tchefuncte River		7	40	8	55	35.02
Tangipahoa River		10	34	4	48	34.87
Natalbany River		5	57	11	73	35.08
Amite River		6	55	6	67	35.00
Thompson Creek		15	94	12	121	34.97
Total		57	335	45	437	34.97

Table 10. Tabular comparison of pigmentation in three species of *Fundulus* from Lake Pontchartrain tributaries.

Pigmentation Characters (Based on Adult Males)	*F. euryzonus*	*F. notatus*	*F. olivaceus*
Lower lip band	Wide; dark	Narrow; light	Narrow to moderately wide; light to dark
Posterior to rictus	Visible patches	None	Few (scattered melanophores)
Dorsal Fin	Many distinct spots from base to margin	Few distinct spots, confined to basal third	Many distinct spots from base to margin
Anal fin	Many distinct spots on basal two-thirds	Fewest spots, scattered near base only	Fewer than *euryzonus*, and restricted to basal third
Caudal fin	Central two rays always dusky along margins; spots over most of fin from base two-thirds	Central 2 or 3 rays may be dusky along margins; spots extend from base to half of upper rays	Central rays not dusky at margins; spots scattered over most of fin
Lateral spotting	Few to moderate number; diffuse	Very few to none; diffuse	Many; distinct
Mid-predorsal area spotting	None	Diffuse spots	None
Lateral stripe	Wide (40% body depth)	Narrow (20% body depth)	Narrow (25% body depth)
Vertical extensions on lateral stripe	None	Present; narrow; few to many	Present; often diamond-shaped; few to many
Prepectoral pigment	Lateral stripe covers at least upper half of pectoral fin base	Lateral stripe above pectoral insertion; narrow bar extends to base of upper 4 rays	Lateral stripe above pectoral insertion; narrow bar extends to base of upper 4 rays

Karyotypes in Populations of the Cyprinodontid Fishes of the *Fundulus notatus* Species-complex: A Geographic Analysis

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ABSTRACT: Howell, W. Mike and Ann Black, Karyotypes in populations of the cyprinodontid fishes of the *Fundulus notatus* species-complex: a geographic analysis. Bulletin Alabama Museum of Natural History, Number 6:19-30, 4 figures, 7 tables, 1981. Chromosome studies were performed on three sibling species of topminnows and hybrids belonging to the *Fundulus notatus* species complex: *F. notatus* (Rafinesque), *F. olivaceus* (Storer) and *F. euryzonus* Suttkus and Cashner. Two thousand sixty-one metaphases were studied from 545 specimens from throughout the ranges of these species. Three basic karyotypes were found: *F. notatus*, 2n = 40; *F. notatus* (Tombigbee River chromosomal race), 2n = 44; and *F. olivaceus* and *F. eruyzonus*, 2n = 48. The most primitive karyotypes were those of *F. olivaceus* and *F. euryzonus*. The karyotypes of these two species were indistinguishable and consisted of 46 acrocentric and 2 small metacentric chromosomes. The Tombigbee River chromsomal race of *F. notatus* had a more derived karyotype of 38 acrocentric, 2 small metacentric and 4 large metacentric chromosomes. All other populations of *F. notatus* had the most derived karyotype of the complex, with 30 acrocentric, 2 small metacentric and 8 large metacentric chromosomes. We hypothesize that the primitive diploid karyotype in this complex consisted of 48 acrocentric chromosomes of graded sizes. Karyotypic evolution probably involved a pericentric inversion and several Robertsonian fusions. The pericentric inversion, involving a small pair of acrocentrics, occurred early in the evolution of the complex and produced the small pair of metacentrics seen in all four karyotypes. This was the only apparent karyotypic change in *F. olivaceus* and *F. euryzonus*. Subsequently, Robertsonian fusions of acrocentrics occurred to form the metacentrics in the derived karyotypes of *F. notatus*.

Zones of hybridization between *F. notatus* and *F. olivaceus* were found in Trussels Creek of the Tombigbee River system, Alabama, and in the Neches River of Texas. In these hybrids, intermediate diploid numbers ranging from 2n = 41 to 2n = 47 were encountered. Karyotypes from hybrids showed some individuals with only one large metacentric, some with as many as seven metacentrics, and others with metacentric counts between one and seven. The hybrid nature of many of these individuals was supported by the presence of trivalents in male meioses. These hybrids probably represented combinations of F_1 hybrids, hybrid-hybrid crosses, and backcrosses to either *F. notatus* or *F. olivaceus* parents. Fertility of both natural and laboratory reared hybrids indicates that Robertsonian fusions in this complex may not serve as effective post-mating isolating mechanisms. Ecological barriers, rather than cytogenetic ones, may act as strong isolating mechanisms to preserve species identities throughout most of their sympatric range.

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Bull. Alabama Mus. Nat. Hist. 6:19-30.

Introduction

Most fish karyotypes which have been documented for a particular species have been based on only one to a few individuals, usually from a single locality or from a small part of the species' geographic range. Fish cytology has now advanced to the stage where more attention should be focused on chromosome studies involving large numbers of individuals from populations throughout the range of a species. Such studies could allow ichthyologists to: (1) determine the extent of intra- and interspecific chromosome variations; (2) delineate possible zones of intergradation, hybridization and/or introgression; (3) detect possible chromosomal races; (4) relate chromosome changes to speciational processes; (5) recognize trends in chromosome evolution; and (6) detect previously unrecognized sibling species.

The following attributes of the cyprinodontid fishes, *Fundulus notatus, F. olivaceus* and *F. euryzonus,* make them ideal subjects for a geographic analysis of chromosome variation: (1) they are sibling species; (2) their distribution and taxonomy are well known (Brown, 1956; Braasch and Smith, 1965; Thomerson, 1965 and 1966; Suttkus and Cashner, 1980); (3) *F. notatus* and *F. olivaceus* are widely distributed throughout much of the Mississippi River system and in coastal stream tributaries to the Gulf of Mexico; (4) their ranges overlap (Thomerson, 1966; Suttkus and Cashner, 1981); (5) reproductive barriers break down in certain areas and hybridization occurs (Thomerson, 1966 and 1967; Setzer, 1970); (6) three different diploid chromosome numbers and karyotypes have been found within the group, i.e., *F. notatus* has 2n=40, *F. notatus* (Tombigbee River chromosomal race) has 2n=44, and *F. olivaceus* and *F. euryzonus* have 2n=48 (Setzer, 1970; Chen, 1971; Cuca, 1976; Black and Howell, 1978; and Howell and Black, this study); and (7) a previously unrecognized sibling species, *Fundulus euryzonus,* has recently been described from the Amite and Tangipahoa river systems of Louisiana and Mississippi (Suttkus and Cashner, 1981).

The primary objectives of this study were to analyze the karyotypes of natural populations *F. notatus, F. olivaceus* and *F. euryzonus* from throughout their ranges and to determine the biological and systematic significance of variations in karyotype.

Acknowledgements

We thank Eugene C. Beckham, Herbert Boschung, Robert Cashner, William A. Cox, Guido Dingerkus, Tomas Keevin, Ronald Lindahl, Stanley Painter, Robert A. Stiles, Royal D. Suttkus, Fred Tatum and James E. Thomerson for helpful advice, suggestions, and aid during field collections. Special thanks go to John Gold, William LeGrande, Kenneth Thompson and Gary Thorgaard, who critically reviewed our manuscript. This study could not have been completed without the generosity of Mr. Greg Cuca who provided us with live hybrids, karyotypes and unpublished data from his research on *Fundulus notatus* and *F. olivaceus.* This research was supported by National Science Foundation grant No. DEB-76-84195 To Howell.

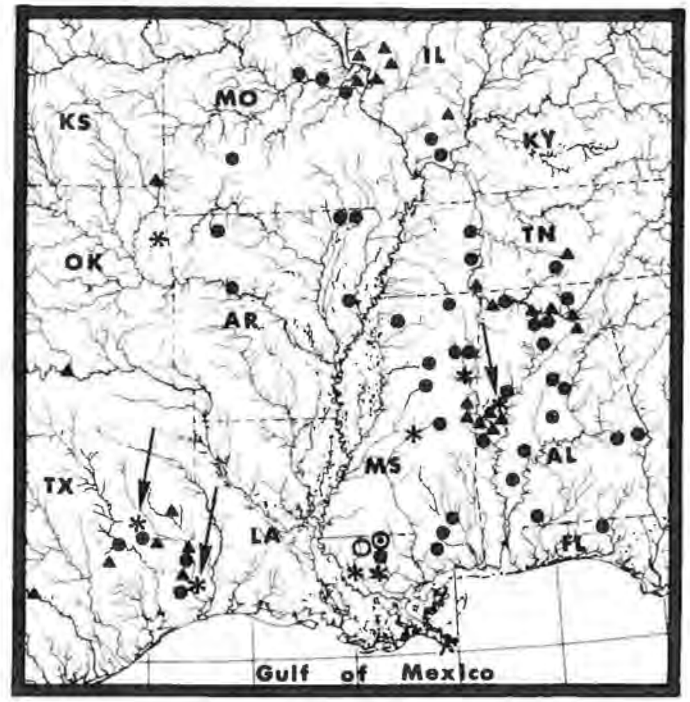

Fig. 1. Collecting sites for study specimens from which karyotypes were obtained: *Fundulus notatus* (triangles); *F. olivaceus* (solid circles); *F. euryzonus* (open circles); sympatric population of *F. euryzonus* and *F. olivaceus* (dot within circle); sympatric populations of *F. notatus* and *F. olivaceus* (asterisks); three populations containing hybrids, *F. notatus* × *F. olivaceus,* are indicated by arrows.

Materials and Methods

Five hundred forty-five specimens belonging to the *Fundulus notatus* species-complex were collected over much of the ranges of the species. Collecting localities are listed at the end of this paper in the Materials Examined section and are plotted on the map in Fig. 1.

The fishes were collected with minnow seines, placed into well-aerated styrofoam containers, and returned to the laboratory for study. Living specimens were injected intraperitoneally with 0.5 ml of 0.1% colchicine and sacrificed 8 to 12 hours later. Tissues from gill, scale and testes (when available) were removed, swollen in 0.4%KCl for one hour and fixed in two changes of 3:1 methanol-glacial acetic acid for 30 minutes each. Prepared tissue was then dabbed onto a microscope slide, air dried, and stained in 6% Giemsa, Harleco Co., Azure Blend Type (Denton and Howell, 1973). Chromosome banding techniques (Sumner et al. 1971; Seabright, 1972) were attempted but were unsuccessful. Because no banding patterns were obtained, chromosome pairings in Figs. 2 and 3 were partially subjective. Since most chromosomes lacked clear-cut size differences, unequivocal pairing was impossible.

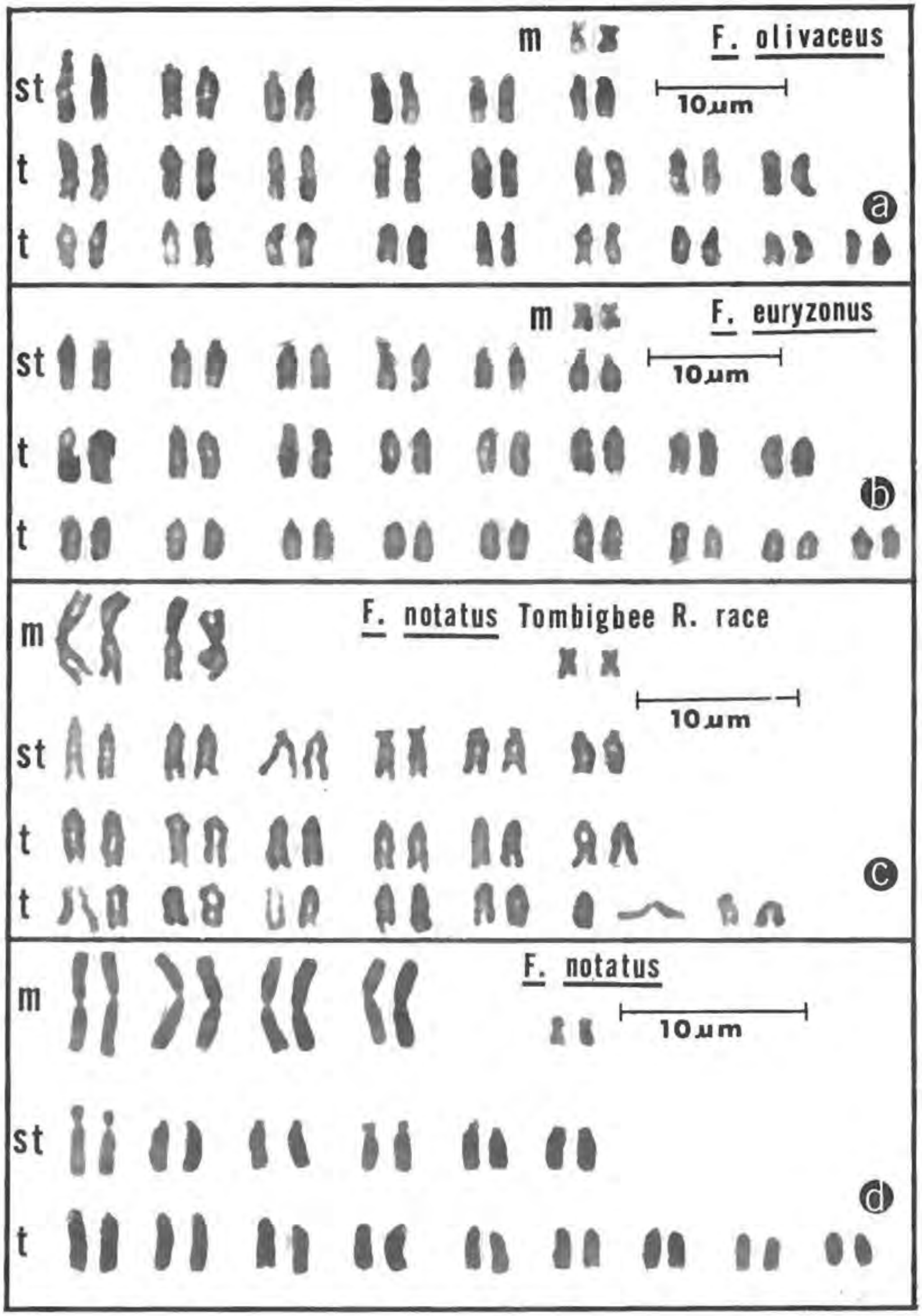

Fig. 2. Mitotic karyotypes of topminnows of the *Fundulus notatus* species-complex: a) *F. olivaceus*, 2n = 48; b) *F euryzonus*, 2n = 48, c) *F. notatus*, 2n = 44, Tombigbee River chromosomal race; d) *F. notatus*, 2n = 40, all populations except Tombigbee River.

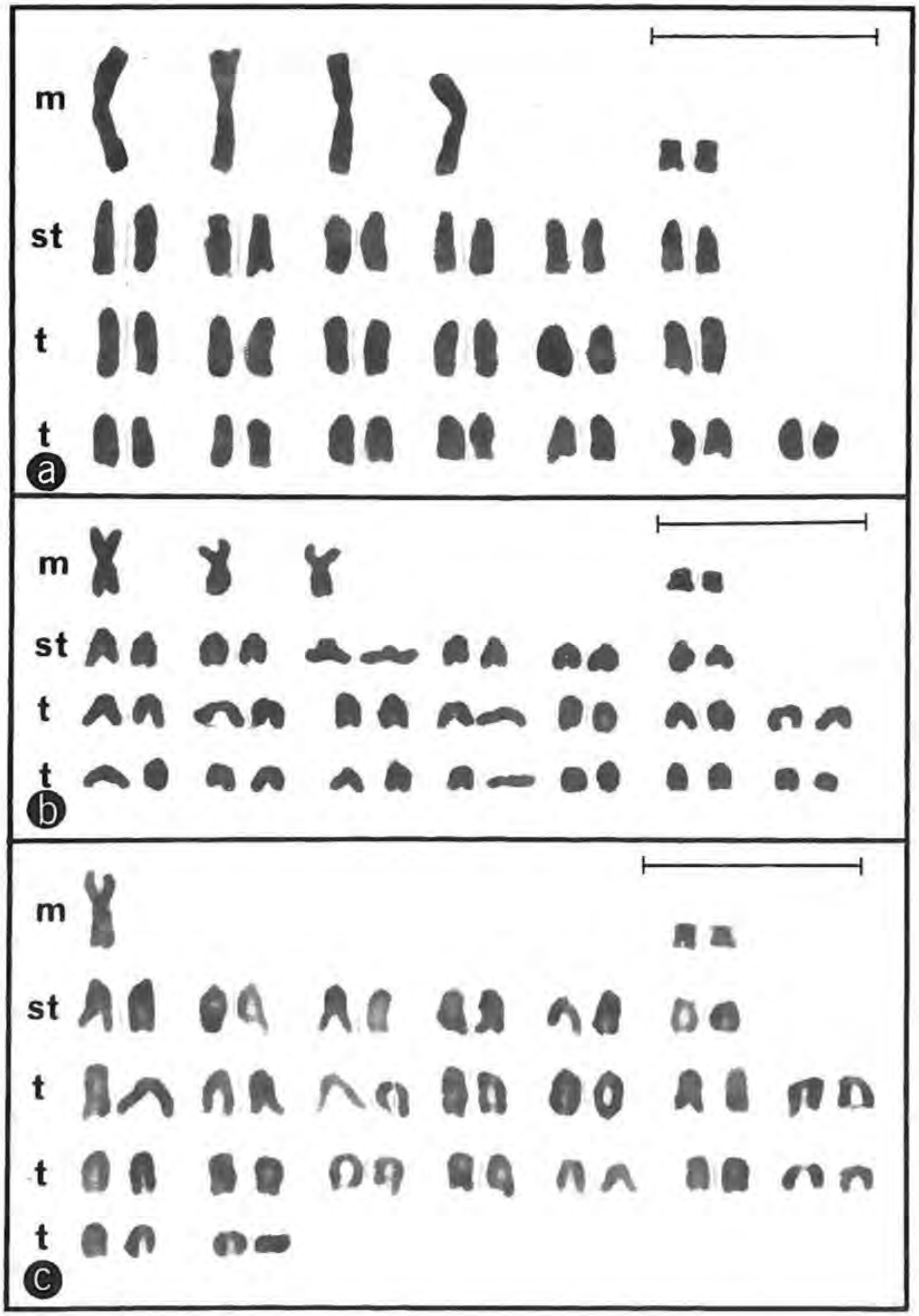

Fig. 3. Karyotypes from hybrids, *F. notatus* × *F. olivaceus: a) F_1 hybrid*, showing 2n = 44 with 4 large metacentrics, Neches River, Texas; b) possible hybrid backcross to *F. olivaceus* parent, 2n = 45 with 3 large metacentrics, Neches River, Texas; c) possible hybrid backcross to *F. olivaceus* parent, 2n = 47 with only one large metacentric, Trussels Creek, Alabama. Scale bars equal approximately 10 microns.

In order to make this study comparable to previous works by fish cytogeneticists, a dual classification of chromosome types was used. Following the conventional system, chromosomes were classified as either acrocentric, submetacentric or metacentric. Additionally, the classification system of Levan *et al.* (1964) was used. According to the Levan system, a centromere was considered to be median (m) in position if the long arm/short arm ratio fell within the range 1.00-1.70; submedian (sm) if between 1.71-2.99; subterminal (st) if between 3.00-6.99; and terminal (t) if between 7.00-∞ . A chromosome with a median (m) centromere is thus equivalent in terminology to the conventional metacentric chromosome, and one with a terminal or subterminal (t or st) centromere is equal to an acrocentric chromosome.

Results and Discussion

Karyotypic Analysis of *Fundulus olivaceus* populations.—Diploid chromosome numbers in 1,158 metaphases examined from 318 specimens of *F. olivaceus* varied from 46 to 48, with strong modal count of 48 for all populations (Table 1). The infrequent counts of 46 and 47 were probably the result of chromosome loss during disruption of the cell membrane during chromosome preparation. The karyotype consisted of 2 small metacentric chromosomes and 46 acrocentric chromosomes of graded sizes. When classified according to centromeric position (Levan et al., 1964), the karyotype consisted of 2 small median, 12 subterminal, and 34 terminal chromosomes (Fig. 2a.). These findings are consistent with those of Setzer (1970), Chen (1971), Cuca (1976) and Black and Howell (1978). The modal haploid count obtained from testicular tissue of 73 specimens was 24, confirming the diploid number (Table 2). The meiotic chromosomes were always arranged into 24 bivalents (Fig. 4a).

Karyotypic Analysis of *Fundulus euryzonus* Populations.—The mitotic karyotype of *F. euryzonus* is indistinguishable from that of *F. olivaceus* (Fig. 2a and b). The karyotype consisted of 2 small metacentric and 46 acrocentric chromosomes of graded sizes (Table 3). When classified according to centromeric position, the karyotype consisted of 2 small median, 12 subterminal and 34 terminal chromosomes (Fig. 2b). Meiotic preparations from testicular tissue showed a modal count of 24 with all of the chromosomes arranged into bivalents (Table 2).

Karyotypic Analysis of *Fundulus notatus* Populations.—Two distinctive diploid karyotypes were obtained for populations of *F. notatus*: the Tombigbee River population had 2n=44; all other populations had 2n=40 (Table 4). The Tombigbee River population has already been noted as constituting a distinctive chromosomal race by Black and Howell (1978).

The karyotype in 50 specimens from the Tombigbee River population was modally 2n=44 and consisted of 2 small metacentrics, 4 large metacentrics, and 38 acrocentrics of graded sizes (Fig. 2c). When classified according to centromeric position, there were 2 small median, 4 large median, 12 subterminal, and 26 terminal chromosomes (Fig. 2c). At meiosis, chromosomes from Tombigbee River specimens formed 22 bivalents (Table 2; Fig. 4c).

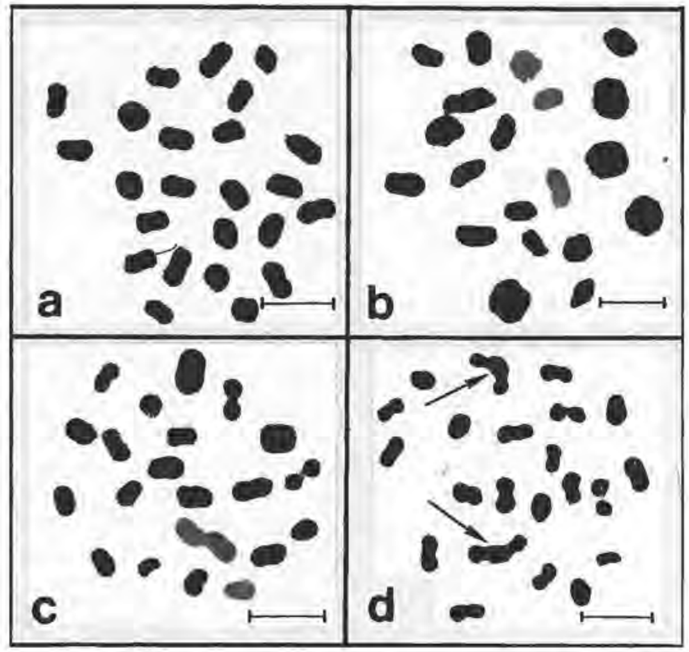

Fig. 4. Meiotic Metaphase I chromosome spreads from top minnows of the *Fundulus notatus* species-complex: a) *F. olivaceus*, 24 bivalents; b) *F. notatus*, 20 bivalents; c) *F. notatus* (Tombigbee River chromosomal race), 22 bivalents; d) hybrid, *F. notatus* (Tombigbee River chromosomal race) × *F. olivaceus*, 20 bivalents and 2 trivalents. Arrows point to trivalents. Scale bars equal approximately 5 microns.

The mitotic karyotype in 129 specimens from all populations of *F. notatus* (except the Tombigbee River) was 2n=40 with 8 large metacentrics, 2 small metacentrics, and 30 acrocentrics of graded sizes. When classified according to centromeric position, the karyotype consisted of 8 large median, 2 small median, 12 subterminal, and 18 terminal chromosomes (Fig. 2d). At meiosis, chromosomes formed 20 bivalents (Table 2; Fig. 4b).

Zones of Hybridization between *F. notatus* and *F. olivaceus*.—Hybrids were found at two localities: Trussels Creek, tributary to Tombigbee River, along County Route 19, N of Boligee, Greene County, Alabama; and Neches River proper, 6 km E of Silsbee, along U.S. Route 96, Hardin and Jasper counties, Texas.

In the lower, more sluggish reaches of Trussels Creek, we collected only *F. notatus* (Tombigbee chromosomal race) with 2n=44 (Fig. 2c). In the swifter headwaters of Trussels Creek, we collected only *F. olivaceus* with a karyotype of 2n=48 (Fig. 2a). At the bridge along County Route 19, in the ecologically intermediate stretches of the creek where the swifter water from the uplands intermixed with the sluggish water of the lowlands, we found a zone of hybridization. In this area, we collected 1 specimen of *F. notatus*, 11 specimens of *F. olivaceus* and 6 hybrids, *F. notatus* × *F. olivaceus* (Table 5). The hybrids were karyotypically intermediate between *F. notatus* and *F. olivaceus*. Chromosome numbers in the hybrids were: 2n = 45 (2 specimens), 2n = 46 (3 specimens), and 2n = 47 (1 specimen). The three hybrids with 2n = 46 had only 2 large metacentrics and were most likely F_1 hybrids between *F. notatus* (2n = 44) and *F. olivaceus* (2n = 48). Likewise, they could have been F_2 hybrids. The two hybrids with 2n = 45 had 3 large metacentrics and could represent an F_1 hybrid (2n = 46) backcrossed to *F. notatus* (2n = 44). Due to random segregation of chromosomes at meiosis, variations from the F_1 karyotype, 2n = 46, may represent either a backcross to one of the parental species or to an F_2. The single hybrid with 2n = 47 had only one large metacentric and could represent an F_1 hybrid (2n = 46) backcrossed to *F. olivaceus* (2n = 48). While the karyotypic variations within three of these specimens, i.e., those with 2n = 45 and 2n = 47, may be explained by F_1 hybrids backcrossing to the parental species, they may also be due to the presence of F_2 individuals and not introgression. If one assumes that trivalent formation with equational segregation occurs for each trivalent at meiosis, then a metacentric would migrate to one pole, and its two homologous acrocentrics would migrate to the opposite pole. This would allow for all possible diploid numbers observed, including the same diploid numbers as the parental forms in an F_2 population.

Meiotic data confirmed the hybrid nature of three of these individuals. In a presumed F_1 individual with 2n = 46, there was a meiotic count of 20 bivalents and 2 trivalents (Fig. 4 d). Apparently, at meiosis in these hybrids, the two metacentrics of *F. notatus* synapse with their homologous acrocentric counterparts contributed by *F. olivaceus*, thus forming two trivalents. The trivalent formation is similar to that observed from meiotic figures on laboratory-reared hybrids (Cuca, 1976). One hybrid (2n = 47) had only a single metacentric (Fig. 3 c), and only a single trivalent was observed in its meiotic figures.

In the Neches River, *F. notatus* predominantly occurred in the slower moving waters of the river proper, while *F. olivaceus* was found in the swifter tributaries emptying into the river. Hybrids between *F. notatus* and *F. olivaceus* were found at the mouths of these tributaries. Of the specimens collected from the Neches River, 36 were *F. notatus*, 17 were *F. olivaceus* and 17 were hybrids (Table 6). The modal chromosome number was 2n = 40 in *F. notatus* and 2n = 48 in *F. olivaceus*. The following modal numbers were obtained for the hybrids: 2n = 43 (2 specimens), 2n = 44 (8 specimens), 2n = 45 (4 specimens), 2n = 46 (2 specimens), and 2n = 47 (1 specimen). Representative hybrid karyotypes for the Neches River population are shown in Fig. 3 a b. The hybrid nature of these individuals can be seen by a comparison of the diploid numbers with the numbers of large metacentrics. Those hybrids with 2n = 44 had four large, unpaired metacentrics (Fig. 3a). Those with 2n = 45 had three large, unpaired metacentrics (Fig. 3b). Those with 2n = 47 had only one metacentric. Unfortunately, the hybrid specimens were collected late in the summer after the breeding season, and no corroborative meiotic data could be gathered.

Literature Reports of Natural Hybridization Between *F. notatus* and *F. olivaceus*.—Setzer (1970) reported a hybrid swarm between *F. notatus* and *F. olivaceus* in Bracken Creek, a tributary to Pedro Creek, in Houston County, Texas. The upstream portion of Bracken Creek contained predominantly *F. olivaceus* while Pedro Creek contained almost pure *F. notatus*. The lower reaches of Bracken Creek contained a mixture of *F. notatus, F. olivaceus*, and their hybrids. Karyotypic data from the hybrid zone showed four fish with 2n = 40 *(F. notatus)*, one fish with 2n = 48 *(F. olivaceus)*, and eight hybrids with the following: 2n = 41 (1 specimen), 2n = 42 (four specimens), 2n = 44 (2 specimens) and 2n = 46 (1 specimen). The total number of chromosome arms was constant at 50 in all karyotypes. A reduction in chromosome number was always correlated with a corresponding increase in the number of large metacentric chromosomes, and *vice versa*, thus lending evidence to the hybrid nature of that population.

In areas of syntopy, hybridization may occur when one species, either *F. notatus* or *F. olivaceus*, outnumbers the other (Thomerson, 1967). In an area of syntopy in Southern Illinois, Thomerson identified three natural hybrids on basis of phenotypic characteristics.

Laboratory Hybridization of *F. notatus* and *F. olivaceus*.—Cuca (1976) produced laboratory hybrids between *F. notatus* (2n = 40) and *F. olivaceus* (2n = 48). These hybrids had 2n = 44 with a chromosome complement of 4 large median, 2 small median, 12 subterminal, and 26 terminal chromosomes. The testicular tissue of Cuca's artificial hybrids surprisingly gave a gross haploid count of 20. However, in those spreads in which the chromosomes were elongated, it was obvious that four trivalents and 16 bivalents were present, and this would be comparable to the expected haploid count of 22 bivalents. Trivalent formation would be expected if two acrocentric chromosomes from the *F. olivaceus* parent paired with their homologous arms of each large metacentric derived from the *F. notatus* parent. Cuca's work on meiotic chromosomes of these hybrids provided strong evidence of homology between some of the acrocentrics in *F. olivaceus* and the metacentrics of *F. notatus*.

The mitotic karyotype of the artificial hybrids produced by Cuca (1976) is identical to that seen in eight presumed F_1 hybrids in the Neches River population (Fig. 3 a). Meiotic behavior was also the same in our Trussels Creek hybrids as evidenced by trivalent formation (Fig. 4 d).

Thomerson (1966) was particularly successful in obtaining laboratory hybrids between *F. notatus* and *F. olivaceus*. This was done simply by placing a ripe male of one species into an aquarium with a ripe female of the other and allowing them to spawn. The resulting hybrids were "vigorous, fertile, sexually active and were spawned successfully with other hybrids and backcrossed with both parent species". This is apparently what happens in nature when ecological barriers are broken down and the two species become syntopic during breeding season.

Ecological Relationships and Isolating Mechanisms

Because freely interbreeding and backcrossing *F. notatus* × *F. olivaceus* hybrids can be produced with ease in the laboratory (Thomerson, 1966; 1967), and because such situations have been found in nature in Trussels Creek, lower Bracken Creek, and in the Neches River, it is apparent that the isolating mechanisms which usually prevent interbreeding of these forms are not cytogenetic ones. One must then look at other possible isolating mechanisms. One possibility is differences in ecological preference. Braach and Smith (1965) studied the ecological interrelationships of *F. notatus* and *F. olivaceus* in Illinois, Kentucky, and Missouri and found that the two species are invariably microgeographically and ecologically separated. *F. notatus* is found in low-gradient streams, ditches, bottomland lakes, and overflow pools of large rivers, while *F. olivaceus* occurs in fast, gravelly streams in upland regions. Water current velocity appears to be the most important single factor separating the species. Indeed, the three zones of hybridization discussed herein are all in areas where swifter, more upland waters intergrade with slower, more lowland waters. Water temperature, substrate, and amount of vegetation also interact to prevent overlap of the two species.

Differences in food habits or competition for the same food resources also may serve as isolating mechanisms. Thomerson and Wooldridge (1970) studied the food habits of both allotopic and syntopic populations of *F. notatus* and *F. olivaceus*. Qualitative analyses of gut contents showed that both species had similar food habits. Terrestrial arthropods, aquatic insects, and algae seemed to make up most of the important diet items. Thomerson and Wooldridge (1970) attributed the rareness of syntopy in these two species to competitive exclusion because of similar food niches.

Robertsonian Fusion and Karyotypic Evolution.—The evolution of the three distinct karyotypes (2n = 48, 44 and 40; see Table 7) within the *F. notatus* species-complex can largely be explained by Robertsonian fusion of acrocentric chromosomes to form metacentric chromosomes. The classic concept of Robertsonian (centric) fusion is that two non-homologous acrocentric chromosomes may fuse at their centromeric ends to form one biarmed chromosome. If each acrocentric or each arm of a biarmed (metacentric) chromosome is considered one unit, then there is no actual change in the number of chromosome arms following Robertsonian fusion. Thus, a karyotype of 20 metacentric chromosomes has the same number of units as a karyotype of 40 acrocentrics. In studies on karyotypic evolution, it is therefore important to determine the fundamental arm numbers within a karyotype. The fundamental arm number in primitive fundulines and other teleosts is believed to be 48, with a karyotype of 48 acrocentric chromosomes (Setzer, 1970; Chen, 1971). We hypothesize that the ancestor of the *F. notatus* species-complex had a karyotype of 48 acrocentric chromosomes of graded sizes. In this ancestor, a pericentric inversion in a pair of small acrocentrics gave rise to the small pair of metacentrics seen in all of the karyotypes presented herein (Figs. 2 and 3). This resulted in 46 acrocentrics and 2 small metacentrics yielding a fundamental arm number of 50. This is exactly the karyotype seen in *F. olivaceus* and *F. euryzonus* (Fig. 2 a and b). Black and Howell (1978) discussed four possible explanations for the origin of the karyotype in the Tombigbee River chromosomal race of *F. notatus*, 2n = 44. While this karyotype could have arisen via hybridization, centric fusion or centric fission, the fundamental arm number remains at 50. With further centric fusions in the Mississippi River population of *F. notatus*, a karyotype was evolved with a 2n = 40 with eight large metacentrics. Again, the fundamental arm number remains 50. Even the karyotypes of the hybrids all have 50 fundamental arms (Fig. 3). Any reduction in chromosome number of the primitive karyotype (2n = 48) is always correlated with the increase in the number of large metacentrics.

Two lines of evidence favor the role of centric fusions in the karyotypic evolution within the *F. notatus* species- complex: production of fertile hybrids and chromosome pairing at meiosis. A rule relating chromosome number to the production of fertile hybrids was proposed by Nicoljukin (1965). This states that in order for fertile hybrids to be produced, the parental types must possess either the same chromosome number or that the chromosomes must be homologous enough to pair and segregate in a normal fashion. If, then, the reduction of chromosome number in *F. notatus* is due to centric fusions, one might expect that pairing at meiosis would take place between an acrocentric chromosome and each arm of a metacentric, i.e., trivalent formation. This being true, segregation could be normal with no reduction in fertility of the hybrids. However, as pointed out by White (1973), certain trivalent orientations at metaphase I of meiosis may lead to inequational segregation, and this could reduce fertility in heterozygotes for simple Robertsonian fusions. In some cases, simple fusions in the heterotygous state can be effective postmating isolating mechanisms. While it is possible that equational segregation could occur, it cannot be assumed *a priori* that in trivalent formation it would occur. Nonetheless, trivalent formation was seen in hybrid meitoic spreads (Cuca, 1976; this study, Fig. 4 d). Additionally, no reduction in fertility was seen in laboratory-produced hybrids (Thomerson, 1966).

Because fertile hybrids and possible backcrosses are readily produced where ecological barriers break down, it is apparent that the marked differences in karyotypes of these fishes may not function as a postmating isolating mechanism. Ecological barriers, rather than cytogenetic ones, appear to maintain species identities in the *F. notatus* species-complex.

Materials Examined

Locality data for specimens from which karyotypic data have been obtained are given in the following order: state; county; number of specimens examined, males followed by females, in parenthesis; locality; and date of collection. The localities for each species are grouped according to the drainage systems. Hybrid populations are indicated by an asterisk.

Fundulus notatus

Tombigbee River. **Alabama:** GREENE COUNTY: (10, 12) Boligee Canal, Hwy 19, 24 June, 8 and 13 July, and 26 August 1976; (0, 1) Trussels Creek, Hwy 19, N Boligee, 6 May 1978*; (1, 0) Roadside pond, N side Hwy 39, ca 0.5 km W Tennessee-Tombigbee Canal, Gainesville, 26 August 1976. SUMTER COUNTY: (1, 3) Turkey Creek, Hwy 21, 13 July 1976; (2, 5) Bodka Creek, Rt 17, 24 July 1976. **Mississippi:** KEMPER COUNTY: (0, 1) unnamed trib to Big Scooba Creek, E Scooba, Rt 15, ca .25 km W jct 45, 26 August 1976. LOWNDES COUNTY: (3, 3) Tombigbee River at Columbia, Hwy 82, 24 July 1976. NOXUBEE COUNTY: (4, 4) Tibby Creek, trib to Noxubee River, 7 km E Macon, Hwy 14, 24 July 1976.

Pearl River. **Mississippi:** LEAKE COUNTY: (1, 1) Pearl River, Hwy 35, S Carthage, 26 and 29 August 1976.

Lake Pontchartrain. Natalbany River. **Louisiana:** TANGIPAHOA PARISH: (4, 5) Natalbany River, 4.2 km W Hammond (I-55) on I-12, 21 April 1978.

Amite River. **Lousisana:** EAST BATON ROUGE PARISH: (1, 6) Amite River, Hwy 190, 2.7 km W Denham Spring, 21 April 1978.

Neches River. **Texas:** HARDIN COUNTY: (7, 5) Right bank Neches River, 6 km E Silsbee, US Hwy 96, 13 July 1979*. Jasper County: (13, 2) Left bank Neches River, 6 km E Silsbee, US Hwy 96, 13 July 1979* TYLER COUNTY: (1, 8) Neches River above Town Bluff Dam at Town Bluff, FM 92, 13 July 1979.

Missouri River. **Missouri:** ST. LOUIS COUNTY: (1, 2) Bonhomme Creek, Hwy CC, 5 August 1978.

Illinois River. **Illinois:** JERSEY COUNTY: (3, 1) unnamed trib to Illinois River ca 1.5 km E of East Hardin, 6 August 1978.

Mississippi River. **Illinois:** MADISON COUNTY: (7, 0) private pond, trib to Cahoika Creek, N Southern Illinois University on Bohm Rd, S Hwy 143, 6 August 1978; (6, 2) Silver Creek, Hwy 4, 6 August 1978.

Tennessee River. **Alabama:** LAUDERDALE COUNTY: (4, 0) Collier's Slough, backwaters to Tennessee River, 11 November 1978. MARSHALL COUNTY: (11, 5) Tennessee River at Brown's Creek, Warrenton, 5 November 1977 and 25 May 1978; (1, 1) Honeycomb Creek, backwaters to Tennessee River, US Hwy 431, 25 May 1978. MORGAN COUNTY: (4, 4) backwaters to Tennessee River, Wheeler Wildlife Refuge, ca 0.5 km N Point Mallard, Decatur, 18 September 1976; (5, 3) Dry Creek along Rt 36, 0.5 km W Lacey Springs, 28 April 1979. **Tennessee,** HARDIN COUNTY: (3, 6) Tennessee River at Pickwick State Park, 28 September 1976.

Duck River. **Tennessee:** BEDFORD COUNTY: (1, 0) trib to Sugar Creek, 200 yds S jct 64 on Hwy 130, 14 June 1979; (1, 4) Sugar Creek ca 1 km W jct 130 on Hwy 64, 14 June 1979.

Fundulus olivaceus

Appalachicola River. **Alabama:** RUSSELL COUNTY: (4, 5) Uchee Creek trib to Chattahoochee River, ca 2.6 km S Marvyn, Hwy 37, 12 August 1979.

Choctawhatchee River. **Florida:** HOLMES COUNTY: (12, 9) Hurricane Creek, trib to Pea River, ca 1 km W Sweetgum Head, Hwy 2, 24 June 1978.

Escambia Bay. **Alabama:** ESCAMBIA COUNTY: (3, 7) Escambia Creek ca 1 km N I-65 on dirt road off Rt 17, 9 October 1977.

Tombigbee River. **Alabama:** CLARKE COUNTY: (8, 10) unnamed trib to Little Bassett Creek, Hwy 43 0.5 km S Thomasville, 11 June 1978. GREENE COUNTY: (4, 4) Trussels Creek, Hwy 19, N Boligee, 24 June and 26 August 1976, and 6 May 1978*; (7, 10) Trussels Creek, N Mt. Olive Church off Hwy 19, 6 May 1978. SUMTER COUNTY: (1, 1) Yellow Creek ca 1 km SE Hwy 11, ca 2 km SW Livingston, 24 July 1976. **Mississippi:** LOWNDES COUNTY: (1, 2) Tombigbee River at Columbus, Hwy 82, 24 July 1976. MONROE COUNTY: (1, 0) Halfway Creek E of river proper ca 1.9 km N Hwy 45, Aberdeen 7 August 1976; (0, 3) Tombigbee River at Bigbee, Hwy 6, 7 August 1976.

Black Warrior River. **Alabama:** CULLMAN COUNTY: (1, 5) Marriott Creek, trib to Mulberry Fork, ca 1 km S jct Hwy 69 on I-65, 27 June 1979.

Alabama River. **Alabama:** CHILTON COUNTY: (1, 0) Mulberry Creek N of Maplesville, 31 March 1979. WILCOX COUNTY: (0, 1) Chilatchee Creek, Hwy 5 ca 0.5 km N Alberta, 10 June 1978.

Cahaba River. **Alabama:** JEFFERSON COUNTY: (5, 5) Cahaba River at Hwy 280 bridge, 5 July 1979.

Coosa River. **Alabama:** SHELBY COUNTY: (3, 6) headwaters to Yellowleaf Creek at jct Hwy 280 and Hwy 47, 5 July 1979.

Tallapoosa River. **Alabama:** MACON COUNTY: (7, 6) unnamed trib to Chewacla Creek 8 km W Auburn on I-85, 21 May 1978.

Pascagoula Bay. **Mississippi:** JONES COUNTY: (5, 6) Leaf River, I-59 bridge, 1 November 1978. LAMAR COUNTY: (4, 3) Boggy Hollow, trib to Pascagoula River, 2.7 km SW Purvis, 31 October 1978.

St. Louis Bay. **Mississippi:** PEARL RIVER COUNTY: (2, 4) Wolf River at I-59 bridge, 1.5 km W Poplarville, Hwy 26, 1 November 1978.

Pearl River. **Mississippi:** LEAKE COUNTY: (4, 0) Pearl River, N Philadelphia on Rt 19 at boat landing, 26 August 1976.

Lake Pontchartrain. Tangipahoa River. **Louisiana:** TANGIPAHOA PARISH: (0, 4) E Fork Big Creek off Hwy 10, 6.8 km E Arcola, 27 March 1978; (1, 0) E Fork Big Creek at Rt. 1054 bridge, 27 March 1978.

Natalbany River. **Louisiana:** TANGIPAHOA PARISH: (10, 9) Natalbany River 4.2 km W Hammond (I-55) on I-12, 21 April 1978.

Amite River. **Louisiana:** EAST BATON ROUGE PARISH: (3, 4) Amite River, Hwy 190 bridge, 2.7 km W Denham Spring, 21 April 1978.

Neches River. **Texas:** HARDIN COUNTY: (6, 2) Neches River, right bank, 6 km E Silsbee, US Hwy 96, 12 July 1979*; (5, 4) Village Creek at US Hwy 96 bridge, 13 July 1979.

Yazoo-Big Black River. **Mississippi:** CALHOUN COUNTY: (4, 7) Big Creek, trib to Yalobusha River, 17 km S Vardaman, 7 August 1976. TATE COUNTY: (4, 5) James Wolf Creek, trib to Coldwater River at Hwy 4 bridge, 1 km E Looxhoma, 8 November 1977. WEBSTER COUNTY: (1, 2) Big Black River, Hwy 82, 7 August 1976.

White River. **Arkansas:** RANDOLPH COUNTY: (1, 3) Eleven Point River at US Hwy 62 access, Imboden, 9 November 1977; (1, 0) unnamed trib to Fourche River on Hwy 115, N Middlebrook, 9 November 1977.

St. Francis River. **Arkansas:** ST. FRANCIS COUNTY: (0, 3) Crow Creek, W Madison on Hwy 70, 8 November 1977.

Hatchie River. **Mississippi:** ALCORN COUNTY: (6, 6) Tarebreeches Creek, trib to Tuscumbia River at Hwy 72 bridge, W Corinth, 8 November 1977.

Missouri River. **Missouri:** MONTGOMERY COUNTY: (4, 7) Dry Fork, trib to Loutre River, Hwy K bridge, W Big Spring, 5 August 1978. ST. LOUIS COUNTY: (2, 4) Wild Horse Creek, ca 2 km S Centaur Station in Babler State Park, Hwy CC, 5 August 1978. WARREN COUNTY: (2, 4) Charrette Creek, Hwy 47 bridge, 5 August 1978.

Ohio River. **Illinois:** MASSAC COUNTY: (2, 0) Tucker Ditch, trib to Main Ditch, Hwy 45, 0.5 km S jct 169, 7 August 1978. WILLIAMSON COUNTY: (3, 0) Little Creek, trib to S Fork Saline River, Hwy 37, Pulleys Mill, 7 August 1978.

Tennessee River. **Alabama:** LAUDERDALE COUNTY: (3, 2) Cypress Creek at Hwy 133 bridge, 0.2 km W jct Hwy 41, 11 Nov. 1978. MORGAN COUNTY: (6, 4) unnamed trib to Cotaco Creek, 0.5 km NE Cotaco School, Rt 36, 28 April 1979; (1, 0) headwaters Town Creek, 0.8 km S Sommerville, 28 April 1979; (0, 1) E Fork Flint Creek, 0.5 km N Morgan-Cullman County line on Hwy 31, 24 May 1978. **Tennessee:** BENTON COUNTY: (3, 4) unnamed trib at Hwy 69, ca 4 km S Camden, 1 July 1978. DECATUR COUNTY: (1, 0) unnamed trib at Hwy 69, ca 9 km S Decaturville, 1 July 1978.

Elk River. **Tennessee:** MOORE COUNTY: (0, 4) E Fork Mulberry Creek, Hwy 55 bridge, 0.5 km N jct 50, S Lynchburg, 30 June 1978.

Paint Rock River. **Alabama:** JACKSON COUNTY: (5, 5) Clear Creek, Hwy 65 bridge, ca 3 km N jct Hwy 72, 27 April 1979.

Fundulus euryzonus

Lake Pontchartrain. Tangipahoa River. **Louisiana:** TANGIPAHOA PARISH: (2, 8) E Fork Big Creek, Rt 1054 bridge, 27 March 1978.

Amite River. **Louisiana;** ST. HELENA PARISH: (7, 7) Darling's Creek, 0.6 km E Chipola on Hwy 38, 21 April 1978.

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Table 1. Distribution of diploid chromosome counts obtained for *Fundulus olivaceus*.

Population	Number of Specimens	Diploid Chromosome Number 46	47	48	Number of Cells Analyzed	Mode
Appalachicola River	9	1	3	32	36	48
Choctawhatchee River	21		3	60	63	48
Escambia Basin	10	4	4	34	42	48
Mobile Basin:						
Tombigbee River	52	3	15	150	168	48
Black Warrior River	6	1	5	25	31	48
Alabama River	2		2	8	10	48
Cahaba River	10	3	3	32	38	48
Coosa River	9	5	5	29	39	48
Tallapoosa River	13	2	7	39	48	48
Pascagoula Basin	18	1	3	57	61	48
St. Louis Basin	6			16	16	48
Pearl River	4		1	11	12	48
Lake Pontchartrain:						
Tangipahoa River	5	1	3	34	38	48
Natalbany River	19	4	12	77	93	48
Amite River	7		1	38	39	48
Neches River	17	2	9	56	67	48
Yazoo-Big Black Rivers	23	1	9	56	66	48
White River	5		4	13	17	48
St. Francis River	3		1	5	6	48
Hatchie River	12	4	4	34	42	48
Missouri River	23		2	71	73	48
Ohio River	5		2	4	6	48
Tennessee River	25		7	91	98	48
Elk River	4			14	14	48
Paint Rock River	10		1	34	35	48

Table 2. Distribution of haploid chromosome counts obtained for *Fundulus notatus*, *F. olivaceus* and *F. euryzonus*.

Species	Number of Specimens	Haploid Chromosome Number 19	20	21	22	23	24	Number of Cells Analyzed	Mode
Fundulus notatus[1]	45	5	170					175	20
Fundulus notatus									
Tombigbee race	4		1	1	20			22	22
Fundulus olivaceus	73					3	308	311	24
Fundulus euryzonus	4					2	34	36	24

[1] Includes all populations of *F. notatus* except Tombigbee River race

Table 3. Distribution of diploid chromosome counts obtained for *Fundulus euryzonus.*

Population	Number of Specimens	Diploid Chromosome Number 46	47	48	Number of Cells Analyzed	Mode
Lake Pontchartrain:						
Tangipahoa River	11	3	7	40	50	48
Amite River	14	1	7	53	61	48

Table 4. Distribution of diploid chromosome counts obtained for *Fundulus notatus.*

Population	Number of Specimens	Diploid Chromosome Number 38	39	40	41	42	43	44	Number of Cells Analyzed	Mode
Tombigbee River	50					1	2	178	181	44
Pearl River	2			6					6	40
Lake Pontchartrain:										
Natalbany River	11	2	3	32					37	40
Amite River	7	1	6	32					39	40
Neches River	36	3	18	104	1				126	40
Missouri River	3			4					4	40
Illinois River	4	1		5					6	40
Mississippi River	15		4	16					20	40
Tennessee River	47	11	20	144					175	40
Duck River	6	1	2	19					22	40

Table 5. Distribution of diploid chromosome counts obtained for *Fundulus notatus, F. olivaceus* and their hybrids, *F. notatus* × *F. olivaceus,* from Trussels Creek, Greene County, Alabama.

Species	Number of Specimens	Diploid Chromosome Number 43	44	45	46	47	48	Number of Cells Analyzed	Mode
Fundulus notatus	1		3					3	44
F. notatus × *F. olivaceus*	6			2	16	4		22	46
F. olivaceus	11					4	29	33	48

Table 6. Distribution of diploid chromosome counts obtained for *Fundulus notatus, F. olivaceus* and their hybrids, *F. notatus* × *F. olivaceus* from the Neches River, Texas.

Species	Number of Specimens	Diploid Chromosome Number 38	39	40	41	42	43	44	45	46	47	48	Number of Cells Analyzed	Mode
Fundulus notatus	36	3	18	104	1								126	40
F. notatus × *F. olivaceus*	2				2	3	15						20	43
"	8				1	4	12	57	1				75	44
"	4						3	4	22	2			31	45
"	2							1	4	13	1		19	46
"	1									1	7	1	9	47
Fundulus olivaceus	17									2	9	56	67	48

Table 7. Summary of the distribution of diploid chromosome counts obtained for *Fundulus notatus, F. olivaceus* and *F. euryzonus*.

Species	Number of Specimens	Diploid Chromosome Number 38	39	40	41	42	43	44	45	46	47	48	Number of Cells Analyzed	Mode
Fundulus notatus[1]	129	19	53	362	1								435	40
Fundulus notatus Tombigbee race	50					1	2	178					181	44
Fundulus olivaceus	318									32	106	1020	1158	48
Fundulus euryzonus	25									4	14	93	111	48

[1] Includes all populations of *F. notatus* except Tombigbee River race

An Isozymic Analysis of Several Southeastern Populations of the Cyprinodontid Fishes of the *Fundulus notatus* Species-complex[1]

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ABSTRACT: Tatum, Fred, Ronald Lindahl, and Herbert Boschung, 1981. An Isozymic Analysis of Several Southeastern Populations of the Cyprinodontid Fishes of the *Fundulus notatus* Species- complex. Bulletin Alabama Museum of Natural History 6:31-35. An electrophoretic analysis of ten enzyme systems coded by sixteen loci was performed to elucidate the phylogenetic relationships of the *Fundulus* species- complex of *notatus*, *olivaceus* and *euryzonus*. The electrophoretic data do not unequivocally distinguish the three species; rather, the data confirm the close biochemical relationship of them. The malate dehydrogenase-2 gene product serves as an isozymic marker that identifies *Fundulus notatus* from the other species of the complex. Genetic identity values obtained were 0.86, and they indicate a high degree of genetic similarity with the complex. Two chromosomal populations of *F. notatus*, 2n = 40 and 2n = 44, were also analyzed. Genetic identity values were 0.95, confirming their designation as chromosomal races of *F. notatus*.

Introduction

Fundulus notatus (Rafinesque), the blackstripe topminnow, *Fundulus olivaceus* (Storer), the blackspotted topminnow, and *Fundulus euryzonus* Suttkus and Cashner (1981), the broadstripe topminnow, are sibling species forming the *Fundulus notatus* species-complex. Morphological, karyotological, and ecological characters that separate the species have been discussed by Moore and Paden (1950), Brown (1956), Braasch and Smith (1965), Thomerson (1966, 1967), Cuca (1976), and Black and Howell (1978). The authors unanimously agree that *F. notatus* and *F. olivaceus* are closely related but distinct species. Suttkus and Cashner (1981) align *F. euryzonus* with *F. notatus* and *F. olivaceus*, and Howell and Black (1981) concur.

Because of the high degree of morphological similarities and interspecific chromosomal homologies resulting in hybrid fertility (Cuca, 1976), an electrophoretic analysis of the *F. notatus* species-complex was undertaken. Principally, the purpose of the research reported herein was to analyze the relationships among the three species by measuring the genetic differentiation that has occurred in their evolution. Specifically, the objectives were: 1) to confirm biochemically the rank of distinct species for *F. notatus*, *F. olivaceus*, and *F. euryzonus*; 2) to determine if *F. euryzonus* is more closely aligned with *F. notatus* or *F. olivaceus*; and 3) to determine if the Tombigbee population of *F. notatus*, reported by Black and Howell (1978), represents a distinct species or a chromosomal race of *F. notatus*.

Materials and Methods*

Specimens of the three species were collected from the following localities: *Fundulus notatus* (2N = 40) from the Tennessee River, Morgan County, Alabama and the Amite River, East Baton Rouge Parish, Louisiana. *F. notatus*

[1]Contribution No. 37 from the Aquatic Biology Program, The University of Alabama.

(2N=44) from Tubb's Creek, tributary to the Tombigbee River, Greene County, Alabama. *F. olivaceus* from the Sipsey River, (Tombigbee River drainage), Tuscaloosa County, Alabama, and from the Natalpany River, Tangipahoa Parish, Louisiana. *F. euryzonus* from Darling's Creek, St. Helena Parish, Louisiana.

After capture, some specimens were placed immediately on dry ice and, upon return to the laboratory, transferred to a −70°C freezer until needed. Others were kept in aquaria until time of analysis.

Horizontal starch gel electrophoresis was conducted on 12% gels (Connaught Laboratories Ltd., Willowdale, Ontario, Canada). Buffer systems employed were:

I. Stock buffer – 900 mM Tris, 500 mM boric acid, and 20 mM EDTA, pH 8.7; cathode buffer-1:5 dilution of stock; anode buffer-1:7 dilution of stock; gel buffer-1:20 dilution of stock (Markert and Faulhaber, 1965);

II. electrode buffer – 135 mM Tris, 45 mM citric acid and 1.2 mM EDTA, pH 7.2; gel buffer – 9 mM Tris, 3 mM citric acid, and 1.2 mM EDTA, pH 7.2 (Ayala and Powell, 1972);

III. electrode buffer – 300 mM boric acid and 5 mM NaOH, pH 8.0; gel buffer – 30 mM boric acid and 1.2 mM NaOH, pH 8.5 (Shaw and Prasad, 1970);

IV. electrode buffer – 233 mM Tris, 80 mM citric acid, pH 7.2; gel buffer – 8.14 mM Tris, 3 mM citric acid, pH 7 2 (modified after Smith, 1968).

Samples of whole heads, skeletal muscles, and viscera were prepared in cold gel buffer IV (33% w/v). Homogenates were centrifuged for 30 minutes at 27,000 g. Fifty microliters of supernatant were drawn off and placed on Whatman No. 3 filter paper wicks (1 × 1 cm) for electrophoresis. Gels were run from 12 to 18 hours at 16 volts/cm (Buffer System I), 8 volts/cm (Buffer System II), 3 volts/cm (Buffer System III), or 3.5 volts/cm (Buffer System IV).

Whole head samples were stained for lactate dehydrogenase, malate dehydrogenase, phosphoglucose isomerase, creatine kinase, and adenylate kinase. Skeletal muscle samples were stained for phosphoglucomutase, glycerol-3-phosphate dehydrogenase, aspartate aminotransferase and superoxide dismutase. Visceral samples were stained for catalase.

Staining procedures modified after Shaw and Prasad (1970), Ayala and Powell (1972) or Harris and Hopkinson (1976), are listed below:

Lactate Dehydrogenase (Buffer System II)

0.4 ml lactic acid, 60 mg NAD, 40 mg NBT, 5 mg PMS, 100 ml 50 mM Tris-HCl, pH 7.4.

Malate Dehydrogenase (Buffer System II)

2.68 grams malic acid, 60 mg NAD, 40 mg NBT, 5 mg PMS, 100 ml 50 mM Tris-HCl. Malic acid and Tris-HCl buffer are mixed and the pH adjusted to 7.5 prior to addition of remaining substances.

Phosphoglucose Isomerase (Buffer System IV)

100 mg fructose 6-phosphate, 25 mg EDTA, 15 mg NADP, 200 mg $MgCl_2$, 20 mg NBT, 5 mg PMS, 0.04 ml (91 units) G6PD, 100 ml 100 mM Tris-HCl, pH 7.2.

Creatine Kinase (Buffer System IV)

350 mg creatine phosphate, 75 mg ADP, 90 mg glucose, 21 mg $MgCl_2$, 25 mg NADP, 3 mg PMS, 20 mg NBT, 0.053 ml (160 units) hexokinase, 0.08 ml (80 units) G6PD, 100 ml 50 mM Tris-HCl, pH 7.1.

Adenylate Kinase (Buffer System IV)

Same as Creatine Kinase but omit creatine phosphate.

Aspartate Aminotransferase (Buffer System IV)

532 mg L- aspartic acid, 73 mg α-ketoglutaric acid, 50 mg pyridoxal phosphate, 200 mg Fast Blue RR, 100 ml 50 mM Tris-HCl, pH 7.1.

Superoxide Dismutase (Buffer System I)

40 mg NBT, 10 mg PMS, 100 ml 50 mM Tris-HCl, pH 8.0.

Glycerol-3-Phosphate Dehydrogenase (Buffer System IV)

50 mg NAD, 30 mg NBT, 5 mg PMS, 500 mg Na glycerol-3-phosphate, 200 mg Na pyruvate, 100 ml 50 mM Tris-HCl, pH 7.2.

Catalase (Buffer System III)

1 ml 3% H_2O_2, 99 ml H_2O, incubate gel in solution for 15 minutes, rinse gel with distilled H_2O, immerse gel in 100 ml 2% potassium ferricyanide and 100 ml 2% ferric chloride.

Results and Discussion

Ten enzyme systems coded by sixteen loci were analyzed in this study (Table 1). A total of six polymorphic loci were detected (PGI-1, PGI-2, GPD, Cat., SOD, and PGM) while the remaining ten loci (LDH-A, LDH-B, LDH-C, MDH-1, MDH-2, CK-1, CK-2, AK, AAT-1, and AAT-2) proved monomorphic. All six polymorphic loci are represented in the pooled populations of *F. notatus* (Table 1):GPD, SOD, Cat., and PGM in the Amite River population; PGI-1, PGI-2, GPD, Cat., and SOD in the Tennessee River population; and PGI-1, GPD, and Cat. in the Tombigbee River Population. *F. euryzonus* and both populations of *F. olivaceus* are polymorphic at the PGI-1, PGM, GPD, Cat. and the PGM, GPD, and Cat. loci respectively.

The MDH-2 locus gene product was a species marker which distinguished both chromosomal races of *F. notatus* from *F. olivaceus* and *F. euryzonus*. Electrophoretic mobilities were identical for MDH-2 of *F. olivaceus* and *F. euryzonus*, while MDH-2 of *F. notatus* exhibited a slower anodal migration. Two unique allozymes were observed in the PGI-2 gene products of the Tennessee River population

* Abbreviations used: EDTA, ethylenediamine tetraacetic acid; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; AAT, aspartate aminotransferase; AK, adenylate kinase; Cat., catalase; CK, creatine kinase; G6PD, glucose-6-phosphate dehydrogenase, GPD, glycerol 3-phosphate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; SOD, superoxide dismutase.

of *F. notatus*, and in *F. euryzonus*. The frequencies of these novel alleles were 0.708 in *F. euryzonus* and 0.337 in the Tennessee River population of *F. notatus*. Also, a unique allele of PGM was present in the Amite River population of *F. notatus* in the frequency of 0.136. The remaining genetic divergence detected in the analysis of the *F. notatus* complex resulted from differences in frequencies of shared alleles.

The pooled data of the three populations of *F. notatus* revealed 37% of their loci were polymorphic, the two populations of *F. olivaceus* was polymorphic at 25% of the surveyed loci, and the one population of *F. euryzonus* was 19% polymorphic (Table 2). These values compare favorably with other studies detailing levels of genetic variation in fishes. Data compiled by Selander (1976) on 14 species of fishes with a mean number of 21 loci showed levels of polymorphism of approximately 30% per population.

The procedure of Nei (1978) was used to estimate the average heterozygosity (Table 2) and genetic identity (Table 3) of the populations examined. The average number of heterozygous loci per individual in the *Fundulus notatus* complex calculated here ($\overline{H} = 0.068$) is very similar to that estimated ($\overline{H} = 0.078$) from the data of several laboratories by Selander (1976). However, within the complex, considerable variability in average heterozygosity is detectable between populations of *F. notatus*, as well as between the three species.

According to Ayala (1975), genetic identities as high as the 0.86 observed here (Table 3), would suggest that members of the *Fundulus notatus* species-complex are subspecies at best. However, in addition to these isozymic data, there are an abundance of behavioral, ecological, and morphological data describing members of the complex (Moore and Paden, 1950; Brown, 1956; Braasch and Smith, 1965; Thomerson, 1966; Suttkus and Cashner, 1981). Karyotypic analyses have been performed on the complex (Setzer, 1970; Chen, 1971; Cuca, 1976; Black and Howell, 1978; Howell and Black, 1981). When evaluated as part of all the available data, the relatively low level of structural gene (genes encoding polypeptide products) differentiation apparent in the members of this complex suggest that mutations in regulatory and/or other genes may have been the primary mechanism responsible for speciation in this complex (Wilson et at. 1974; King and Wilson, 1975; Ayala, 1976). Such mutations could result in speciation without being associated with the structural gene differentiation that often accompanies these events.

From the evidence available, we conclude that *F. euryzonus*, *F. olivaceus* and *F. notatus* are distinct species. Furthermore, we conclude that *F. euryzonus* is more closely aligned to *F. olivaceus* than to *F. notatus*. Our conclusion are based on karyotypic data and the identical electrophoretic mobilities of the MDH-2 gene product. Moreover, the genetic identities of *F. euryzonus*, *F. olivaceus*, and *F. notatus* ($I \geq 0.86$) confirm the suggestion that the mechanisms separating these forms are largely behavioral and/or ecological, and not genetic (Howell and Black, 1981). Our results also indicate that the karyotypic changes observed within the *F. notatus* complex (Howell and Black, 1981) have not as yet resulted in significant structural gene changes. The very high genetic identities (≥ 0.95) and the identical MDH-2 gene products in the three *F. notatus* populations is consistent with the conclusion of Black and Howell (1978) that the Tombigbee population of *F. notatus* represents a distinctive chromosomal race and not a separate species.

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Table 1. Allele frequencies of 16 enzyme loci in the *Fundulus notatus* species-complex.

Locus	Allele	*F. olivaceus*		*F. euryzonus*		*F. notatus* 2N = 44		*F. notatus* 2N = 40		*F. notatus* pooled	
LDH-A	a	1.000[1]	140[2]	1.000	76	1.000	72	1.000	140	1.000	212
LDH-B	a	1.000	140	1.000	76	1.000	72	1.000	140	1.000	212
LDH-C	a	1.000	140	1.000	76	1.000	72	1.000	140	1.000	212
MDH-1	a	1.000	120	1.000	80	1.000	76	1.000	138	1.000	214
MDH-2	a	1.000	120	1.000	80	0.000	76	0.000	138	0.000	214
	b	0.000	120	0.000	80	1.000	76	1.000	138	1.000	214
CK-1	a	1.000	136	1.000	74	1.000	72	1.000	134	1.000	206
CK-2	a	1.000	136	1.000	74	1.000	72	1.000	134	1.000	206
AAT-1	a	1.000	66	1.000	18	1.000	66	1.000	84	1.000	150
AAT-2	a	1.000	66	1.000	18	1.000	66	1.000	84	1.000	150
AK	a	1.000	136	1.000	74	1.000	66	1.000	134	1.000	200
PGI-1	a	0.047	148	0.861	72	0.212	52	0.765	136	0.575	188
	b	0.953	148	0.139	72	0.788	52	0.235	136	0.425	188
PGI-2	c	0.000	148	0.708	72	0.000	52	0.000	136	0.000	188
	d	0.000	148	0.000	72	0.000	52	0.169	136	0.127	188
	e	1.000	148	0.292	72	1.000	52	0.831	136	0.873	188
PGM	a	0.014	138	0.000	48	0.000	84	0.093	114	0.071	198
	b	0.986	138	1.000	48	1.000	84	0.847	114	0.884	198
	c	0.000	138	0.000	48	0.000	84	0.060	114	0.045	198
SOD	a	1.000	114	1.000	72	1.000	64	0.899	98	0.938	162
	b	0.000	114	0.000	72	0.000	64	0.101	98	0.062	162
GPD	a	0.047	106	0.964	28	0.125	32	0.293	92	0.270	124
	b	0.953	106	0.036	28	0.875	32	0.707	92	0.730	124
Cat	a	0.625	48	1.000	24	0.750	24	0.949	78	0.889	102
	b	0.375	48	0.000	24	0.250	24	0.051	78	0.111	102

[1]allele frequency. [2]number of genes sampled.

Table 2. Average heterozygosity per locus ($\bar{H}$) and percent polymorphism (P) for *Fundulus notatus*, *Fundulus olivaceus* and *Fundulus euryzonus*.

Species and locality	$\bar{H}$	P
Fundulus notatus (Tombigbee River, 2N = 44)	0.0623	0.1875
Fundulus notatus (Amite River, 2N = 40)	0.0690	0.2500
Fundulus notatus (Tennessee River, 2N = 40)	0.1125	0.3125
Fundulus notatus (pooled, 2N = 40 and 2N = 44)	0.1032	0.3750
Fundulus euryzonus (2N = 48)	0.0459	0.1875
Fuldulus olivaceus (2N = 48)	0.0460	0.2500

Table 3. Genetic identities between *Fundulus olivaceus*, *Fundulus euryzonus*, and *Fundulus notatus* populations.

	1	2	3	4	5	6
1. *Fundulus notatus* (Tombigbee River, 2N = 44)	———	0.95018	0.98655	0.98803	0.82637	0.93534
2. *Fundulus notatus* (Amite River, 2N = 40)		———	0.96112	0.98469	0.86003	0.86059
3. *Fundulus notatus* (Tennessee River, 2N = 40)			———	0.99446	0.86455	0.90749
4. *Fundulus notatus* (pooled, 2N = 4 0 and 2N = 44)				———	0.86561	0.90720
5. *Fundulus euryzonus* (2N = 48)					———	*0.86018*
6. *Fundulus olivaceus* (2N = 48)						———