A COMPARATIVE BIOSYSTEMATIC STUDY OF *FUNDULUS NOTATUS* AND *FUNDULUS OLIVACEUS* (PISCES: CYPRINODONTIDAE)

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ABSTRACT

Western populations of *Fundulus notatus* (Guadalupe, Colorado, Brazos, San Jacinto, Trinity, Neches and Sabine rivers) tend to have more dorsal (average 9.9) and anal (average 12.8) rays. Eastern populations (Mississippi Valley, Lake Pontchartrain drainage, Pearl River and Tombigbee River) tend to have fewer dorsal and anal rays (average 9.2 and 12.1 respectively). Trends in caudal ray number are roughly parallel. Lateral-scale number is generally 34 or 35. Northern fish tend to have broader heads and shorter snouts than southern fish. Dorsolateral spots, if present, are irregular, or diffuse, or both.

The westernmost population of *Fundulus olivaceus* (Navasota River, Texas) has fewer dorsal and anal rays (9.4 and 12.0 respectively). Populations from the Trinity River east to the Mississippi Gulf drainage average 9.8 dorsal rays. Dorsal ray number decreases clinally to the east to a low average of 8.9 for Choctawhatchee Bay drainage samples. Trinity and Neches river populations average 12.5 anal rays. Anal ray number decreases clinally to the east to a low average of 11.2 for Choctawhatchee Bay drainage samples. Caudal ray number tends to be high from the Alabama River west, and low to the east. But average lateral-scale number is greatest (35.7) for the Chattahoochee sample. Northern fish tend to be more robust than southern fish. Dorsolateral spots are the best character to separate *F. notatus* from *F. olivaceus*. With rare exceptions, *F. olivaceus* has discrete, regular, black dorsolateral spots.

Reproductive isolation is primarily ecological and is reinforced by homospecific mating preference. The two species are interfertile and produce fertile and distinctive hybrids in the laboratory. Only two natural hybrids are known. The two species have broadly overlapping ranges but are seldom syntopic. Syntopic associations are unstable and character displacement is not demonstrated. Ecological preferences are not uniform throughout the ranges of *F. olivaceus* and *F. notatus*, but *F. notatus* seldom occurs in "blackwater".

INTRODUCTION

Fundulus notatus (Rafinesque) and *F. olivaceus* (Storer) are two of the most widely distributed species of the North American cyprinodontid genus *Fundulus* (Fig. 1). Both sexes of these two species have a single black, more or less solid, lateral stripe originating at the tip of the snout and terminating at the distal end of the caudal peduncle or on the caudal fin. This character separates them from all other presently recognized species of *Fundulus*.

Both *F. notatus* (personal communication, Clark Hubbs) and *F. olivaceus* (Miller, 1955) have been reported from brackish water near the Gulf of Mexico, but they ordinarily swim at the surface of the water near the margins of freshwater lakes, rivers, ponds and streams. Both species occur in many different types of environment and both are quite variable. Almost all characters that have been used to separate them have considerable overlap and are unreliable over large parts of their sympatric range.

Most early workers considered them to be conspecific (Garman, 1895; Jordan and Evermann, 1896; Forbes and Richardson, 1920; Hubbs and Ortenburger, 1929).

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Figure 1. Known ranges of *Fundulus notatus* and *Fundulus olivaceus* and distribution of material examined. The dashed line outlines the range of *F. notatus* and the dotted line outlines the range of *F. olivaceus.* Solid circles are *F. olivaceus* localities and open circles are *F. notatus* localities. Solid triangles are *F. olivaceus* localities and open triangles are *F. notatus* localities plotted from data given by Brown (1956).

Kuhne (1939) used *F. n. notatus* and *F. n. olivaceus* as subspecific names in his study of the fishes of Tennessee.

Moore and Paden (1950) considered *F. notatus* and *F. olivaceus* as separate species and presented evidence that they can occur together without interbreeding in the Illinois River of Oklahoma and Arkansas. Jurgens and Hubbs (1953) and Knapp (1953) recognized both species in Texas. Brown (1956) compared specimens from several areas and concluded that both species are valid. Braasch and Smith (1965) reached the same conclusion from their study of the two forms in the upper Mississippi Valley. Their paper includes a synonomy for both species.

This study was undertaken in an attempt to answer the following questions: How do various systematic characters vary from population to population? Do populations in the area of sympatry exhibit character displacement (Brown and Wilson, 1956)? Are *F. notatus* and *F. olivaceus* capable of hybridizing? Do they hybridize in nature? If the two forms are in fact valid species, what isolating mechanisms keep them separate? The results of this study indicate that *F. notatus* and *F. olivaceus,* though completely interfertile, are valid species separated in nature primarily by different ecological requirements and secondarily by behavioral reproductive isolation.

Range

Fundulus notatus

The westernmost Gulf drainage inhabited by *F. notatus* is the Guadalupe River system in southern central Texas. Hubbs, Kuehne, and Ball (1953) considered Landa Park Lake in Comal County as closely defining the upstream limit of the species in the Guadalupe River. It is abundant in the mainstream of the Colorado River at Austin in Travis County and is known from tributaries from Colorado County (Clark Hubbs, personal communication) upstream to Llano County (TU uncatalogued). *Fundulus notatus* is distributed generally throughout the Brazos (upstream to Palo Pinto and Eastland counties), San Jacinto, Trinity, and Neches rivers (Clark Hubbs, personal communication). It also occurs in isolated bayous and creeks draining into the Gulf between the Brazos and Sabine rivers. I have no records of *F. notatus* from any of the rivers between the Sabine River and the various eastern tributaries of the Mississippi River. This area is hereafter referred to as "western Louisiana drainage".

The southernmost record of *F. notatus* in the Mississippi Valley is approximately 20 miles west of New Orleans, Louisiana (TU 387). The species ranges north to southeastern Wisconsin, southern Michigan, northeastern Iowa, and western and central Ohio; west to eastern Oklahoma and Kansas (Brown, 1956); and east to Jackson County, Tennessee (UMMZ 125113).

A few specimens have been taken from the Lake Pontchartrain tributaries: Amite (TU 35697), Natalbany (UMMZ 182345), and Tangipahoa (UMMZ 182344) rivers. *Fundulus notatus* has been collected from the Pearl River from the vicinity of Jackson, Mississippi (UMMZ 170728) and downstream.

The species has not been recorded from

any of the small drainages between the Pearl River and the Tombigbee River (hereafter called Mississippi Gulf drainage), but is known from the Tombigbee drainage of the Alabama system (Brown, 1956). I have no record of *F. notatus* from the Alabama River.

Fundulus olivaceus

The range of *F. olivaceus* overlaps the range of *F. notatus* in large part but is displaced to the east (Fig. 1). The westernmost population known is from the Navasota River, Texas (see *Material Examined*), an eastern tributary of the Brazos River. No records are available from the rest of the Brazos system. The species is abundant along the Gulf Coast eastward to the Choctawhatchee Bay drainage of western Florida and Alabama. In the Apalachicola (Chattahoochee) drainage *F. olivaceus* occurs in a limited area south of the fall line in Alabama and Georgia. This distribution is strikingly similar to that given by Suttkus (1955) for *Notropis euryzonus.* Fowler (1945) recorded *F. notatus* (=*F. olivaceus?*) from the Okefenokee Swamp. Since I have been unable to verify this record or obtain additional records of *F. olivaceus* east of the Apalachicola drainage, I doubt that the species occurs east of that drainage. In the Mississippi Valley, *F. olivaceus* ranges north to southern Missouri and Illinois, west to eastern Oklahoma, and east to western Kentucky (Brown, 1956) and to Anderson County, Tennessee (Cornell University 19148 cited by Brown, 1956).

Geographic Variation

Methods

Dorsal and anal rays were counted at their bases. All rays were counted, rather than considering the last 2 rays as a single ray as is often done. This is the same method used by Brown (1956) and I have incorporated Brown's counts into my data. Caudal ray count is total branched rays plus 2. Since both species show early ontogenetic increase in the number of branched caudal rays this character was not recorded for individuals smaller than 30 mm standard length. Lateral-scale counts were made as described by Brown (1956). Head width was measured at the rear of the orbits, and body depth was taken as the vertical distance from the origin of the anal fin to the dorsal surface of the body. Caudal peduncle depth and length of depressed dorsal fin were taken as described by Hubbs and Lagler (1947).

Color pattern was recorded for specimens over 30 mm standard length. No attempt was made to quantify such patterns in more than a general manner except to estimate the number of dorsolateral spots for *F. olivaceus.* This estimate was obtained by counting the number of spots on the left side of the dorsum, above the lateral stripe, below the dorsal midline and within the standard length. Dorsum pattern was recorded as uniform, intermediate, or cross-hatched. Lateral stripe shape was recorded as smooth, rough, or with few, several or many vertical extensions. Tone of the band and extensions was recorded as dark, intermediate, or light. Dorsum spotting in *F. notatus* was recorded as none, few, many, or blotched. Spots in the dorsal, caudal and anal fins were recorded as many, normal, few, very few, and none. Predorsal line was recorded as absent, light, dashed, partial, or complete. These categories were not particularly satisfactory because of the high variability in coloration from sample to sample. However, their use helped guard against forming false impressions of the distribution of color patterns in a given sample.

Methods of statistical comparison follow those given by Cazier and Bacon (1949) and Mayr, Linsley and Usinger (1953). Meristic characters were compared with the use of a standard 2 x n Chi square test in addition to graphical comparison following Hubbs and Hubbs (1953).

Fundulus notatus

Number of dorsal rays (Table 1, Fig. 2) and number of anal rays (Table 2, Fig. 2) serve to separate *F. notatus* into two groups: a western group which includes populations from the Guadalupe, Colorado, Brazos, San Jacinto, Trinity, Neches, and Sabine rivers; and an eastern group composed of populations from the Mississippi and Great Lakes drainages, Lake Pontchartrain drainage, Pearl River, and Tombigbee River. The differences between these two groups do not warrant the recognition of subspecies.

Samples from populations making up the western group have 10 as the modal dorsal ray number and an observed range of variation of 8 to 12. The modal dorsal ray number is 9 in all samples from the eastern group and the observed range of variation

TABLE 1
Number of Dorsal Rays in Fundulus notatus

Populations	Number of Dorsal Rays 8	9	10	11	12	N	M	S.D.	2S.E.
Guadalupe R., Tex.		8	39	20		67	10.2	.64	.16
Colorado R., Tex.		21	44	6		71	9.8	.59	.14
Brazos R., Tex.		25	68	7		100	9.8	.54	.11
San Jacinto R., Tex.		11	54	34	1	100	10.2	.66	.13
Trinity R., Tex.		15	32	3		50	9.8	.57	.16
Neches R., Tex.		40	60			100	9.6	.49	.09
Sabine R., Tex.-La.	2	38	54	6		100	9.6	.63	.13
Total Western	2	158	352	76	1	588	9.9	.66	.06
Mississippi R.	8	116	47			171	9.2	.45	.07
L. Pontchartrain Dr.	2	33	5			40	9.1	.41	.13
Pearl R., La.-Miss.	3	68	29			100	9.3	.50	.10
Tombigbee R., Ala.	3	24	9			36	9.2	.57	.19
Total Eastern	16	239	90			345	9.2	.50	.05

is from 8 to 10. A line drawn between 9 and 10 dorsal rays separates 73% of 588 western specimens from 74% of 345 eastern specimens; average separation 73.5%; coefficient of divergence 0.60.

The two groups are similar in observed range of variation (11 to 14) in anal ray number but have different modes. All samples from western populations except those from the Brazos River (mode 12) are modal at 13. All samples from eastern populations are modal at 12. A line drawn between 12 and 13 anal rays separates 63% of 588 western specimens from 85% of 337 eastern specimens; average separation 74%; coefficient of divergence 0.60.

Caudal ray number (Table 3) varies from 11 to 17. With the exception of the San Jacinto sample (mean 13.3, mode 13), the western group samples have a high average number of caudal rays (13.8 to 14.2). The Mississippi River sample (mean 14.2, mode 14) resembles the western group for this character. The rest of the eastern group samples are modal at 13. The Lake Pontchartrain drainage sample of 16 specimens (mean 13.4) is significantly different from the Mississippi River sample (Chi square 14.2, 4 degrees of freedom, P less than 0.01), but not from the Pearl River sample (mean 12.9) or the Tombigbee River sample (mean 13.4). The pattern of caudal ray number distribution roughly parallels the patterns seen in dorsal and anal ray number with the exception of the Mississippi River sample which has high mean caudal ray number and low mean dorsal and anal ray numbers.

Observed range of variation in lateral

TABLE 2
Number of Anal Rays in Fundulus notatus

Populations	Number of Anal Rays 11	12	13	14	N	M	S.D.	2S.E.
Guadalupe R., Tex.		6	39	20	67	13.2	.58	.14
Colorado R., Tex.		26	41	4	71	12.7	.53	.12
Brazos R., Tex.	2	53	37	8	100	12.5	.67	.13
San Jacinto R., Tex.		30	62	8	100	12.8	.58	.12
Trinity R., Tex.	1	16	29	4	50	12.7	.64	.16
Neches R., Tex.		44	51	5	100	12.6	.58	.12
Sabine R., Tex.-La.	2	35	57	6	100	12.7	.62	.12
Total Western	5	210	316	51	588	12.8	.65	.05
Mississippi R.	7	124	16		167	12.0	.51	.08
L. Pontchartrain Dr.	3	34	2		39	12.0	.36	.12
Pearl R., La.-Miss.	3	68	29		100	12.3	.48	.10
Tombigbee R., Ala.	2	23	10	1	36	12.3	.94	.52
Total Eastern	35	249	57	1	337	12.1	.42	.05

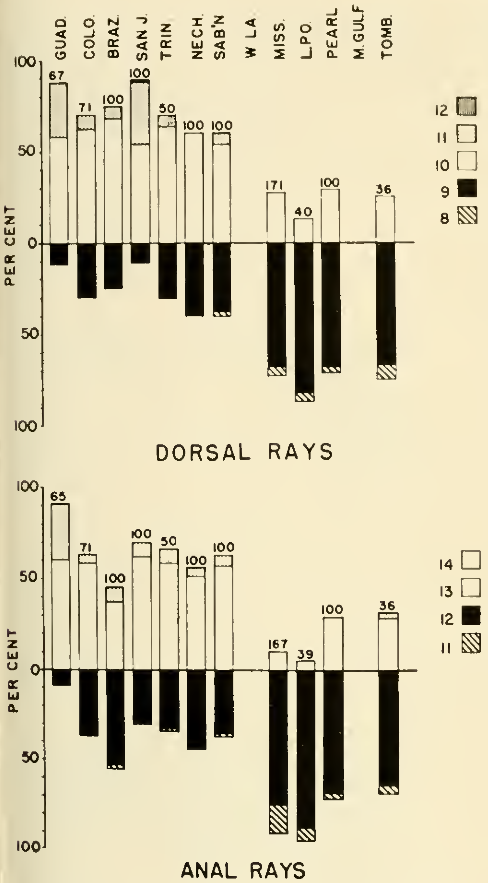

Figure 2. Distribution of dorsal and anal ray numbers in *Fundulus notatus*. Each bar represents 100% of the sample and is aligned on the break between 9 and 10 (dorsal rays) and 12 and 13 (anal rays), the most common numbers. Numbers above each bar are total sample size. The samples are arranged in order from west to east. These data are tabulated in tables 1 and 2.

scale number is 32 to 37 (Table 4); Braasch and Smith (1965, Fig. 2), however, reported a variation of 30 to 38 in their samples from the upper Mississippi Valley. Their method of counting gave counts 1 scale greater than the method I used. Most samples are modal at 34 or 35, but the Colorado River sample is modal at 36 and the Mississippi River sample is modal at 33. There is no broad pattern of geographic variation, such as those seen in ray number for the dorsal, anal and caudal fins.

Specimens from the upper Mississippi Valley and Great Lakes drainage tend to have broader heads, shorter snouts, and deeper caudal peduncles in comparison to specimens from other populations, but these characters are highly variable in all samples and do not seem to warrant the recognition of northern and southern groups. The Mississippi Valley and Great Lakes drainage populations seem to be similar throughout on the basis of dorsal, anal, and caudal ray numbers as well as lateral scale number.

In general, *F. notatus* may be divided into an eastern group characterized by a high percentage of low dorsal, anal and caudal ray numbers and a western group characterized by a high percentage of high numbers of dorsal, caudal and anal rays. No such pattern is seen in lateral scale number. The Mississippi River populations are characterized by low dorsal and anal ray numbers, high caudal ray number and low lateral scale lateral scale number.

Fundulus olivaceus

Differences in dorsal ray number (Table 5, Fig. 3) may be used to divide *F. olivaceus* into three population groups. The most common dorsal ray number in samples from

TABLE 3
Number of Caudal Rays in Fundulus notatus

	Number of Caudal Rays										
Populations	11	12	13	14	15	16	17	N	M	S.D.	2S.E.
Guadalupe R., Tex.		2	5	17	5	1		30	14.0	.88	.32
Colorado R., Tex.		2	6	30	14	3		55	14.1	.89	.24
Brazos R., Tex.		4	5	27	13	1	1	51	14.1	.99	.28
San Jacinto R., Tex.	1	13	25	21	8			68	13.3	1.00	.25
Trinity R., Tex.		2	4	14	14	1		35	14.2	.91	.31
Neches R., Tex.		2	9	13	6			30	13.8	.86	.34
Sabine R., Tex.-La.		2	9	21	10			43	13.9	.80	.24
Mississippi R.		2	6	21	20	1		50	14.2	.85	.27
L. Pontchartrain Dr.			10	6				16	13.4	.50	.25
Pearl R., La.-Miss.	1	13	28	7	2			51	12.9	.81	.23
Tombigbee R., Ala.		2	9	7	2			20	13.4	.89	.40

TABLE 4
Number of Lateral Scales in Fundulus notatus

Populations	Number of Lateral Scales 32	33	34	35	36	37	N	M	S.D.	2S.E.
Guadalupe R., Tex.		2	24	11	1		38	34.3	.57	.19
Colorado R., Tex.		3	7	17	23	2	52	35.3	.95	.26
Brazos R., Tex.		2	26	14	7	1	50	34.6	.61	.20
San Jacinto R., Tex.		1	13	21	1		36	34.6	.61	.20
Trinity R., Tex.	1	6	19	8			34	34.0	.70	.24
Neches R., Tex.	1	6	20	12			39	34.1	.76	.25
Sabine R., Tex.-La.		5	11	21	6	2	45	34.8	.99	.30
Mississippi R.	6	24	9	3	1		53	33.0	.94	.26
L. Pontchartrain Dr.			4	14			18	34.8	.44	.21
Pearl R., La.-Miss.		18	32	1			51	33.7	.47	.23
Tombigbee R., Ala.		9	13	3			25	33.8	.44	.18

the Navasota River population is 9. Samples from the Trinity, Neches, Sabine, western Louisiana drainage, Mississippi, Lake Pontchartrain drainage, Pearl, and Mississippi Gulf drainage are modal at 10 dorsal rays. There is clinal intergradation in this character from the Mississippi Gulf drainage population (mode 10) into the populations at the eastern limits of the range, where the mode is 9. Observed range of variation is from 8 to 11.

A line drawn between 9 and 10 dorsal rays separates 64% of 100 Navasota River specimens from 77% of 970 Trinity to Mississippi Gulf drainage specimens; average separation 70.5%; coefficient of divergence 0.37. A line drawn between 9 and 10 separates 77% of 970 Trinity to Mississippi Gulf specimens from 93% of 110 Choctawhatchee Bay specimens; average separation 85%; coefficient of divergence 0.89. The mean dorsal ray number of the Apalachicola population (9.2) is significantly higher than that of the Choctawhatchee Bay populations (8.9); Chi square (2 x 3, 2 degrees of freedom) 11.2, P less than 0.01.

A sample of 100 specimens from the southern Mississippi Valley is significantly different from a sample of 70 specimens from the northern Mississippi Valley populations; Chi square (2 x 4, 3 degrees of freedom) 11.7, P less than 0.01. A sample of 100 specimens from middle Mississippi tributaries (White River, Tennessee River) is intermediate between the northern and southern Mississippi Valley samples. These data indicate a clinal intergradation from low average dorsal ray number (9.6) in the southern Mississippi Valley to a higher average number (9.9) near the northern limits of the range in the upper Mississippi Valley. However Braasch and Smith (1965, Fig. 2)

TABLE 5
Number of Dorsal Rays in Fundulus olivaceus

Populations	Number of Dorsal Rays 8	9	10	11	N	M	S.D.	2S.E.
Navasota R., Tex.	2	62	35	1	100	9.4	.54	.11
Trinity R., Tex.		13	68	19	100	10.1	.56	.11
Neches R., Tex.	1	20	70	9	100	9.9	.56	.11
Sabine R., Tex.-La.		22	70	8	100	9.9	.53	.11
Western La. Dr.		20	73	7	100	9.9	.50	.11
S. Mississippi R.	1	40	53	6	100	9.6	.61	.12
M. Mississippi R.		22	73	5	100	9.8	.45	.09
N. Mississippi R.		11	56	3	70	9.9	.44	.11
L. Pontchartrain Dr.		19	77	4	100	9.8	.56	.11
Pearl R., La.-Miss.	1	30	64	5	100	9.7	.56	.11
Miss. Gulf Dr.		25	73	2	100	9.8	.47	.09
Total Trin. R.-Miss. G.	3	222	677	68	970	9.8	.53	.03
Alabama R.	4	132	103	2	241	9.4	.55	.07
Pensacola Bay Dr.		63	37		100	9.4	.49	.10
Choctawhatchee Bay Dr.	18	84	8		110	8.9	.48	.10
Apalachicola R.	5	69	26		100	9.2	.52	.10

give a mean dorsal ray number between 9.6 and 9.7 for upper Mississippi Valley *F. olivaceus.* This suggests that Mississippi Valley populations are reasonably homogenous for this character. Further study is needed to clarify this point.

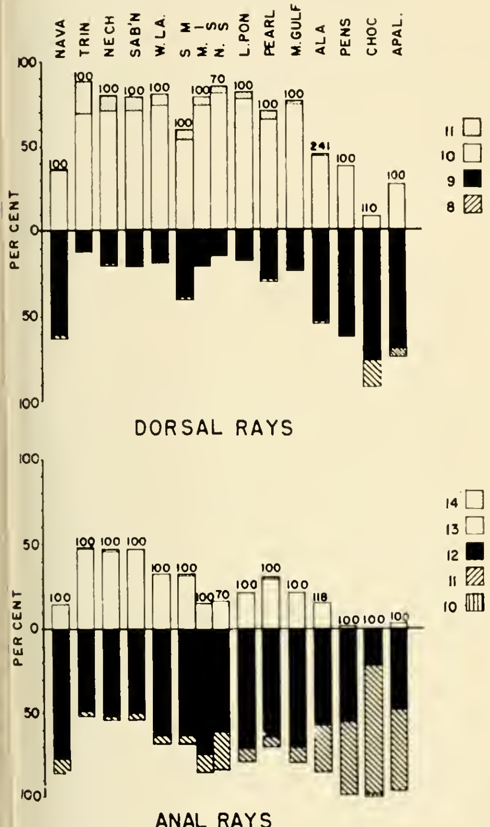

Figure 3. Distribution of dorsal and anal ray numbers in *Fundulus olivaceus.* Drawn as in fig. 2. These data are tabulated in tables 5 and 6.

Samples from eastern tributaries to the Mississippi were compared with those from the western tributaries. No significant differences were found.

The total observed range in variation in anal ray number is from 10 to 14 (Table 6, Fig. 3). Number of anal rays shows a pattern of variation similar to that seen in dorsal ray number, but the samples from the Mississippi Valley do not differ significantly. The sample from the Navasota River is modal at 12 as is the sample from the Trinity River, but a line drawn between 12 and 13 anal rays separates 86% of 100 Navasota specimens from 48% of 100 Trinity specimens; average separation 67%; coefficient of divergence 0.48.

Average number of anal rays drops from a high of 12.5 in the Trinity and Neches samples to a low of 11.2 in the Choctawhatchee Bay sample. This trend does not continue to the east. Although the average number (11.6) of the Apalachicola population is low, it is higher than the Choctawhatchee Bay average. The difference between the Choctawhatchee Bay and Apalachicola populations are probably real; Chi square (2 x 4, 3 d. f.) 13.0, P less than 0.01.

Two different rates of clinal intergradation are involved: (1) a change of average number of anal rays from 12.5 in the Trinity and Neches rivers into an average number of 12.1 for samples from the Mississippi Gulf drainage. This change in average number is the result of an eastward increase in percent of specimens with 12 anal rays and

Table 6
Number of Anal Rays in Fundulus olivaceus

	Number of Anal Rays								
Populations	10	11	12	13	14	N	M	S.D.	2S.E.
Navasota R., Tex.		9	77	14		100	12.0	.48	.10
Trinity R., Tex.		3	49	47	1	100	12.5	.56	.11
Neches R., Tex.		2	52	45	1	100	12.5	.56	.11
Sabine R., Tex.-La.		4	50	46		100	12.4	.59	.12
Western La. Dr.		5	63	32		100	12.3	.55	.11
Mississippi R.		32	180	57	1	270	12.1	.58	.07
L. Pontchartrain Dr.		8	71	21		100	12.1	.51	.10
Pearl R., La.-Miss.		6	64	29	1	100	12.2	.57	.11
Miss. Gulf Dr.		9	70	21		100	12.1	.53	.11
Alabama R., Ala.		33	67	18		118	11.9	.65	.12
Pensacola Bay Dr.		44	55	1		100	11.5	.52	.10
Choctawhatchee Bay Dr.	2	76	22			100	11.2	.45	.09
Apalachicola R.		50	48	2		100	11.5	.54	.11

TABLE 7
Number of Caudal Rays in Fundulus olivaceus

Populations	Number of Caudal Rays 11	12	13	14	15	16	17	N	M	S.D.	2S.E.
Navasota R., Tex.			10	31	17	2		60	14.2	.75	.20
Trinity R., Tex.		4	6	22	28		1	61	14.3	.94	.24
Neches R., Tex.		1	1	10	36	12	1	61	15.0	.65	.16
Sabine R., Tex.-La.			3	18	18	1		40	14.4	.71	.23
Western La. Dr.		1	9	11	29	2		52	14.4	.89	.25
Mississippi R.			11	35	86	9	3	144	14.3	.69	.11
L. Pontchartrain Dr.		1	13	32	38	4		82	14,3	.79	.17
Pearl R., La.-Miss.		4	15	24	27	4	2	76	14.2	1.08	.25
Miss. Gulf Dr.			8	22	28	1		59	14.4	.73	.19
Alabama R., Ala.		2		12	14			28	14.4	1.07	.41
Pensacola Bay Dr.		5	26	2	6			39	13.2	.87	.28
Choctawhatchee Bay Dr.		5	32	11	2			50	13.5	.74	.21
Apalachicola R.	1	1	16	13	11			42	13.8	.96	.30

a decrease in percent of specimens with 13 anal rays. The percentage of fish with 11 anal rays is reasonably constant in all these samples. (2) A reduction of average number of anal rays from 12.1 in the samples from the Mississippi Gulf drainage to an average number of 11.2 in the samples from the Choctawhatchee Bay drainage. This drop is the result of an eastward trend toward an increase in percent of specimens having 11 anal rays and a concurrent decrease in percent of specimens with 13 or 12 anal rays. A line drawn between 11 and 12 anal rays separates 91% of 100 specimens from the Mississippi Gulf drainage from 78% of 100 specimens from Choctawhatchee Bay drainage; average separation 84.5%; coefficient of divergence 0.92.

Observed range of variation in caudal ray number is 11 to 17 (Table 7). The Navasota River *F. olivaceus* sample is modal at 14, samples from the Trinity River east to the Alabama River are modal at 15, and samples from drainages east of the Alabama River are modal at 13. Though the modal numbers of the Trinity and Navasota samples are different, the means, 14.3 and 14.2 respectively, are not significantly different. A line drawn between 13 and 14 separates 93% of 28 specimens from the Alabama River from 79% of 39 specimens from the Pensacola Bay sample; average separation 86%; coefficient of divergence 0.64.

Lateral scale number ranges from 32 to 37 (Table 8) and 34 or 35 lateral scales are most common. Distribution of lateral scale number varies considerably from population to population. The Navasota, Trinity, Neches, and Sabine River samples are similar (mean: 34.4, 34.8, 34.4, 34.8 respectively). With the exception of the Neches sample, bimodal at 34 and 35, these samples are

Table 8
Number of Lateral Scales in Fundulus olivaceus

Populations	Number of Lateral Scales 32	33	34	35	36	37	N	M	S.D.	2S.E.
Navasota R., Tex.	1	6	19	22	2		50	34.4	.75	.21
Trinity R., Tex.		2	15	27	8		52	34.8	.75	.21
Neches R., Tex.		2	15	15			32	34.4	.56	.20
Sabine R., Tex.-La.		5	11	21	6	2	45	34.8	.98	.29
Western La. Dr.	2	14	19	8	2		45	33.9	.92	.28
Mississippi R.	6	37	107	41	3	1	194	34.0	.78	.11
L. Pontchartrain Dr.	2	15	26	28	3		74	34.2	.91	.21
Pearl R., La.-Miss.		5	32	29	7		73	34.5	.78	.18
Miss. Gulf Dr.	1	17	31	11	4		64	34.0	.78	.20
Alabama R., Ala.		12	21	2			35	33.7	.53	.18
Pensacola Bay Dr.		2	15	23	9		49	34.8	.79	.23
Choctawhatchee Bay Dr.			4	37	11		52	35.1	.55	.15
Apalachicola R.			2	17	30	3	52	35.7	.66	.18

modal at 35. The western Louisiana drainage sample is modal at 34 as is the Mississippi River sample. The Lake Pontchartrain drainage sample is almost bimodal at 34 and 35 (26 and 28 specimens respectively). These 3 samples have significantly lower means (33.9, 34.0 and 34.2 respectively) than the 4 western samples. There is a clinal decrease in average number of lateral scales from the Pearl River (34.5) east to the Alabama River (33.7) and a clinal increase in average lateral scale number from the Alabama River to a high of 35.7 for the Apalachicola River sample.

Brown (1956) contrasted Mississippi Valley specimens with specimens from the Gulf Coast. The former were said to have deeper and wider bodies and deeper caudal peduncles than the latter. He did not examine specimens from the lower Mississippi Valley. Southern Mississippi Valley fish resemble Gulf Coast drainage specimens and do not have the deep, wide body and deep caudal peduncle characteristic of northern specimens.

The following generalizations may be made. There is some correlation in variation in fin ray number in the unpaired fins. With the exception of the Navasota River population, western populations have a higher percentage of high fin ray numbers than eastern populations. The average dorsal ray number is nearly uniform from the Trinity River population east to the Mississippi Gulf drainage and drops off clinally to the east. Intergradation from high average anal ray number in the west to low average anal ray number in the east involves a low rate of clinal intergradation from the Trinity population to the Mississippi Gulf drainage population followed by a rapid rate of clinal intergradation eastward. Western populations (Navasota included) have high average caudal ray number and populations east of the Alabama River have low average caudal ray number. The Alabama River sample has the lowest average lateral scale number and the Apalachicola River sample has the highest, a reverse trend to those shown by fin ray numbers.

Ecology

The published information available on the ecology and distribution of *F. olivaceus* and *F. notatus* is somewhat confusing, in part because each statement has been based on a study of either or both species in a limited area. In a study of the fishes of Tennessee, Kuhne (1939) stated, "Two subspecies occur in Tennessee, the northern (and more upland) *F. n. notatus* and the southern lowland *F. n. olivaceus* (Putnam). The latter has a flatter head and the body is marked by strong blackish spots." Moore and Paden (1950) noted that the two species sometimes occurred together in the Illinois River. Knapp (1953), in regard to *F. olivaceus* in Texas, stated: "Where its range overlaps with *F. notatus* the two are usually ecologically separated, *F. olivaceus* being typically a quiet water form. Near the coastal plain this species inhabits swifter water." He also stated, "In Texas *F. notatus* is to be expected in headwaters and fast streams." Braasch and Smith (1965) stated that *F. notatus* is more likely to be found in still water and *F. olivaceus* in fast water in the upper Mississippi Valley, and that the two seldom occur together in that area.

My examination of the hundreds of collections of the 2 species catalogued at Tulane University, University of Michigan Museum of Zoology and the Texas Natural History Collection at the University of Texas shows that in fact the two species are seldom collected at the same locality at the same time. When collections lumping specimens from several different habitats are excluded, it is clear that *F. notatus* and *F. olivaceus* are rarely syntopic (Rivas, 1964: 43).

Braasch and Smith (1965) reported mixed collections from the same general area taken 64 years apart, but my observations of two areas where syntopy has occurred indicate that syntopic associations at a particular locality are unstable and transient. One area is in the mainstream of the Amite River in Louisiana and the other is in a small tributary of the Navasota River in Texas. The first area was visited 4 times and the second 3 times (Table 9). Where numbers are given, all *Fundulus* seen were collected and the sample is probably an accurate reflection of the *Fundulus* population at that time. Where catalogue numbers are not given, specimens were retained alive and mixed with specimens from other localities for use in another study.

The differences in ecological preferences that are responsible for this separation of the two species are not clear at present. *Fundulus olivaceus* is abundant in the

Table 9
Variation in relative abundance of Fundulus notatus and Fundulus olivaceus in 2 areas of occasional syntopy

Drainage and Date	*F. notatus*	*F. olivaceus*	Water Level
Amite River			
28 Nov. 1963	abundant	0	low
14 May 1964	— about equal numbers —		high
3 July 1964	2	0	high, rising
25 Oct. 1964	19 (TU 35697)	22 (TU 35691)	low
Navasota River			
30 March 1963	7	5	low
19 June 1964	abundant	0	low
19 Nov. 1964	1 (TU 37114)	7 (TU 37116)	high, dropping

"blackwater" streams along the Gulf Coast and *F. notatus* is rare in this type of stream, but neither species seems to show any preference for a particular type of habitat over a large area. Knapp (1953) has characterized *F. notatus* as a headwater form, but in the Guadalupe and Colorado rivers it is excluded from the headwaters. In the Pearl River drainage *F. notatus* is generally excluded from the small tributaries and is most abundant in the main channel of the river, but all the records I have from the Tombigbee River drainage are from small tributaries. Clark Hubbs (1957) pointed out that *F. olivaceus* in Texas is an eastern element of the Texas fauna whose western limits correspond primarily with the western limits of the Mixed Pine Oak Region. Exceptions to this statement are the records from the Navasota River in Brazos and Grimes counties (see *Material Examined*). These records are slightly west of the Mixed Pine Oak Region but from a drainage with previously recognized eastern faunal affinities (Knapp, 1953).

Where only one of the two species is present, it is able to occupy suitable habitats along the margins of lakes, rivers, ponds, and streams. Either species may inhabit large or small bodies of water and in the southern part of their ranges either *F. notatus* or *F. olivaceus* may be found at the edges of still or rapidly flowing water. Ordinarily neither species is found in riffle areas, although either may occur in quieter areas at the margins of riffles. In the area of sympatry the suitable habitats are divided between the two species and contact between the two is rare. With the exception of the general exclusion of *F. notatus* from "blackwater" areas, it appears that the macrohabitat occupied by *F. notatus* does not exhibit any consistent difference from the macrohabitat occupied by *F. olivaceus*.

In general, *F. notatus* is abundant in Texas and in the western and northern Mississippi Valley, and relatively rare in the southern Mississippi Valley and east of the Mississippi. *F. olivaceus* may be less abundant than *F. notatus* in Texas, but is by far the more common in the south, and is about as abundant as *F. notatus* in the middle Mississippi Valley. The abundance of *F. olivaceus* in the southern tier of states from Louisiana to western Florida is to some extent correlated with the occurrence of "blackwater" streams and swamp lakes in that area, but *F. olivaceus* is also abundant in other types of habitat.

Experimental Hybridization

In reference to *F. notatus* and *F. olivaceus* Bailey, Winn, and Smith (1954) suggested: "It is possible that a more thorough study may prove them to be the genetic variants of a single species." Many individuals of both species have an intermediate appearance suggestive of either hybrid origin or introgression. The spotted individuals of *F. notatus* and the individuals of *F. olivaceus* which have very few, or very small spots could be interpreted as hybrids if opposing evidence were not available.

The most fruitful approach to this type of problem seemed to be the production of known hybrids for comparison with suspected natural hybrids. These experiments were intended to help determine the nature of the isolating mechanisms (if any) existing between the two forms in question. At the beginning of this study I believed *F. notatus* and *F. olivaceus* to be conspecific but the results of these experiments render this position untenable.

Materials and Methods

In February, March and April, 1964, 3 sets of hybridization experiments were performed: (1) a female *F. olivaceus*, from the Bogue Falaya River at the Delta Regional Primate Center near Covington, Louisiana, was crossed with a male *F. notatus* from the Colorado River at Austin, Texas; (2) a female *F. notatus*, from Hildebrandt Bayou near Beaumont, Texas, was crossed with a male *F. olivaceus* collected with the *F. olivaceus* female used in experiment one; and (3) a female *F. olivaceus* from a tributary to the Cahaba River in Alabama was crossed with a male *F. notatus* from Hildebrandt Bayou. The choice of fishes used in these experiments was governed by the necessity of using individuals unquestionably belonging to one species or the other.

Each cross was carried out in a 5 gallon Metaframe brand aquarium filled with aged New Orleans tap water. Some of the water used in changing or adding water came from a temporary rainwater pond near Lake Pontchartrain. This was necessary because New Orleans tap water was lethal to these fishes during late January and February, 1964. A nylon yarn mop weighted with a lead sinker was placed in a bottom inside corner of the aquarium to provide a spawning site. Both green and white mops were used with good results. Filtration and aeration were provided by a standard small inside box filter in each aquarium. These filters were charged with glass wool, charcoal and fine gravel. The fish were usually fed twice a day and at least once a day with Tetra-Min brand flake dry food, frozen raw beef liver, or frozen adult Artemia. Live mosquito larvae and cladocerans were fed when available. Each aquarium was illuminated with a 15 watt incandescent bulb in addition to daylight from an east window. A timer turned the lights on at 0530 and off at 2030 to simulate a 15 hour day.

The fishes spawned readily, generally in the late afternoon. Each morning the mops were removed from the aquarium, squeezed partially dry and inspected visually while running the strands of the mop through the fingers. Eggs were removed with the fingers and placed in clean aged aquarium water in a plastic container (11 cm square and 2 cm deep). Acriflavine dye was added to the incubation water to serve as a fungicide. Dead eggs were discarded and newly hatched fry (F-1) were transferred to small rearing tanks and fed twice daily on live newly hatched *Artemia* nauplii. Dead *Artemia* and detritus were removed from the rearing tanks periodically and the water was partially replaced with aged aquarium water.

When the fry began to show signs of crowding (differential and decreased growth rates) they were transferred to 10 or 20 gallon Metaframe brand aquaria and had scraped frozen beef liver, frozen adult *Artemia* and Tetra-Min added to their diet. The fry were distributed at the rate of about one fish per gallon of water.

The F-1's were transferred to outdoor concrete pools. The fry from experiment one were transferred on 28 May and the fry from experiments two and three on 22 July 1964. They were from 4 to 5 months old and averaged about 30 mm total length when transferred. The offspring from experiment one were removed from the pools on 20 September 1964, 21 were preserved on 21 September, and 5 pairs were retained for further experiments. The fish from experiment two were removed and preserved on 7 December 1964, and the fish from experiment three were transferred to a laboratory aquarium on 28 September 1964 and preserved 13 December 1964.

While in the pools, fish were fed daily with Tetra-Min or frozen adult *Artemia* to supplement natural foods present in the pools.

Results

Experiment one resulted in 34 F-1 offspring; 2 died before reaching maturity, 1 was lost in transfer, 2 have been kept alive and 29 preserved for study. A comparison of the F-1 hybrids from experiment one with samples from the parent populations is given in Table 10. These hybrids are intermediate and distinctive in general appearance (Fig. 4). F-1 hybrids from experiment one were able to produce F-2 hybrids and backcrosses to both parent species with no sign of reduced sex drive or fertility. These experiments will be discussed further in a later paper.

Though the hybrids are intermediate in general appearance, analysis of individual characters (Table 10) shows that they are intermediate only in pattern of the dorsum and in number and shade of dorsolateral

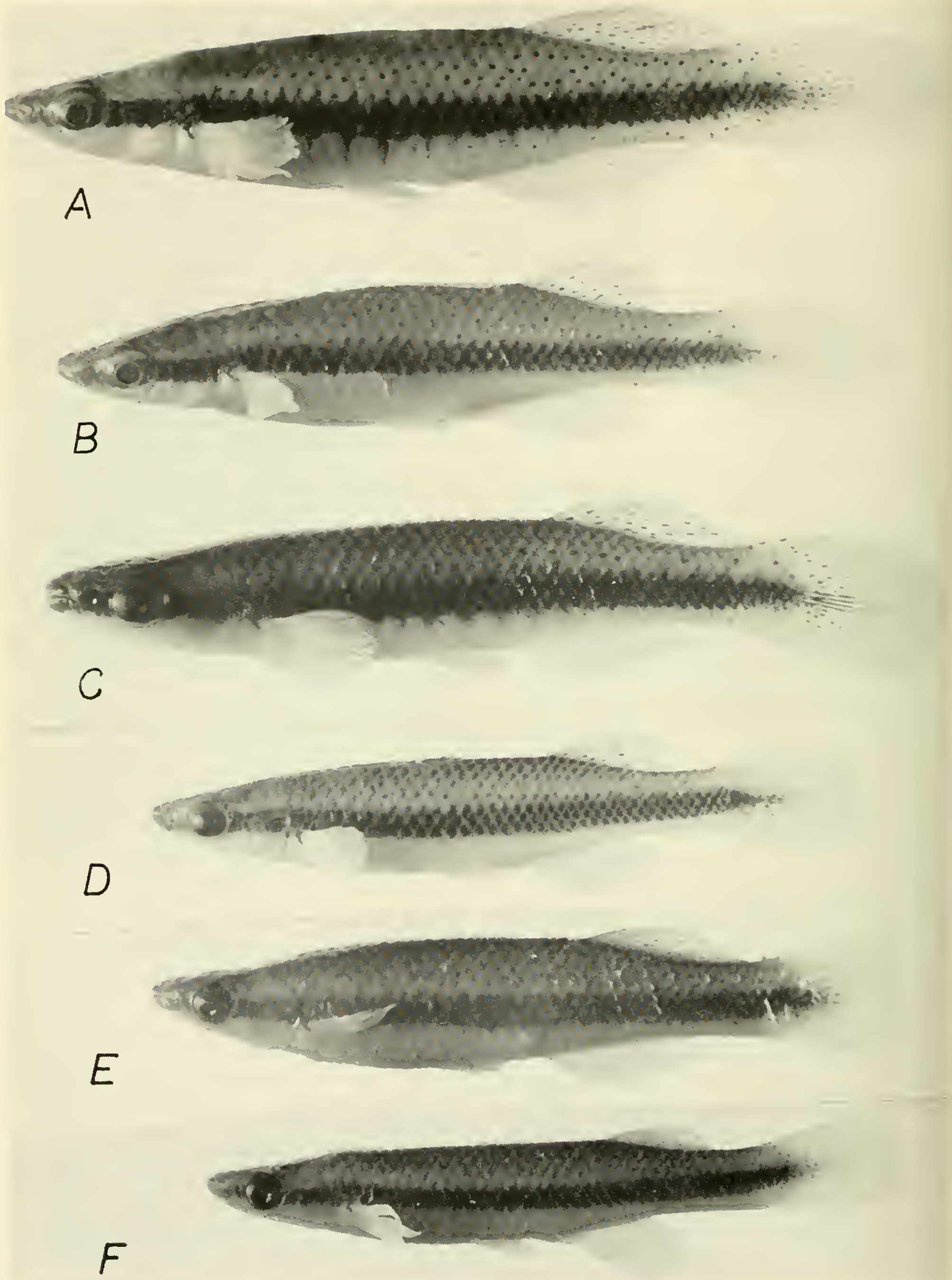

Figure 4. A. male *Fundulus olivaceus* (SL 54.9 mm) and B. a female *F. olivaceus* (SL 47.8 mm), from the Bogue Falaya River. C. a male, and D. a female F-1 hybrid from experiment one (SL 54.6 mm and 45.0 mm respectively). E. a male, and F. a female *Fundulus notatus* from the Colorado River (SL 49.4 mm and 42.8 mm respectively). Photographs by Forrest Jack Hurley.

spots. Other characters resemble one parent species, both parent species, or neither. The parent population samples and the F-1 hybrids do not show significant differences in dorsal ray number, caudal ray number, body depth, caudal peduncle depth of both males and females, or male dorsal fin length (see Fig. 5). The F-1 hybrids resemble Colorado River *F. notatus* in anal ray number and lateral scale number, but resemble Bogue

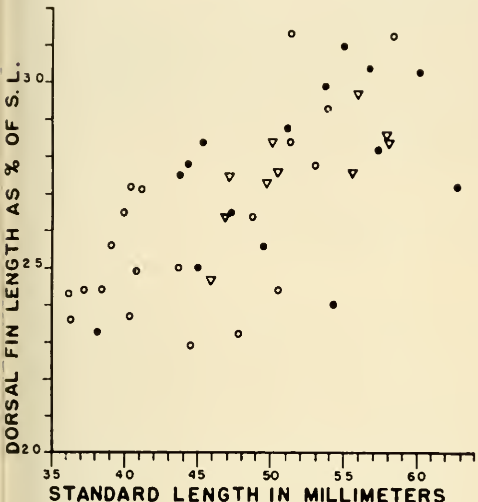

Figure 5. Comparison of dorsal fin length in *Fundulus notatus* (open circles), *Fundulus olivaceus* (solid circles), and F-1 hybrid (triangles) males. See Table 10 for comparison of females.

Falaya River *F. olivaceus* in female dorsal fin length, female anal fin spotting, and number of lateral band extensions in males. Hybrid males have more heavily spotted anal fins than males from either parent population. The 29 F-1 hybrids are significantly broader-headed than samples from either parent population. This character seems to be intrinsic in the hybrids and not an artifact caused by laboratory conditions. Specimens of both parent species raised under similar conditions have not shown a similar effect. Of 16 characters studied, the F-1 hybrids from experiment one are intermediate in 3, extreme in 2, similar to both parent populations in 6, similar to *F. notatus* in 2 and similar to *F. olivaceus* in 3.

Discussion

Fundulus olivaceus generally has discrete, regular black spots on the dorsolateral surface of the body. *Fundulus notatus* may be immaculate or may have dusky spots or blotches. Though other characters may be of use in separating these species in a given area, this is the only one of the many proposed by Hubbs (in Moore and Paden, 1950: 88) and by Brown (1956: 131) which will serve to separate the two species everywhere. Head shape, snout length, body depth, caudal peduncle depth, shape of male dorsal and anal fins, shape and number of spots in the unpaired fins, color of the dorsum, predorsal stripe, and male lateral band extensions may be different for the two species at a given locality, but these characters are not conservative and their use as key characters confuses rather than clarifies.

The spots characteristic of *F. olivaceus* are generally more numerous in males than in females and large males tend to have more spots than small males. The spots vary from less than 0.2 mm to over 1.0 mm in diameter. They are usually round but may be dash-shaped or slightly irregular. The most striking development of these spots was seen on large males from the Pensacola Bay drainage samples. An attempt to use numbers of spots to define subpopulations of *F. olivaceus* failed because variation in most samples is too great.

This spotting characteristic, though the most reliable for use in separating *F. olivaceus* and *F. notatus*, breaks down in rare instances. A few collections from the southern states, from Texas to Florida, have included individuals of *F. olivaceus* with few dorsolateral spots. Braasch and Smith (1965) reported similar collections from the upper Mississippi Valley. Males in these collections have as few as 15 spots. Females usually have between 5 and 15 spots and some females have 4, 3, 2, 1, or none. These rare immaculate females may be morphologically indistinguishable from *F. notatus* females, and are identified as extreme variants of *F. olivaceus* rather than *F. notatus* because: (1) they sometimes occur outside the range (as presently known) of *F. notatus*, (2) they are not associated with immaculate males, and (3) they are associated with *F. olivaceus* which have a low number of dorsolateral spots. One of these females taken alone would certainly be identified as *F. notatus* and records of *F. notatus* based on single females should be critically examined.

F. notatus may have an immaculate dorsum or may have dusky spots or blotches on

TABLE 10

Comparison of Parental Stocks of Fundulus notatus and Fundulus olivaceus with Artificially Reared F-1 Hybrids

	Colorado *F. notatus*				F-1 Hybrids				Bogue Falaya *F. olivaceus*				Hybrids
Character	N	x̄	S.D.	2S.E.	N	x̄	S.D.	2S.E.	N	x̄	S.D.	2S.E.	Resemble
Dorsal Rays	71	9.8	.59	.14	29	9.9	.49	.19	100	9.8	.56	.11	Both Parents
Anal Rays	71	12.7	.53	.12	29	12.7	.49	.19	100	12.1	.51	.10	*F. notatus*
Caudal Rays	55	14.1	.89	.24	29	14.4	.61	.23	82	14.3	.79	.17	Both Parents
Lateral Scales	52	35.3	.95	.26	29	35.0	.87	.32	74	34.2	.91	.21	*F. notatus*
HW/SL as %	33	13.3	.33	.11	29	15.1	.68	.25	30	14.3	.43	.16	Neither
BD/SL as %	20	18.4	1.34	.60	29	19.1	.92	.29	29	18.2	1.34	.50	Both Parents
CPD/SL ♂	16	11.5	.80	.40	10	10.9	.30	.19	16	11.2	.58	.28	Both Parents
CPD/SL ♀	15	11.0	.37	.20	19	10.7	.49	.22	14	10.4	.59	.32	Both Parents
DL/SL ♂	No significant difference between samples, fig. 5.												Both Parents
DL/SL ♀	14	20.5	1.32	.71	19	22.0	.69	.31	12	22.2	.48	.28	*F. olivaceus*
Anal Fin Spots ♂	very few or none				many				few				Neither
Anal Fin Spots ♀	none				few				few				*F. olivaceus*
Stripe Extensions	5 or less				10 to 15				5 to 15				*F. olivaceus*
Dorsum Pattern	cross-hatched				intermediate				uniform, dusky				Intermediate
Dorsum Spots, ♂ and ♀	♂ - none to few very light, ♀ - none				♂ - intermediate ♀ - few, dusky				♂ - many dark ♀ - few to many dark				Intermediate

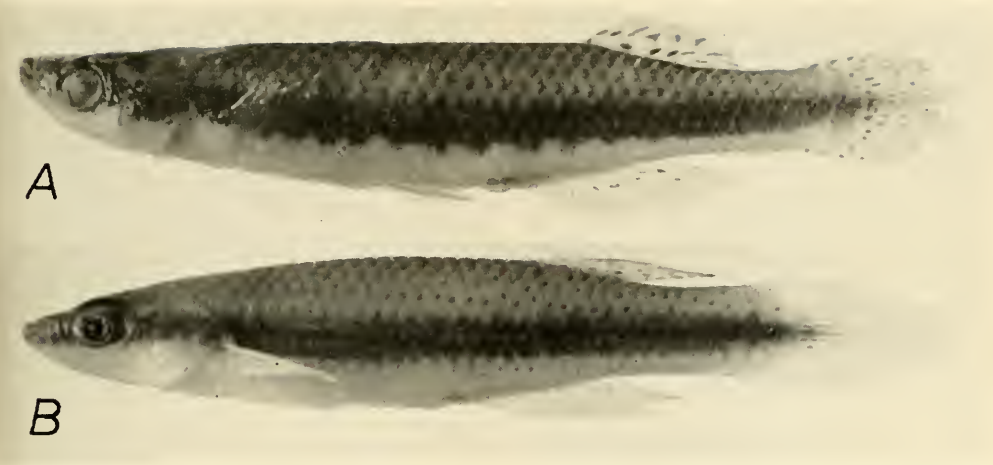

Figure 6. Two natural hybrids, both males. UMMZ 161253 (SL 39.5 mm, above) was the only *Fundulus* in the collection. TU 37120 (SL 31.2 mm, below)) was taken with both *Fundulus notatus* and *F. olivaceus*. Photographs by Forrest Jack Hurley.

the dorsum. Females are often more heavily spotted than males. Usually individuals with spots or blotches also have strong development of the predorsal line, which may be broken up into dashes or spots which complement the rest of the dorsal pattern. In contrast, *F. olivaceus* seldom has strong development of the predorsal stripe.

As has been pointed out by Clark Hubbs (1963) and others, closely related species generally are easier to cross than more distantly related ones. The ease with which these two species hybridize in the laboratory supports the idea that their close morphological similarity reflects close phylogenetic relationship. As the hybrids are intermediate in several characters and resemble one parent or the other in other characters, it is clear that the hybrids are not of gynogenetic origin nor are they "false hybrids" in which the paternal chromatin has no effect. The sex ratio of the hybrids (experiment one), 10 males to 21 females, is not significantly different from a theoretical 50:50 sex ratio of 15 males and 15 females. Hubbs and Drewry (1959) have discussed the pitfalls encountered in interpreting results of hybrid experiments.

That *F. notatus* and *F. olivaceus* are interfertile in the laboratory is not necessarily evidence that they are conspecific, but is evidence that isolating mechanisms (if any) which separate them in nature are ecological or behavioral rather than cytological.

My examination of some 12,000 specimens of *F. notatus* and *F. olivaceus* has uncovered two natural hybrids, both males (TU 37120 and UMMZ 161253, Fig. 6). The extreme rarity of *F. notatus* x *F. olivaceus* hybrids in nature is evidence of the effectiveness of isolating mechanisms. Paucity of natural hybrids may be due to hybrid non-survival but several lines of evidence point against this. Moore and Paden (1950) collected breeding pairs of *F. notatus* and *F. olivaceus* from an area of syntopy and found no heterospecific pairs. This observation indicates that when the ecological barriers which ordinarily separate the two species break down, isolation is maintained by the preference of the fish for mates of their own species. Heterospecific matings are easily obtained in the laboratory but seem to occur rarely in nature. The robust nature of the laboratory hybrids suggests that natural hybrids are probably able to compete with either or both parent species.

The paucity of natural F-1 hybrids suggests that introgressive hybridization is not at present an important contributor to variation in either species. Thus *F. olivaceus* with small spots, or few spots, or both, are probably not of hybrid origin. The same is true of heavily spotted or blotched *F. notatus*.

The two species are similar in range of variation in dorsal. anal and caudal ray numbers and in lateral scale number. Although there is considerable intraspecific variation in average number of caudal rays, populations of *F. notatus* are not significantly different from populations of *F. olivaceus* from the same area, with the exception of the populations from the Pearl River (compare Tables 3 and 6). Populations of the two species have significantly different distributions of lateral scale numbers in the Trinity, Mississippi, and Pearl rivers. Lateral scale number does not show a coordinated pattern of geographic variation in the 2 species. It is thus probable that local interspecific differences in this character are fortuitous and of little importance.

With the exception of the Navasota River population of *F. olivaceus,* western populations of *F. notatus* and *F. olivaceus* tend to have high dorsal ray number, and this character does not serve to separate them. The trend to high dorsal ray number extends to the Mississippi Gulf drainage in *F. olivaceus.* High dorsal ray number "western group" *F. olivaceus* and low dorsal ray number "eastern group" *F. notatus* show statistically significant differences in the Mississippi River (see also Braasch and Smith, 1965), Lake Pontchartrain drainage, and Pearl River. In the Alabama system populations of the two forms are not distinguishable on this character because *F. olivaceus* dorsal ray numbers are beginning to drop clinally toward the east.

With the exception of the Navasota River *F. olivaceus* both species tend to have high anal ray numbers in the west and low anal ray numbers in the east. Although there seems to be a slight difference in pattern of variation between the two species, this character serves only to separate the Navasota River *F. olivaceus* population from the Brazos River *F. notatus* population.

Fundulus notatus and *F. olivaceus* are two closely related species which are sympatric and occasionally syntopic over a large area. Both species have completely allopatric populations, *F. notatus* in the west and *F. olivaceus* in the east (Fig. 1). In this kind of distribution the phenomenon of character displacement is often seen. Brown and Wilson (1956) defined character displacement as follows: "Character displacement is the situation in which, when two species of animals overlap geographically, the differences between them are accentuated in the zone of sympatry and weakened or lost entirely in the parts of their ranges outside this zone." The probable causes were said to be reinforcement of reproductive isolating mechanisms and ecological displacement. Character displacement would thus be the result of a long term process, and would be most pronounced where it was the result of relatively stable contact of populations of two closely related species over a period encompassing a large number of generations. My observations on areas of occasional syntopy do not support the hypothesis that *F. olivaceus* and *F. notatus* populations maintain stable contact over the period of time necessary for character displacement to occur. I suspect that individuals or populations of both species may migrate considerable distances. Individuals collected syntopically were perhaps allotopic the day before and vice versa.

Braasch and Smith (1965) were unable to show statistically significant differences between allopatric and sympatric samples from the upper Mississippi Valley, but they felt that apparent parallel divergence of two independent characters (dorsal ray number and lateral scale number) indicated some interspecific interaction between sympatric populations. My data do not support this conclusion because the patterns of geographic variation in either species seem to be independent of the presence or absence of the other species. I have been unable to see evidence of character displacement, either between syntopic populations and allotopic populations in the same drainage or between populations of one species inside and outside the range of the other. The possibility of character displacement in species recognition ability is presently being studied but results to date are inconclusive.

Material Examined

Complete locality data are given for only those collections which represent range extensions or other significant distributional data. Other collections are listed by drainage, state, county and museum number. Complete data on most collections are given by Thomerson (1965). The following museum abbreviations are used: University of Alabama Ichthyological Collection, UAIC; Florida State University, FSU; Private collection of William Smith-Vanez, WSV; Texas Natural History Collection, University of Texas, TNHC; Tulane University, TU; University of Michigan, Museum of Zoology, UMMZ. In addition to standard abbreviations for states and compass directions, the following are used: Co(s).—County(ies), Par(s).—Parish(es), Cr.—Creek, Dr.—Drainage, Hwy.—Highway, mi.—mile(s), R.—River, and trib.—tributary. In addition to the specimens listed here all specimens of the two species catalogued TNHC and TU (to

May 31, 1965), most of the specimens catalogued by UMMZ, and a few UAIC specimens were examined for verification of identification. Some 12,000 specimens of the two species were examined and counts were made on 933 *F. notatus* and 1621 *F. Olivaceus* from the material listed below. Distribution of these collections is shown in Fig. 1.

Fundulus notatus

Guadalupe Dr.—Texas: Medina Co.; TNHC 1865 (12)—Bexar Co.; TNHC 5336 (43)—Gonzales Co.; TNHC 301 (5)—Caldwell & Guadalupe Cos.; TNHC 86 (13), TNHC 177 (1), TNHC 235 (3), TNHC 330 (3)—Hays Co.; TNHC 3037 (13).

Colorado Dr.—Texas: Bastrop Co.; TNHC 3733 (11), TNHC 3795 (7), TNHC 5266 (16)—Travis Co.; TNHC 225 (4), TNHC 526 (2), TNHC 540 (22), TNHC 1078 (1), TNHC 1098 (2), TNHC 1895 (5), TNHC 2552 (2)—Llano Co.; Honey Cr. on Ray Smith Ranch midway between Llano and Marble Falls, TU uncatalogued (19).

Brazos Dr.—Texas: Grimes Co.; TU 37115 (1)—Bell Co.; TNHC 1021 (2), TNHC 3773 (12), TU 35996 (41)—McLennan Co.; TNHC 3467 (1), TNHC 4165 (4), TU 36002 (17)—Bosque & Hill Cos.; TNHC 4246 (1)—Bosque Co.; TU 2989 (9)—Erath Co.; TNHC 1992 (39)—Eastland Co.; TNHC 993 (20).

San Jacinto Dr.—Texas: Montgomery Co.; TNHC 1118 (42), TNHC 1199 (18)—San Jacinto Co.; TNHC 2377 (36), TNHC 2843 (4).

Trinity Dr.—Texas: Polk Co.; TNHC 5445 (3)—Madison & Walker Cos.; TU 4891 (13)—Ellis Co.; UMMZ 92385 (6), UMMZ 92388 (2)—Dallas Co.; UMMZ 120152 (4)—Tarrant Co.; UMMZ 97503 (7), UMMZ 170048 (1), UMMZ 170060 (5).

Neches Dr.—Texas: Anderson Co.; TU 3479 (6)—Cherokee Co.; TU 17686 (46), TU 17788 (11)—Rusk & Shelby Cos.; TU 3854 (6)— Shelby Co.; TU 3315 (20).

Sabine Dr.—Louisiana: Sabine Par.; TU 33312 (2), TU 33798 (1), TU 33938 (34), TU 34005 (6), TU 34078 (21), TU 35461 (11), TU 35567 (2)—De Soto Par.; TU 35544 (11)—*Texas:* Panola Co.; TNHC 6063 (10)—Harrison & Panola Cos.; TNHC 3190 (6).

Mississippi and Great Lakes Dr.—Louisiana: St. Charles Par.; Ponds in Bonnet Carre Spillway S of Hwy. 61, 20 mi. W New Orleans, TU 387 (5)—La Salle Par.; UMMZ 181940 (12)—*Oklahoma:* Nowata Co.; TU 15532 (39)—*Tennessee:* Lake Co.; UMMZ 103367 (2)— Davidson Co.; UMMZ 88106 (6), UMMZ 104498 (3), UMMZ 177568 (7)—Marshall Co.; UMMZ 121387 (13)—Jackson Co.; UMMZ 125113 (10)—*Michigan:* Washtenaw Co.; (Huron R.) UMMZ 72255 (19).

Lake Pontchartrain Dr.—Louisiana: East Baton Rouge Par.; Amite R. at Hwy. 190, 7 mi. E of intersection Hwy. 190 & Hwy. 61, TU 35697 (19)—Tangipahoa Par.; Tangipahoa R. at Hwy. 35, 1.5 mi. E Amite, UMMZ 182344 (1)—Tangipahoa Par. (?); Trib. to Natalbany R., UMMZ 182345 (20).

Pearl Dr.—Louisiana: Washington Par.; TU 37118 (54)—*Mississippi:* Marion Co.; TU 37119 (36)—Lawrence Co.; TU 26408 (29)—Hinds & Rankin Cos.; UMMZ 170728 (3).

Tombigbee Dr.—Alabama: Sumter Co.; TU 7497 (3), UMMZ 163741 (12)—*Mississippi:* Kemper Co.; UMMZ 157774 (3)—Noxubee Co.; TU 3755 (11), UMMZ 113885 (6).

Fundulus olivaceus

Navasota Dr.—Texas: Grimes Co.; Trib. to Navasota R. 5.1 mi. NE Navasota at Hwy. 190, TU 37116 (7)—Brazos Co.; Wickson Cr. UMMZ 129747 (3), Peachtree Cr. borrow pits 12 mi. S College Station, UMMZ 129764 (1), UMMZ 129951 (16), and UMMZ 138235 (5); Peach Cr. at Hwy. 6, UMMZ 129807 (80), Sand Cr. trib. to Navasota R. E of Kurten, UMMZ 129916 (3), Navasota R. and borrow pits 4 mi. E Kurten, UMMZ 129935 (7).

Trinity Dr.—Texas: San Jacinto Co.; TNHC 1169 (9)—Polk Co.; TNHC 5446 (29), TNHC 2415 (40), TNHC 6039 (20)—Anderson Co.; TU 3792 (2).

Neches Dr.—Texas: Hardin Co.; TU 21615 (57), TU 21761 (28)—Tyler Co.; TU 21686 (23).

Sabine Dr.—Texas: Newton Co.; TU 14045 (3)—Panola Co.; TU 3496 (10)—*Louisiana:* Vernon Par.; TU 777 (6) TU 5136 (6)—Sabine Par.; TU 793 (7), TU 5075 (21), TU 33764 (18), TU 33824 (13), TU 33941 (2), TU 3717 (1).

Western Louisiana Dr.—Louisiana: Allen Par.; TU 1327 (16), TU 14458 (4)—Rapides Par.; TU 1344 (11), TU 2059 (5), TU 2065 (13), TU 3459 (23), TU 4982 (19), TU 5769 (1), TU 5831 (11), TU 5871 (4).

Southern Mississippi Dr.—Louisiana: East Feliciana Par.; TU 5231 (19) TU 30884 (43)—*Mississippi:* Lincoln Co.; TU 28889 (7)—Holmes Co.; TU 3695 (10). *Middle Mississippi Dr., (Arkansas and Tennessee rivers)—Arkansas:* Garland Co; TU 14249 (12)—Yell Co.; TU 15487 (73)—Pope Co.; TU 13770 (13), TU 26925 (3)—*Mississippi:* Tishomingo Co.; TU 4197 (4)—*Tennessee:* Benton Co.; TU 14715 (5). *Northern Mississippi Dr.—Illinois:* Massac Co.; TU 3530 (25)—Johnson Co.; UMMZ 175870 (7).

Lake Pontchartrain Dr.—Louisiana: East Baton Rouge Par.; TU 35691 (22)—East Feliciana Par.; TU 4656 (39)—St. Tammany Par.; TU 967 (33), TU 9811 (14), TU 14512 (18).

Pearl Dr.—Louisiana: St. Tammany Par.; TU 25823 (25)—*Mississippi:* Pearl River Co.; TU 14107 (20), TU 23549 (40)—Marion Co.; TU 28415 (44)—Copiah Co.; TU 28872 (22).

Mississippi Gulf Dr.—Mississippi: Hancock Co.; TU 4771 (20), TU 7653 (6), TU 27078 (12)—Harrison Co.; TU 27094 (3)—Jackson Co.; TU 28087 (7)—Stone Co.; TU 16348 (21), TU 28095 (1)—Lamar Co.; TU 1622 (5)—Perry Co.; TU 1231 (5)—Covington Co.; TU 28582 (9), TU 28593 (1)—Lauderdale Co.; TU 7484 (3).

Alabama (including Tombigbee) Dr.—Alabama: Washington Co.; TU 33910 (2), UMMZ 163597 (84)—Clarke Co.; TU 32522 (11)—Monroe Co.; TU 2644 (15)—Crenshaw Co.; TU 2586 (8)—Wilcox Co.; TU 3065 (9), TU 3431 (14) Marengo Co.; TU 24544 (1)—Perry Co.; TU 25969 (19)—Bibb Co.; TU 19423 (6), TU 24688 (17)—Talapoosa Co.; TU 12174 (7)—Tuscaloosa Co; TU 27548 (4), TU 30192 (3), TU 30212 (6)—Blount Co.; UMMZ 168616 (18)—Marion Co.; TU 30235 (21)—*Mississippi:* Lee Co.; TU 2443 (10).

Pensacola Bay Dr.—Florida: Santa Rosa Co.; TU 20941 (7)—Okaloosa Co.; TU 23691 (30)—Walton Co.; TU 24762 (57).

Choctawhatchee Bay Dr.—Florida: Holmes Co.; TU 2277 (37)—*Alabama:* Dale Co; TU 2348 (28)—Henry Co.; TU 3902 (60).

Apalachacola (Chatahoochee) Dr.—Alabama: Russell Co.; UAIC 1097 (1), FSU 6591 (15), FSU 6656 (77), TU 10717 (3), WSV 6423-1 (2), WSV 6438-1 (3), WSV 6445-1 (44)—Lee Co.; FSU 6643 (11), TU 12099 (3), WSV 6414-3 (1)—*Georgia:* Early Co.; UAIC 1134 (1).

Fundulus notatus x *F. olivaceus*

Mississippi Dr.—Louisiana: Jackson Par.; Castor Cr. trib. to Little River at Chatham, UMMZ 161253 (1).

Pearl Dr.—Mississippi: Copiah Co.; Pearl River 3 mi. E Georgetown, TU 37120 (1).

Conclusions

1. *Fundulus notatus* and *F. olivaceus* are highly interfertile in the laboratory and produce fertile and vigorous hybrids. Their ranges broadly overlap in nature and they are occasionally syntopic. The rare occurrence of natural hybrids (about 1 in 6000) indicates that strong isolating mechanisms separate the two species.

2. The exact nature of the isolating mechanisms is not known. The rare occurrence of syntopy indicates that isolating mechanisms are primarily ecological. However, no general statement of ecological parameters seems to have more than local validity and either species seems to occupy all favorable habitats when the other is absent.

3. Observations of syntopic populations indicate that strong behavioral isolation is operative. No evidence is available to suggest intergradation or introgressive hybridization.

4. Morphological character displacement is not demonstrated and is probably rare or absent as a result of the unstable nature of the syntopic associations.

5. A single character, the presence or absence of distinct, regular, dark black dorsolateral spots, separates specimens of *F. olivaceus* from specimens of *F. notatus* except in rare instances. Other characters may be used to separate local populations, but do not have range-wide utility. There is broad intraspecific overlap in all characters studied and the recognition of subspecies is not warranted.

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