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SEPARATION OF THE SUBSPECIES OF LARGEMOUTH BASS *MICROPTERUS SALMOIDES SALMOIDES*, AND *M. S. FLORIDANUS* AND INTERGRADES BY USE OF MERISTIC CHARACTERS

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ABSTRACT

Meristic variation was examined among samples of northern largemouth bass, *Micropterus salmoides salmoides*, from Arkansas and compared with variation found in samples of the Florida largemouth, *Micropterus salmoides floridanus*. Meristic characters employed in this investigation included five scale counts and number of pyloric caeca. Statistical analyses of the data involved analysis of variance, discriminant function analysis, and Hotelling's T^2 test statistic. The primary objective of this study was to establish the best criteria for the practical separation of the two subspecies and, if possible, their intergrades. Number of pyloric caeca, which had not been previously compared for these two subspecies, proved to be the best single character for their separation. Utilizing two scale characters and number of pyloric caeca, discriminant function analyses permit classification of unknown specimens to one or the other subspecies with a high degree of certainty. A graph, containing two overlapping ellipses, constructed using Hotelling's T^2 test statistic, enables a rapid classification of *M. s. salmoides, M. s. floridanus*, and possibly intergrades by plotting original values for number of lateral-line scales and number of pyloric caeca on the axes.

INTRODUCTION

Micropterus salmoides floridanus was described from peninsular Florida by Bailey and Hubbs (1949). It was found to differ from *Micropterus salmoides salmoides* in terms of coloration, scale counts, and larger maximum size. Scale counts, the most reliable of these key characters, permitted separation of most of the specimens of *M. s. floridanus* from most of those of *M. s. salmoides* except in the belt of intergradation where ranges of the two forms overlap.

Since the original subspecific distinction made by Bailey and Hubbs, other investigators have sought to detect or substantiate differences between *M. s. salmoides* and *M. s. floridanus* using various techniques.

Bryan (1964) noted differences in the serum electropherograms of the two taxa. Miller (1965) conducted comparative immunogenetic studies on samples of *M. s. salmoides* and *M. s. floridanus* in California. Although he found immunological differences among the fish examined, few were characteristic of one or the other subspecies. Miller suggested that his study and the work of other investigators indicate that genetic differences between *M. s. salmoides* and *M. a. floridanus* are not great. He also questioned the merit of separate condideration of these two subspecies in fishery management.

C. F. Bryan (1969) suggested that a modal count of the ratio of abdonminal to caudal vertebrae will distinguish *M. s. salmoides* from *M. s. floridanus*. His specimens of *M. s. floridanus* usually had 14 abdominal vertebrae whereas specimens of *M. s. salmoides* usually had 15.

The objective of the present study was to analyze the meristic variation between Arkansas populations of *M. s. salmoides* and Florida populations of *M. s. floridanus*. If possible, specimens representing intergrades between the two subspecies would be examined for morphological intermediacy and criteria established for the practical separation of *M. s. salmoides* and *M. s. floridanus* and their intergrades.

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METHODS AND MATERIALS

Totals of 117 *M. s. salmoides* from eight localities in Arkansas and 121 *M. s. floridanus* from Florida were utilized. In addition, the California Department of Fish and Game provided two samples (60 specimens) utilized by Bottroff (1967) and identified by him as intergrades between introduced California *M. s. salmoides* and introduced *M. s. floridanus*.

A sample of largemouth bass was obtained from Beulah Island Lake in Mississippi. This lake, which already contained a population of *M. s. salmoides*, was stocked with 1,710 six- to eight-inch *M. s. floridanus* by the Arkansas Game and Fish Commission in January 1966. The sample, taken on August 18, 1967, consisted of 7 adult largemouth bass and 14 young-of-year. Gear used in collecting specimens consisted of seine, electro-shocker, rotenone, trammel-net, and hook-and-line.

Meristic examination of the specimens originally consisted of the five scale counts found by Bailey and Hubbs (1949) to show the greatest degree of difference between the two forms. During the course of the study, a sixth character was added, number of pyloric caeca. The six characters examined and their abbreviations are: number of scales along the lateral line (LLS), scale rows above the lateral line (SALL), scale rows below the lateral line (SBLL), scale rows around the caudal peduncle (CPS), scale rows on the cheek (CS), and number of pyloric caeca (PC). The preceding characters were counted according to the method outlined by Hubbs and Lagler (1958).

Statistical Procedure

The data were processed on an IBM 7040 computer at the University of Arkansas. Each character, obtained from known specimens of *M. s. salmoides* and *M. s. floridanus,* was subjected to a one-way analysis of variance to detect significant differences. The more divergent characters were used in further analyses. To establish a practical method of allocating specimens to either *M. s. salmoides* or *M. s. floridanus,* the discriminant function analysis (Fisher 1936) was applied to the datausing two and three characters as variables. This method was also applied by Amos, Anas, and Pearson (1963), and Pearson (1964) in the separation of Asian and North American races of pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Nelson (1968) also utilized the discriminant function analysis for separation of *Catostomus commersonii, C. macrocheilus,* and their hybrids in British Columbia.

One disadvantage involved in the use of the standard discriminant function analysis is that it always classified an individual to one of the target populatins involved. To account for the possibility that an individual to be classified did not belong to either of the target populations, Hotelling's T^2 statistic (Rao 1965) in inverted form was used. A program based on the T^2 statistic enabled the computer to calculate values for selected characters, which were used to construct confidence ellipses about the population sample means. The two characters exhibiting greatest contrast between the subspecies involved were utilized. This procedure facilitated intergrade designation on a test group of unknowns.

RESULTS

Meristic Analysis

Table 1 contains means for the six characters and calculated F–values from the analysis of variance tests for the *M. s. salmoides* and *M. s. floridanus* examined. As reported by Bailey and Hubbs (1949), *M. s. floridanus* are found to have higher scale counts than *M. s. salmoides.* Number of pyloric caeca, however, have not been previously compared for these two subspecies. Applegate (1966) used number of pyloric caeca to separate fry and fingerling *M. s. salmoides* and *M. punctulatus.* In this study, pyloric caeca were counted in 58 specimens of *M. s. salmoides* and 54 *M. s. floridanus* with a mean difference found of 13.6 caeca (Table 5). When this character was examined in two samples of 30 specimens, each identified by Bottroff (1967) as intergrades between *M. s. salmoides* and *M. s. floridanus* from California, sample means were 29.2 and 30.0. These values for number of pyloric caeca would seem to substantiate the intermediacy of these specimens identified as intergrades between *M. s. salmoides* and *M. s. floridanus.*

Highly variable meristic counts on fish in the sample from Beulah Island Lake, Mississippi, indicated that both *M. s. salmoides* and *M. s. floridanus* were present, and possibly intergrades between the two (Table 2). As a result, this sample was used as a test group to represent unknowns in applying statistical techniques designed to distinguish between *M. s. salmoides, M. s. floridanus,* and their intergrades.

Discriminant Function Analysis

Two discriminant function analyses were computed. The first utilized the lateral-line and caudal peduncle scale characters, chosen because of higher F-values in the analysis of variance (Table 1). This discriminant function analysis utilized all 238 of the *M. s. salmoides* and *M. s. floridanus* examined. By means of the two equations given below, each fish was classified as either *M. s. salmoides* or *M. s. floridanus* depending on which equation resulted in the larger value. In each equation, the two calculated coefficients are multiplied respectively by the number of lateral-line scales and number of caudal peduncle scales from a single unknown specimen. From the sum of the products, the calculated constant is then subtracted. If X^1 is larger than X^2, the fish is allocated to *M. s. salmoides*, and if X^2 is larger than X^1, it is allocated to *M. s. floridanus*.

Discriminant Function 1 (*salmoides*)

10.91328 (no. lateral-line scales) + 12.12798 (no. caudal peduncle scales) - 518.02170 = X^1.

Discriminant Function 2 (*floridanus*)

11.97298 (no. lateral-line scales) + 12.82365 (no. caudal penduncle scales) - 608.94987 = X^2.

When 117 *M. s. salmoides* were subjected to the above equations, 108 were classified as *M. s. salmoides*, and 9 (7%) were classified as *M. s. floridanus*. When 121 *M. s. floridanus* were analyzed, 110 were correctly classified, whereas 11 (9%) were classified as *M. s. salmoides*.

In the second analysis, pyloric caecal counts were employed as a third variate in addition to the two scale characters used in the first analysis. Since caecal counts had not been made on all of the specimens, the sample size in this analysis consisted of 58 *M. s. salmoides* and 54 *M. s. floridanus*. When the second analysis was computed, the 58 *M. s. salmoides* were all correctly classified, as were 53 of the 54 *M. s. floridanus*. Thus, 111 of 112 (99.9%) specimens were correctly classified by the discriminant function analysis when the data from the three characters were used as variates.

By utilizing the two simple equations previously given and the calculated values for the second discriminant function analysis, the data from the three characters make it possible to classify an unknown fish to either *M. s. salmoides* or *M. s. floridanus* with a high degree of certainty. However, it must be assumed that the unknown specimen was derived from one of the populations represented by the samples examined in this study or from similar populations of *M. s. salmoides* or M. s. *floridanus*.

When the 21 fish from Beulah Island Lake were subjected to the second set of discriminant function equations, 5 adults were classified as *M. s. salmoides* and 2 adults as *M. s. floridanus*. Ten of 14 young-of-year were classified as *M. s. salmoides* and 4 of 14 as*M. s. floridanus*. The three character values and discriminant functions for these specimens are given on Table 3. The larger of the two functions is underlined and indicates the group (*M. s. salmoides* or M. s. *floridanus*) to which the specimen best fits based on the data from the three characters.

Hotelling's T^2 Statistic

The program based on Hotelling's T^2 test statistic was applied using number of lateral-line scales and number of pyloric caeca as the selected characters. The calculated values, when plotted graphically and connected, formed two overlapping ellipses (Fig. 1). Number of lateral-line scales and pyloric caeca are represented, respectively, by the vertical and horizontal axes of the graph.

The elliptical figure functions by enveloping points representing, in this case, test-group specimens from Beulah Island Lake into the area representing the population, either *M. s. salmoides* or *M. s. floridanus*, which it best fits. The axes and elliptical confidence belts were generated from the data taken from the known subspecies.

To classify a fish by this method involves plotting the original values for both characters on the respective axes. The two ellipses represent the acceptance regions for the two populations constructed at the .01 level of significance. Aberrant specimens could fall outside the range of either ellipse, making it impossible to determine the origin of the specimen. The zone of overlap formed by the two ellipses represents an area of intermediacy between the two theoretical populations, and if intergrades were present in a population, they might well be recognized by this region.

When the 21 fish were classified by plotting on the elliptical figure (Fig. 1), 4 adults were classified as *M. s. salmoides* and 2 as *M. s. floridanus*, with 1 fish falling in the zone of overlap. Eight of 14 young-of-year were classified as *M. s. salmoides* and 2 of 14 as *M. s. floridanus*, with 4 falling in the zone of overlap. Table 4 compares the classification of the 21 fish by both the discriminant function analysis and the T^2 figure.

DISCUSSION

The present data for five scale characters agree well with those of Bailey and Hubbs (1949) in the original distinction of *M. s. floridanus* from *M. s. salmoides.* Bailey and Hubbs present a mean meristic index (sum of five scale counts) of 125.0 (116-132) for *M. s. salmoides* (N = 155) as compared to a mean of 125.9 (124-130) for 117 *M. s. salmoides* used in this study. For 72 specimens of *M. s. floridanus* they found a mean index of 137.9 (129-145) as compared to a mean index of 136.8 (135-141) for 121 *M. s. floridanus* used in this study.

The number of pyloric caeca provides the best single character for separation of the two subspecies. Johnson (1907) examined the variation in number of pyloric caeca in six species of centrarchids and found *M. s. salmoides* to be the only form of the genus having branched caeca. He described the caeca as comprising 9-13 bases which secondarily divide to form as many as 28 caeca. When numbers of pyloric caeca were used to separate fry and fingerling spotted and largemouth basses in Arkansas, Applegate (1966) found a range of variation of 20-33 caeca in 50 *M. s. salmoides* with the highest frequency being 24. In this study, 58 *M. s. salmoides* had caecal counts ranging from 14-35 (x = 23.2), with the highest frequency being 20. For 54 *M. s. floridanus*, caecal counts ranged from 26-53 (x = 36.8). Caecal counts for two samples of California intergrades between *M. s. salmoides* and *M. s. floridanus* were intermediate between those obtained on 54 *M. s. salmoides* and 58 *M. s. floridanus.* The means for the three groups are compared in Table 5.

Addison and Spencer (1971) examined caecal counts in 20 *M. s. salmoides*, 10 *M. s. floridanus*, and 56 F^1 progeny obtained in their study and reported similar results to those described above. For the three groups, mean caecal counts and ranges were: *M. s. salmoides* 22.7 (18-29); *M. s. floridanus* 39.0 (30-47); and F^1 progeny 28.0 (17-41). Because of the overlap and variation of the F^1 counts, Addison and Spencer concluded that it would be difficult to separate F^1 fish from *M. s. salmoides* and *M. s. floridanus* in mixed population.

To identify pure *M. s. salmoides* and *M. s. floridanus* and their intergrades where the possiblity of a mixed population is present, two techniques have been offered. The discriminant function analysis employs three characters and involves elementary calculations for identification. Only two characters are involved in the elliptical figure derived from Hotelling's T^2 statistic. This method involves no calculations in identifying specimens if the figure is available. The figure also allows for aberrant specimens to be classified outside the range of either *M. s. salmoides* or *M. s. floridanus*, and an overlapping or intermediate zone is present for possible recognition of intergrades. It is also possible for certain specimens representing pure *M. s. salmoides* or *M. s. floridanus* to be placed in this overlapping or intermediate zone simply because ranges of variation for scale and caecal counts overlap in the two forms (Table 6).

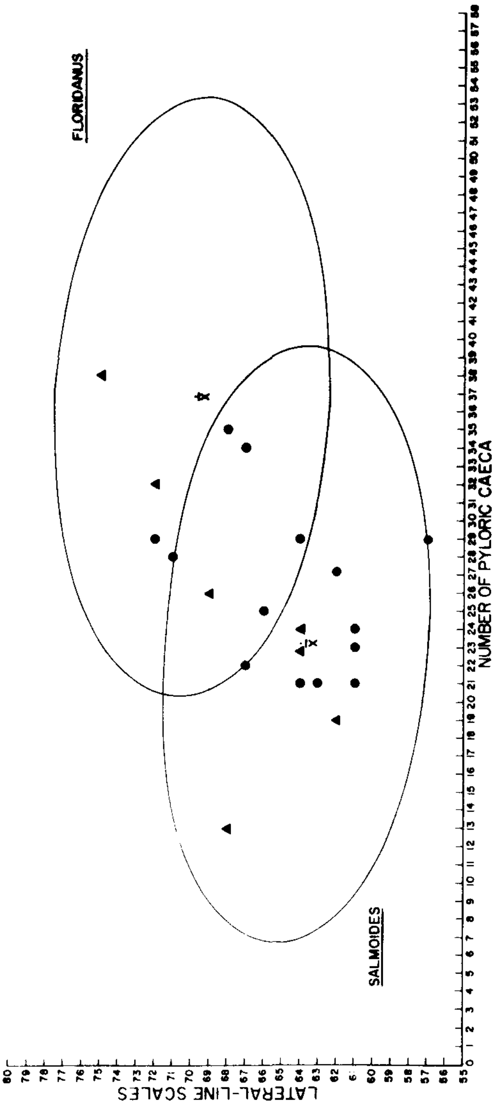

Figure 1. Graph containing overlapping ellipses constructed using Hotelling's T^2 test statistic. Seven adult ( ) and 14 young-of-year (•) *M. salmoides* from Beulah Island Lake plotted for identification as *M. salmoides salmoides*, or *M. salmoides floridanus* by use of number of lateral-line scales and number of pyloric caeca.

Table 1. Pooled means for five scale characters and pyloric caeca. Calculated F-values and tabular F's significant at the five percent level are given from the test for analysis of variance.

Character	*salmoides* $\bar{x}$	No. fish	*floridanus* $\bar{x}$	No. fish	F-value	Sig. F .05
Lateral-line scales	63.9	117	69.8	121	403.5	3.88
Scales above lateral-line	7.6	117	8.1	121	36.5	3.88
Scales below lateral-line	15.8	117	16.8	121	63.9	3.88
Cheek scales	10.6	117	12.2	121	122.1	3.88
Caudal peduncle scales	27.8	117	29.0	121	129.1	3.88
Pyloric caeca	23.2	58	36.8	54	195.5	3.93

Table 2. Counts from five scale characters, number of pyloric caeca, and standard lengths for adult, and young-of-year *M. salmoides* collected in Beulah Island Lake on August 18, 1967.

ADULTS							
Fish No.	LLS	SALL	SBLL	CPS	CS	PC	SL
1.	64	7	15	26	12	24	282
2.	64	7	17	26	13	23	267
3.	62	7	17	28	13	19	260
4.	68	7	16	28	13	13	270
5.	75	8	19	31	12	38	295
6.	69	8	18	28	13	26	287
7.	72	9	20	32	12	32	360

YOUNG-OF-YEAR							
Fish No.	LLS	SALL	SBLL	CPS	CS	PC	SL
1.	66	7	18	27	12	25	121
2.	63	7	16	27	11	21	97
3.	61	8	15	27	12	24	101
4.	61	7	16	26	11	23	71
5.	64	8	15	27	11	21	75
6.	67	8	16	29	12	22	115
7.	62	8	15	26	11	27	73
8.	61	7	15	26	12	21	76
9.	71	8	17	30	13	28	87
10.	57	8	14	27	11	29	77
11.	72	9	17	28	12	29	65
12.	67	9	19	31	12	34	66
13.	64	7	17	28	11	29	60
14.	68	8	16	30	14	35	67

Table 3. Constants and coefficients from second discriminant function analysis applied to 21 *M. salmoides* from Beulah Island Lake for classification as *M. salmoides salmoides* or *M. salmoides floridanus*.

Three characters used as variables:
Lateral-line scales (LLS)
Caudal peduncle scales (CPS)
Pyloric caeca (PC)

Discriminant Function 1 (floridanus)

Coefficients	*Constant*	
10.79237 (LLS) + 14.80185 (CPS) + 2.28062 (PC)	- 640.69295	= Value 1

Discriminant Function 2 (*salmoides*)

Coefficients	*Constant*	
9.80543 (LLS) + 13.81034 (CPS) + 1.69294 (PC)	- 528.11039	= Value 2

BEULAH ISLAND LAKE SPECIMENS (21)

Adults (7)

Fish No.	LLS	CPS	PC	*floridanus*	*salmoides*
1.	64	26	24	489.60171	499.13653
2.	64	26	23	487.32109	497.44359
3.	62	28	19	486.21757	498.68165
4.	68	28	13	537.28807	547.35659
5.	75	31	38	714.25571	699.74912
6.	69	28	26	577.72850	579.17024
7.	72	32	32	682.99673	673.98553

Number classified

salmoides	*floridanus*
5	2

Young-of-year (14)

Fish No.	LLS	CPS	PC	*floridanus*	*salmoides*
1.	66	27	25	528.26892	534.38347
2.	63	27	21	486.76933	498.06262
3.	61	27	24	472.02645	483.53058
4.	61	26	23	454.94398	468.02730
5.	64	27	21	497.56170	507.86805
6.	67	29	22	561.82313	566.59796
7.	62	26	27	474.85883	484.60449
8.	61	26	21	450.38274	464.64142
9.	71	30	28	633.47818	629.70766
10.	57	27	29	440.26007	452.77356
11.	72	28	29	616.94747	613.66535
12.	67	31	34	618.79427	614.53392
13.	64	28	29	530.60851	535.20191
14.	68	30	35	617.06541	612.22195

Number classified

salmoides	*floridanus*
10	*4*

Table 4. Comparison of number of specimens from Beulah Island sample classified as *M. s. salmoides* or *M. s. floridanus* by the discriminant function analysis, with number classified as *M. s. salmoides*, *M. s. floridanus* or in intermediate zone by plotting on elliptical figure (Fig. 1).

		salmoides	*floridanus*	*intermediate zone*
Discriminant Function Analysis:				
		salmoides	*floridanus*	
Adults	(7)	5	2	
Young-of-year	(14)	10	4	
Elliptical Figure (Fig. 1):				
		salmoides	*floridanus*	*intermediate zone*
Adults	(7)	4	2	1
Young-of-year	(14)	8	2	4

Table 5. Comparison of means of pyloric caeca counts from 58 *salmoides*, 54 *floridanus*, and 60 intergrades from California.

Form	Number Examined	Location	Mean Number of Pyloric Caeca
M. s. salmoides	58	Arkansas	23.2
Intergrades	30	California (Sutherland Reservoir)	29.2
Intergrades	30	California (El Capitan Reservoir)	30.0
M. s. floridanus	54	Florida	36.8

Table 6. Frequency tables for three characters (lateral-line scales, caudal peduncle scales, and pyloric caeca) to illustrate overlap in ranges of variation between *M. s. salmoides* and *M. s. floridanus*.

LATERAL-LINE SCALES

	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
(117) *salmoides*	1	2	6	5	17	18	20	22	12	9	5									
(121) *floridanus*								2	4	16	18	16	20	18	11	9	3	2	1	1

CAUDAL PEDUNCLE SCALES

	24	25	26	27	28	29	30	31	32	33	34
(117) *salmoides*	1	5	7	25	50	19	4	5	1		
(121) *floridanus*				2	17	30	37	26	8	0	1

PYLORIC CAECA

	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43-53
(58) *salmoides*	1	0	1	0	3	2	12	7	2	6	3	5	2	5	2	3	1	1	0	1	0	1								
(54) *floridanus*													3	0	1	2	2	4	2	2	2	5	5	3	3	3	1	3	2	11

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