To the Graduate Council:

I am submitting herewith a thesis written by John Raymond Shute, IV entitled "A Systematic Evaluation of the Waccamaw Darter, Etheostoma perlongum (Hubbs and Raney), with Comments on Relationships Within the Subgenus Boleosoma (Percidae: Etheostomatini)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

David Etnier, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

The Graduate School

A SYSTEMATIC EVALUATION OF THE WACCAMAW DARTER, *ETHEOSTOMA PERLONGUM* (HUBBS AND RANEY), WITH COMMENTS ON RELATIONSHIPS WITHIN THE SUBGENUS *BOLEOSOMA* (PERCIDAE: ETHEOSTOMATINI)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

John Raymond Shute, IV

December 1984

ACKNOWLEDGMENTS

I would like to thank the members of my graduate committee, David Etnier, Gary McCracken, and Arthur Echternacht for their helpful suggestions, comments, and inspirations regarding my research. The following persons are acknowledged for collecting and/or providing specimens critical to this study: Noel Burkhead; Brooks Burr; Larry Greenberg; Robert Jenkins; David Lindquist; Edward Menhinick; and Fred Rohde. Their assistance was greatly appreciated. In addition to assistance in the collection of specimens, David Lindquist provided useful assistance during the earlier phase of this study.

Many others provided assistance in various aspects of this study. John McNeill provided the use of his cabin on Lake Waccamaw, and this was greatly appreciated. Ronald Watson provided unpublished information on darters from the Potomac drainage of Virginia. For his advice regarding statistical questions, I would like to acknowledge Rob Wainberg. I would especially like to thank Kitty Gustin for the many hours she spent assisting me in the lab, and Joe Schiller for the hours he spent assisting me with the computer analysis of my data.

Above all, I would like to thank my wife, Peggy, for nearly all of the above reasons, plus the continued support and encouragement she gave me.

Partial funding was provided by Sigma XI.

ABSTRACT

A study was conducted to determine systematic relationships between the Waccamaw darter, Etheostoma perlongum, and other closely related members of the subgenus Boleosoma (primarily E. olmstedi) using horizontal starch-gel electrophoresis and standard morphological techniques. Darter populations were sampled from Lake Waccamaw, the Waccamaw River, and several additional major drainages. Also, one population each of E. longimanum and E. podostemone were included for biochemical comparison. A total of 46 alleles were resolved for the 18 loci examined.

Populations from the Waccamaw and Pee Dee drainages clustered phenetically with an average identity of 0.983. Populations from the Cape Fear, Santee, and Neuse drainages were somewhat more divergent, and the single population from the Oswego drainage of New York, clustered apart from all others.

Several unique alleles were present in the Waccamaw drainage; however, this condition was also evident in E. olmstedi populations from various other drainages. In addition, both morphological and biochemical data indicated clinal variation between darter populations in Lake Waccamaw and those in the Waccamaw River. This clinal variation, coupled with the high degree of genetic similarity between darters from the Waccamaw and other drainages sampled in the southeast, suggests that E. perlongum and E. olmstedi are conspecific. The Waccamaw darter is recognized as a unique ecomorph. Morphological

characteristics distinguishing the Waccamaw darter from other Boleosoma (primarily scale patterns and numbers) are likely controlled to a large degree by environmental conditions.

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CHAPTER I

INTRODUCTION

Hubbs and Raney (1946) were among the first to recognize that Lake Waccamaw, in southeastern North Carolina, contained a unique assemblage of freshwater fishes (see Shute et al., 1981 for a description of Lake Waccamaw). In their publication, Hubbs and Raney described three species of fishes determined to be endemic to the lake. Among these was the Waccamaw darter, Etheostoma (Boleosoma) perlongum. They also described as Lake Waccamaw endemics the Waccamaw killifish, Fundulus waccamensis (Fundulidae), and the Waccamaw silverside, Menidia extensa (Atherinidae).

These fishes received little attention from ichthyologists until recently, when Williams (1977) proposed that they be considered for protection under the Federal Endangered Species Act of 1973. In order to update information concerning these endemic fishes, a three-year baseline biological study was conducted to determine their current status (Lindquist and Yarbrough, 1982). The present study arose as a result of information gathered during that three-year study regarding the taxonomy and distribution of the Waccamaw darter.

Shute et al. (1982a) presented evidence suggesting that E. perlongum was introgressively hybridizing with the tesselated darter, E. (Bolesoma) olmstedi, in the upper reaches of the Waccamaw River. Hubbs and Raney (1946) indeed considered E. perlongum to be a lacustrine derivative of E. olmstedi (at that time regarded as a

subspecies of E. nigrum), but that the two were readily separable based on "a convincing array of . . . characters" (Hubbs and Raney, 1946). Hubbs and Raney, however, failed to examine speciments of E. olmstedi from the Waccamaw drainage in their comparisons. Similarly, Cole (1957, 1967), in his studies on the systematics of the darter subgenus Boleosoma, also failed to examine material from the Waccamaw drainage, and concurred with Hubbs and Raney in the recognition of E. perlongum as a distinct and valid species. As a result, the problem of separating E. perlongum from E. olmstedi in the Waccamaw River headwaters has only recently become evident (Lindquist and Yarbrough, 1982; Shute et al., 1982a).

The preliminary study indicating the apparent taxonomic confusion between E. olmstedi and E. perlongum (Shute et al., 1982) was based upon morphological data collected from specimens within Lake Waccamaw, the Waccamaw River, and surrounding drainages including the Pee Dee, Cape Fear, and Santee. These data suggested clinal differences between Lake Waccamaw and Waccamaw River darter populations (Shute et al., 1982a). Whether the observed polymorphisms were the result of differing ecological conditions, or whether there was a genetic basis for these differences was unclear at the time of the study.

Biochemical studies using gel electrophoresis of proteins have proven useful in the evaluation of systematic relationships of vertebrate populations (Avise, 1975; Selander and Johnson, 1973; and others). Avise and Selander (1972) were perhaps the first to

determine the degree of genetic variability in natural populations of fishes (Characidae: Astyanax) using a "random" sampling of structural gene loci. Numerous papers have appeared in recent years employing electrophoretic techniques to delineate relationships among darters, including: McAllister et al. (1972); Page and Whitt (1973a, 1973b); Echelle et al. (1975); Echelle et al. (1976); Wiseman et al. (1978); Wolfe et al. (1979); Buth et al. (1980); Falls (1982); and McKeown et al. (1984).

The purpose of this study is to determine the degree of genetic differentiation among populations of Boleosoma through the use of gel electrophoresis. In addition, these data are correlated with existing morphological data (most of which is presented for the first time in this thesis) in order to clarify systematic relationships between Etheostoma perlongum in Lake Waccamaw and E. olmstedi populations in the Waccamaw River and elsewhere. Relationships between populations of E. olmstedi, both within and among various drainages, are also evaluated.

CHAPTER II

MATERIALS

A total of 464 and 344 specimens were used in the morphological and the electrophoretic analyses, respectively. These were collected with minnow seines, and, on several occasions in Lake Waccamaw, with a small otter trawl. Fish to be used for the morphological study were preserved in 10% formalin and later transferred to either 70% ethanol or 40% isopropanol. Specimens collected for use in the electrophoretic analysis were frozen (while still alive) in the field, either on dry ice or in liquid nitrogen. These were then transported to the laboratory where they were stored at a temperature of -80°C.

Darter populations from Lake Waccamaw, the Waccamaw River, the Pee Dee, Cape Fear, and Santee drainages were represented in both aspects of the study. Sampling localities for the electrophoretic study were, where possible, nearly the same as those used in the morphological study (Figure 1). In addition, specimens of Etheostoma olmstedi from the Oswego drainage in New York and the Neuse drainage in North Carolina were examined electrophoretically. Etheostoma podostemone from the Roanoke drainage of Virginia and E. longimanum from the James drainage in Virginia served as outgroups in the electrophoretic analysis.

Collecting localities and the number of specimens collected (in parenthesis) follow. An asterick (*) denotes those used for

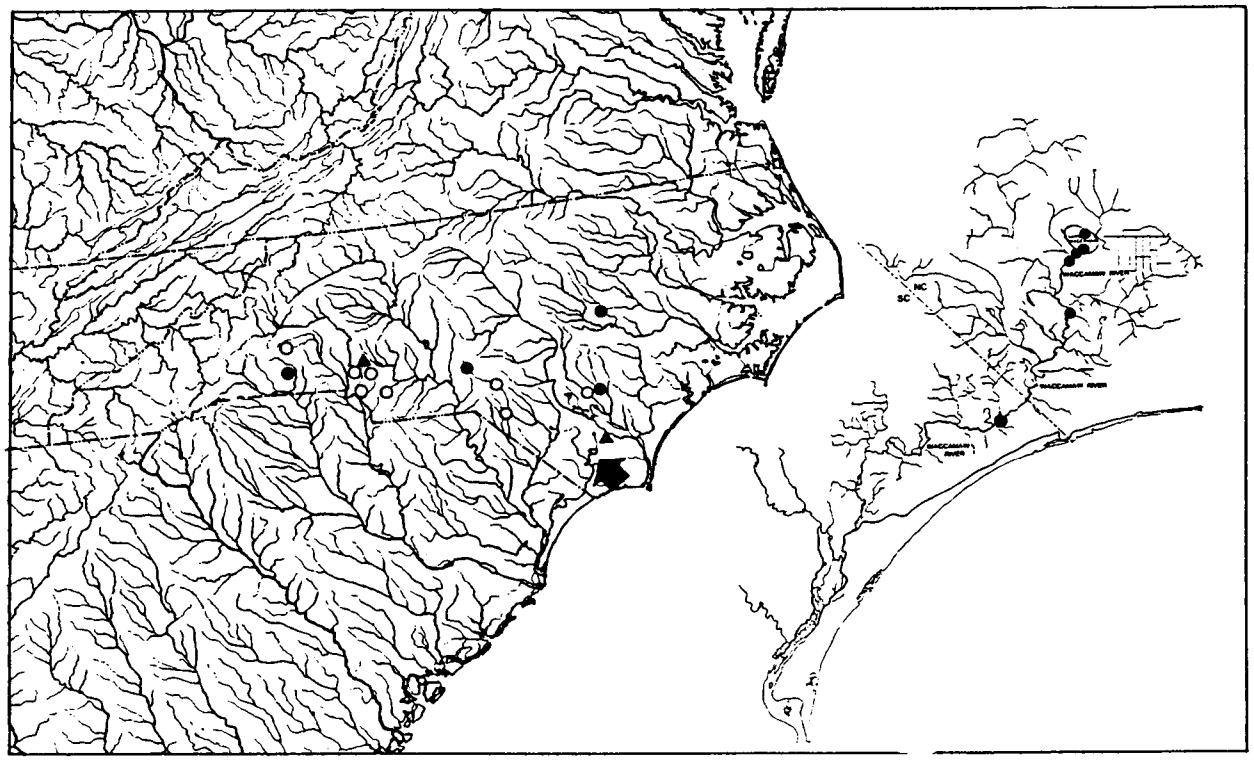

FIGURE 1. Map of localities sampled. Closed circles represent populations sampled for electrophoretic analysis only. Open circles represent populations sampled for morphological analysis only. Triangles represent populations that were analyzed both electrophoretically and morphologically. Populations sampled from Lake Waccamaw and the Waccamaw drainage (see arrow) are plotted on the insert map. Only those analyzed electrophoretically are plotted. Shute et al. (1981) provide a map of the many localities throughout the Waccamaw drainage where specimens were collected for morphological analysis. Etheostoma podostemone, E. longimanum and the Cayuga Inlet, N.Y. populations are not shown.

morphological analysis. Numbers without an asterick denote specimens used for electrophoretic analysis. Specimens used for morphological examination are housed at the University of North Carolina at Wilmington fish collection (UNCW), and the University of North Carolina at Charlotte (UNCC).

Etheostoma olmstedi (including those darters from Lake Waccamaw and the Waccamaw River). WACCAMAW DRAINAGE: Lake Waccamaw (Collections throughout lake), Columbus County, NC (134*, 56) UNCW 77-34, 78-62, 78-73, 78-74, 79-15, 79-20, 79-69, 79-75, 79-84, 79-86, 79-94, 79-117, 79-130, 79-131, 79-152, 79-154, 79-176, 79-177, 79-178, 79-180, 79-181, 79-182, 79-189, 79-191, 79-193, 79-217, 79-226, 79-229, 80-67, 80-68, 80-69, 80-71, 80-72, 80-75, 80-84, 80-94 80-103, 80-104, 80-108; Waccamaw River, directly below dam at Lake Waccamaw (Population R-1), Columbus County, NC (50*, 31) UNCW 76-97, 76-106, 79-167, 79-173, 79-220; Waccamaw River, from approximately 1 km below dam to approximately 7 km below dam (Population R-2), Columbus County, NC (54*, 29) UNCW 79-99, 79-100, 79-168, 79-169, 79-170, 79-196, 79-197, 79-200, 79-245, 79-259, 80-99; Waccamaw River, vicinity of County road 1928 to NC highway 904 (Population R-3) Columbus and Brunswick counties, NC (57*, 30) UNCW 79-10, 79-82, 79-119, 79-255; Waccamaw River, from vicinity of SC highway 9 to Conway (Population R-4), Horry County, SC (30*, 23) UNCW 79-73, 79-125, 79-127, 79-161, 79-230. PEE DEE DRAINAGE: Goose Creek (Yadkin System), county road 1547, Union County, NC (30*) UNCC 67-40; Back Creek (Yadkin System), at county road 2827 crossing, Mecklinburg County, NC (16*) UNCW 81-30;

Mallard Creek (Yadkin System), northeast of Charlotte, Mecklinburg County, NC (27); Drowning Creek (Lumber System), at U.S. highway 15 crossing, Hoke/Scotland county line, NC (5*) UNCW 80-283; Drowning Creek (Lumber System), at NC highway 73 crossing, Montgomery/Moore County line, NC (16); Big Shoe Heel Creek (Lumber System), at U.S. highway 74 crossing, Scotland County, NC (21*) UNCW 79-326, 80-284, 81-32.

CAPE FEAR DRAINAGE: South River, below NC highway 701 south of White Lake Community, Bladen County, NC (30*) UNCC 76-112; Coharie Creek (South River System), at county road 1134 crossing, Sampson County, NC (16) Livingston Creek at U. S. highway 74 crossing, Columbus County, NC (17*, 11) UNCW 80-129, 80-254.

SANTEE DRAINAGE: Hinton Creek (Broad System), at NC highway 226 crossing, Cleveland County, NC (19); tributry to Catawba River along county road 1150, McDowell County, NC (5*) UNCC 76-125; Brier Creek (Catawba System), at Sharon Road, Charlotte, Mecklinburg County, NC (9*) UNCC 66-13; Long Creek (Catawba System), at U.S. highway 21, Charlotte, Mecklinburg County, NC (10*) UNCC 66-8, 75-1.

NEUSE DRAINAGE: Little River 1.6 km west of Goldsboro at NC highway 581, Wayne County, NC (25).

OSWEGO DRAINAGE: Cayuga Inlet, Tompkins County, NY (28).

Etheostoma longimanum. JAMES DRAINAGE: Catawba Creek at VA highway 311 bridge, Roanoke County, VA (15).

Estheostoma podostemone. ROANOKE DRAINAGE: Little Creek, tributary to Blackwater River at county road 735 bridge, Franklin County, VA (18).

CHAPTER III

METHODS

Morphological Analysis

All counts and measurements were made in accordance with Hubbs and Lagler (1958). In addition, the degree of squamation of the nape, cheek, breast, and belly was determined for each specimen. The exact delineation of these areas follows that of Cole (1967). For each area, the degree of squamation was estimated, and a number from zero to four was assigned (0 = no scales on the area, 1 = approximately 25% scaled, 2 = approximately 50% scaled, 3 = approximately 75% scaled, and 4 = completely scaled).

Preoperculomandibular (= operculomandibular, Hubbs and Cannon, 1935) and infraorbital cephalic canal pores were counted as described by Hubbs and Cannon (1935). Graphical analyses of Hubbs and Hubbs (1953) were used to demonstrate the amounts of morphological variation within and among the populations of darters. Only those specimens 30 mm standard length or larger were used in any of the morphological analyses. All measurements were made to the nearest 0.1 mm using Helios® dial calipers.

Electrophoretic Analysis

Whole darters were eviscerated and finely minced immediately after being taken from freezer storage. The minced tissues were then mixed with grinding buffer (Selander et al., 1971) at a ratio of

1:1 (grams tissue:ml grinding buffer). This mixture was centrifuged at 13,500 rpm for 40 minutes at 4°C. The supernate from this was stored at -80°C until used.

Horizontal starch gel electrophoresis techniques were used, employing methods described by Selander et al. (1971). Four hundred milliliter gels were prepared using 30 g of Electrostarch (lot #392; Otto Hiller Co., Madison, WI, 53701) plus 17 g of Sigma starch (Sigma Chemical Co., St. Louis, MO, 63178).

Staining procedures follow recipes modified from Brewer (1970), Selander et al. (1971) and Harris and Hopkinson (1978). Appendix, page 75, gives complete stain recipes. Enzyme systems examined, and buffer systems used are listed in Table 1. Those listed were found to be the most consistently scoreable for these fishes, from a group of many enzyme systems initially surveyed.

Alleles were scored and lettered according to their relative anodal mobility (i.e., the most anodal allele might be lettered "a" while an allele of lesser mobility might be lettered "b" or "c," etc.). In the majority of cases, the allele "b" was designated as the most common allele at each locus in the Lake Waccamaw populations. Exceptions to this are as follows: Phosphoglucomutase (PGM-1), most common allele in Lake Waccamaw = "c"; Indophenoloxidase (IPO), most common allele in Lake Waccamaw = "f."

Genetic divergence between populations was determined using indices of genetic similarity (I) and genetic distance (D) (Nei, 1972). A phenogram was constructed from the genetic identity (I)

TABLE 1. Protein Systems Examined and Buffer Systems Employed

Protein	Locus	Number of Alleles Resolved	Buffer System*
General Protein	GP-1	1	C
	GP-2	1	C
	GP-3	2	C
	GP-4	1	C
Glucose-6-phosphate dehydrogenase	G-6-PD	1	A
Glucosephosphate isomerase	GPI-1	3	B
	GPI-2	3	B
Glutamate-oxaloacetate transaminase	GOT-1	3	C
	GOT-2	4	C
α-Glycerophosphate dehydrogenase	α-GPD-1	4	D
	α-GPD-2	1	D
Indophenyloxidase	IPO	5	B
Isocitrate dehydrogenase	IDH	3	C
Octanol dehydrogenase	ODH-1	1	B
Pepdidase	PEP-1	2	A
	PEP-2	3	A
Phosphoglucomutase	PGM-1	4	A
	PGM-2	4	A

*A = Lithium hydroxide; B = Poulik; C = Tris-versene-borate; D = Tris-citrate pH 8.0 (Selander et al., 1971).

values employing the unweighted pair-group method using arithmetic means (UPGMA, Sneath and Sokal, 1973). Expected genotype frequencies were calculated using Levene's (1949) formula. Significant departure from Hardy-Weinberg equilibrium at each polymorphic locus was detected using a G-test for goodness of fit adjusted using William's correction (Sokal and Rohlf, 1981). Heterogeneity G-tests (Sokal and Rohlf, 1969) were employed using highly polymorphic loci to determine the degree of heterogeneity among selected darter populations. In addition, for all polymorphic loci, heterozygote deviation was calculated using Selander's (1970) D-statistic for detecting the excess or deficiency of heterozygotes.

CHAPTER IV

RESULTS

Morphological Analysis

Darters from 10 separate localities (including Lake Waccamaw) were examined to determine the degree of morphological differentiation present between these populations. Of the 24 different characters analyzed for each specimen, number of lateral-line scales, number of scales above and below the lateral line, and degree of scalation of the nape and cheek proved to be the most diagnostic for delineating populations of Boleosoma.

Specimens from the Waccamaw drainage (Lake Waccamaw, in particular) had a tendency toward a greater degree of scalation, including more lateral-line scales (Table 2, Figure 2), scales above the lateral line (Table 3), scales below the lateral line (Table 4), and degree of nape (Table 5, Figure 3), cheek (Table 6, Figure 4), breast (Table 7), and belly (Table 8) scalation. This degree of scalation decreased down-river from Lake Waccamaw, so that darters farthest downstream demonstrated scalation patterns nearly comparable to those found in adjoining drainages. Outside of Waccamaw drainage, lateral-line scale numbers varied, with no apparent correlation to elevation. They numbered somewhat lower in the Pee Dee drainage and higher in the Santee drainage (see Table 2, Figure 2). Cape Fear populations were somewhat intermediate between the two. In contrast, the degree of scalation of the nape and cheek was somewhat higher in the Pee Dee and lower in the Cape Fear and Santee drainages.

TABLE 2. Numbers of Lateral-Line Scales for Ten *Boleosoma* Populations[a]

	Number of Lateral Line Scales																													
AREA	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
1										1				1		2	6	6	15	22	13	20	14	8	15	6	2	3			1
2									1	1		3	9	1	3	6	9	4	3	3	1	3	1	2							
3										1	1	8	8	10	1	5	5		3												
4							2	9	5	4	8	9	3	5	2																
5								1	3	3	2	6	7	3	2	2	1														
6				1	4	2	6	3	4	1		2	4	1	1																
7			1		2	1	4	2	8	5	3	2	1	1																	
8	1	1	3	1	5	7	3	1	3					1																	
9		1		1	5	6	8	6	5	9	2		1	1	1																
10					2	1	3	2	7	3	3	4	3	1			1														

[a]Localities designated by area numbers are as follows: Lake Waccamaw; (2) Waccamaw R., directly below dam at Lake Waccamaw; (3) Waccamaw R., from below area 2 to approximately 6 river km below Lake Waccamaw; (4) Waccamaw R., from below area 3 to South Carolina state line; (5) Waccamaw R., from South Carolina state line to Conway, South Carolina; (6) Livingston Cr., Cape Fear drainage in North Carolina; (7) South R., Cape Fear drainage in North Carolina; (8) combined populations from Lumber R. System, Pee Dee drainage in North Carolina; (9) combined populations from Yadkin R. system Pee Dee drainage in North Carolina; (10) combined populations from Catawba R. system, Santee drainage in North Carolina.

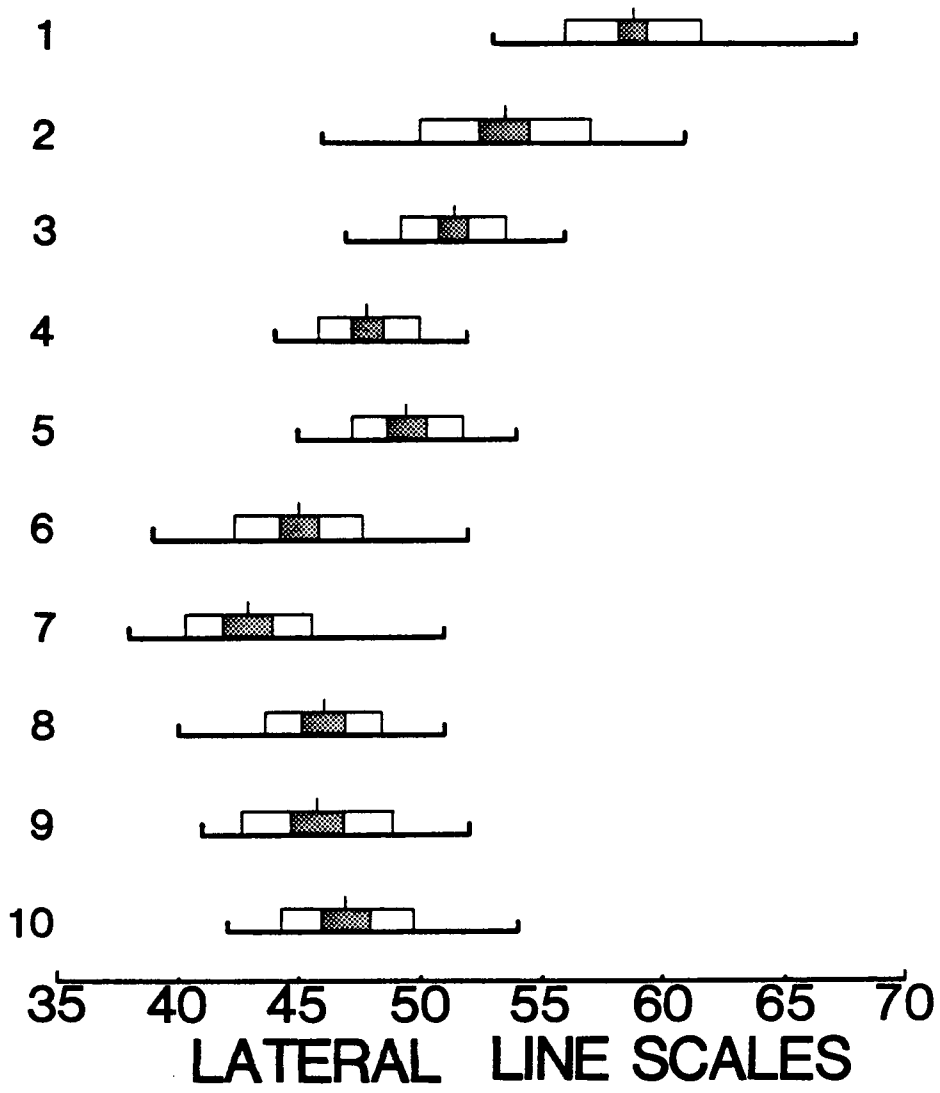

FIGURE 2. Lateral-line scale numbers for ten populations of Boleosoma. The horizontal lines represent the range, the vertical line represents the mean, one standard deviation on either side of the mean is represented by open rectangles and two standard errors on either side of the mean are represented by shaded rectangles. For explanation of area numbers, refer to Table 2.

TABLE 3. Numbers of Scales Above Lateral Line for Ten Boleosoma Populations[a]

AREA	N	Number of Scales Above Lateral Line					
		2	3	4	5	6	7
1	134			1	64	68	1
2	50			2	35	13	
3	42			14	26	2	
4	47		1	28	18		
5	30			23	7		
6	29		2	24	2	1	
7	30			17	13		
8	26		1	23	2		
9	46		3	27	15	1	
10	30	1	1	15	13		

[a]For explanation of area numbers, refer to Table 2.

TABLE 4. Numbers of Scales Below Lateral Line for Ten Boleosoma Populations[a]

AREA	N	Number of Scales Below Lateral Line						
		6	7	8	9	10	11	12
1	134		2	21	47	45	17	2
2	50		1	14	19	12	2	2
3	42		6	19	12	4		1
4	47		13	24	10			
5	30	2	12	12	4			
6	29	7	15	6	1			
7	30	3	14	12	1			
8	26	4	9	13				
9	46	5	19	19	3			
10	30	2	13	10	5			

[a]For explanation of area numbers, refer to Table 2.

TABLE 5. Index of Nape Squamation for Ten Boleosoma Populations[a]

AREA	N	Nape Squamation Index 0	1	2	3	4
1	134	1	4	18	36	75
2	50	13	1	9	14	13
3	42	5	7	12	9	9
4	47	23	9	6	3	6
5	30	15	7	5	3	
6	29	26		2	1	
7	30	29	1			
8	26	26				
9	46	46				
10	30	29	1			

[a]An index value of 0 = completely naked; a value of 1 = approximately 25% scaled; a value of 2 = approximately 50% scaled; a value of 3 = approximately 75% scaled; a value of 4 = fully scaled. For explanation of area numbers, refer to Table 2.

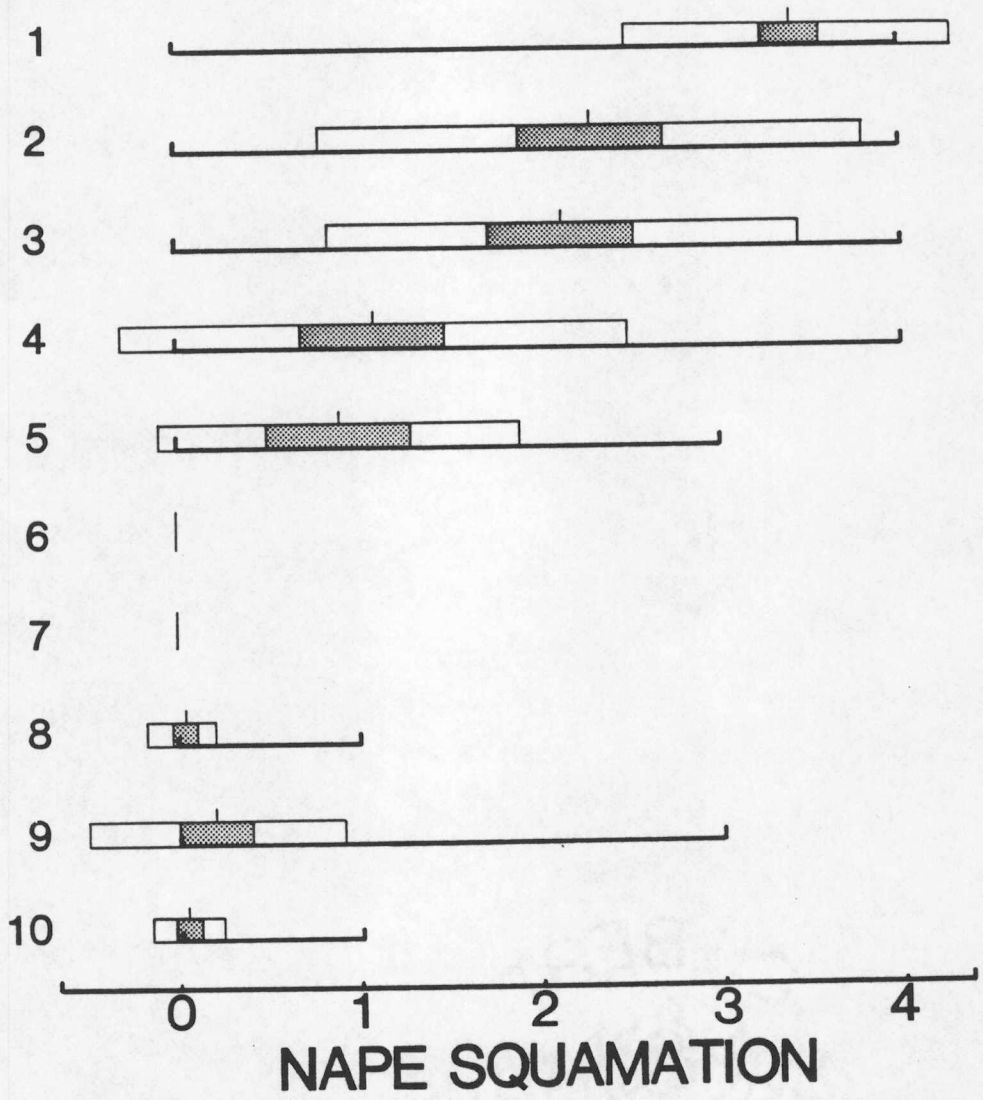

FIGURE 3. Nape squamation indices for ten populations of Boleosoma. The horizontal lines represent the range, the vertical lines represent the mean, one standard deviation on either side of the mean is represented by open rectangles, and two standard errors on either side of the mean are represented by shaded rectangles. For explanation of area numbers, refer to Table 2.

TABLE 6. Index of Cheek Squamation for Ten Boleosoma Populations[a]

AREA	N	Cheek Squamation Index				
		0	1	2	3	4
1	134	3	4	15	42	70
2	50	7	5	9	14	15
3	42	5	5	10	16	6
4	47	12	9	14	11	1
5	30	5	13	7	5	
6	29	20	2	3	4	
7	30	14	7	5	4	
8	26	22	2	2		
9	46	46				
10	30	30				

[a]An index value of 0 = completely naked, a value of 1 = approximately 25% scaled; a value of 2 = approximately 50% scaled; a value of 3 = approximately 75% scaled; a value of 4 = fully scaled. For explanation of area numbers, refer to Table 2.

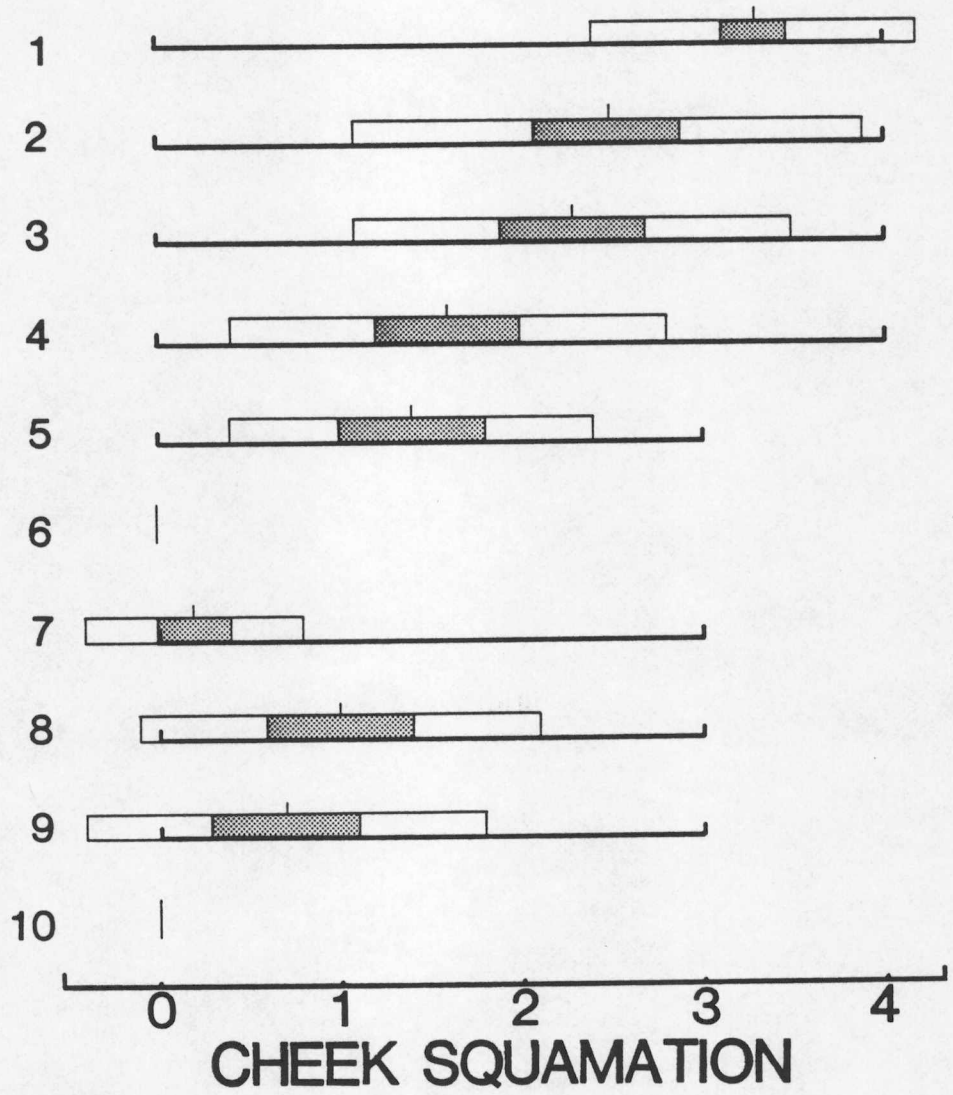

FIGURE 4. Cheek squamation indices for ten populations of Boleosoma. The horizontal lines represent the range, the vertical lines represent the mean, one standard deviation on either side of the mean is represented by open rectangles, and two standard errors on either side of the mean are represented by the shaded rectangles. For explanation of area numbers, refer to Table 2.

TABLE 7. Index of Breast Squamation for Ten Boleosoma Populations[a]

AREA	N	Breast Squamation Index 0	1	2	3	4
1	134	84	26	14	10	
2	50	37	7	3	3	
3	42	29	9	3	1	
4	47	45	2			
5	30	29	1			
6	29	28		1		
7	30	30				
8	26	25	1			
9	46	46				
10	30	30				

[a]An index value of 0 = completely naked; a value of 1 = approximately 25% scaled; a value of 2 = approximately 50% scaled; a value of 3 = approximately 75% scaled; a value of 4 = fully scaled. For explanation of area numbers refer to Table 2.

TABLE 8. Index of Belly Squamation for Ten Boleosoma Populations[a]

AREA	N	Belly Squamation Index 0	1	2	3	4
1	134	42	34	19	24	15
2	50	21	10	5	7	7
3	42	16	10	8	3	5
4	47	35	5	6	1	
5	30	23	5	2		
6	29	17	9	2	1	
7	30	28	2			
8	26	25	1			
9	46	33	13			
10	30	20	3	3	2	2

[a]An index value of 0 = completely naked; a value of 1 = approximately 25% scaled; a value of 2 = approximately 50% scaled; a value of 3 = approximately 75% scaled; a value of 4 = fully scaled. For explanation of area numbers, refer to Table 2.

Fin ray and spine counts were somewhat useful as diagnostic characters, at least in separating the Waccamaw drainage specimens from those of other drainages. For example, dorsal soft rays usually numbered 14 or 15 in the Waccamaw drainage and 13 or 14 in other drainages, except the Lumber/Pee Dee, which resembled those of the Waccamaw drainage (Table 9). Number of dorsal spines was modally 10 in Lake Waccamaw and 9 in the Waccamaw River and other drainages (Table 10). The number of anal spines was modally two in all populations examined (Table 11). Number of anal soft rays varied, however, and decreased from a mode of nine throughout the Waccamaw drainage to a mode of seven or eight in the other drainages (Table 11).

Cephalic canal pores were useful in that Waccamaw drainage specimens normally possessed 11 to 12 preoperculomandibular canal pores, where specimens from other drainages are characterized as having 9 to 11 (Table 12). Infraorbital canal pores were modally eight in all populations (Table 13), and the canal was generally uninterrupted. Although many proportional measurements were taken, they appeared to be of little diagnostic value in this study. Table 14 summarizes these data.

Biochemical Analysis

Darter specimens from 15 populations were analyzed for gene products present at 18 presumptive loci. Of these 18 loci examined, 6 were monomorphic for all populations. A total of 46 alleles were

TABLE 9. Numbers of Dorsal Fin Soft Rays for Ten Boleosoma Populations[a]

AREA	N	Number of Dorsal Rays					
		11	12	13	14	15	16
1	124		3	25	50	45	1
2	50			4	23	21	2
3	42			2	15	23	2
4	22			2	10	9	1
5	30			3	6	21	
6	29		4	15	9	1	
7	30		1	5	16	8	
8	26		1	1	11	12	1
9	46	10	25	10	1		
10	30		18	11	1		

[a]For explanation of area numbers, refer to Table 2, page 13.

TABLE 10. Numbers of Dorsal Fin Spines for Ten *Boleosoma* Populations[a]

AREA	N	Number of Dorsal Spines				
		7	8	9	10	11
1	125			41	83	1
2	50			30	20	
3	42			28	14	
4	22			18	4	
5	30			24	6	
6	29		4	23	2	
7	30		5	23	2	
8	26		3	20	3	
9	46		12	31	3	
10	30	1	9	17	3	

[a]For explanation of area numbers, refer to Table 2, page 13.

TABLE 11. Numbers of Anal Fin Spines and Soft Rays for Ten *Boleosoma* Populations[a]

AREA	N	Anal Spines			Anal Soft Rays				
		1	2	3	6	7	8	9	10
1	126	13	113			5	41	71	9
2	50	7	43			2	9	28	11
3	42	6	36			1	16	21	4
4	22	8	14				10	11	1
5	30	12	18			1	11	17	1
6	29	8	21		1	8	17	3	
7	30	6	24		3	14	12	1	
8	26	5	21			2	12	12	
9	46	15	30	1	12	28	5	1	
10	30	5	25			16	13	1	

[a]For explanation of area numbers, refer to Table 2, page 13.

TABLE 12. Numbers of Preoperculomandibular (POM) Cephalic Canal Pores for Ten *Boleosoma* Populations[a]

AREA	N	Preoperculomandibular Pores								
		5	6	7	8	9	10	11	12	13
1	126						26	85	15	
2	50						7	37	5	1
3	42						2	35	5	
4	22						6	14	2	
5	30						7	19	3	1
6	29					2	20	7		
7	30					15	14	1		
8	26				2	7	15	2		
9	46					2	22	22		
10	30	3	3	1	4	3	9	6	1	

[a]For explanation of area numbers, refer to Table 2, page 13.

TABLE 13. Numbers of Infraorbital (IO) Cephalic Canal Pores for Ten Boleosoma Populations[a]

AREA	N	Infraorbital Pores		
		7	8	9
1	126	2	81	43
2	50	1	40	9
3	42	1	34	7
4	22		21	1
5	30	1	27	2
6	29		27	2
7	30		29	1
8	26		26	
9	46		46	
10	30		28	2

[a]For explanation of area numbers, refer to Table 2, page 13.

TABLE 14. Summary of Body Proportional Measurements for Ten Boleosoma Populations Expressed as a Percentage of Standard Length[a]

Area No.	1	2	3	4	5	6	7	8	9	10
n =	134	50	42	47	30	29	30	26	46	30
Body Depth	11.3-19.0 (15.0)	14.0-17.4 (15.8)	13.8-18.7 (15.3)	15.3-19.2 (17.2)	13.3-18.4 (15.8)	12.1-16.8 (14.7)	13.9-16.5 (14.9)	14.2-19.4 (16.1)	15.3-21.9 (17.7)	14.0-18.1 (16.2)
Head Length	13.6-27.4 (24.8)	21.4-30.3 (26.5)	23.7-31.1 (26.2)	24.4-28.6 (26.5)	21.5-29.1 (27.0)	14.6-29.4 (25.6)	24.6-27.3 (26.5)	23.8-26.8 (25.7)	23.7-29.5 (26.6)	23.7-27.6 (25.8)
Head Depth	8.3-16.4 (13.1)	11.8-16.0 (14.2)	10.9-15.2 (12.9)	13.0-16.7 (15.0)	11.0-16.7 (13.7)	10.7-13.0 (11.7)	10.1-11.9 (10.8)	10.8-14.7 (12.3)	10.7-14.0 (12.2)	10.0-13.2 (11.5)
Head Width	10.3-15.7 (13.5)	12.5-16.4 (14.4)	12.0-16.0 (13.8)	13.0-16.7 (15.2)	11.2-17.5 (14.5)	13.0-16.1 (14.1)	12.8-15.9 (14.5)	11.7-16.4 (15.0)	13.9-18.4 (15.9)	12.1-17.3 (15.6)
Snout Length	4.6-9.7 (6.8)	5.8-9.0 (7.2)	5.6-8.2 (6.9)	4.9-9.1 (7.3)	5.6-9.1 (7.0)	6.1-8.4 (7.2)	6.6-7.7 (7.1)	6.8-8.9 (7.8)	5.9-8.5 (7.1)	6.6-8.9 (7.4)
Upper Jaw Length	5.5-11.3 (7.5)	5.8-11.3 (8.3)	5.8-10.8 (7.3)	6.5-9.8 (8.1)	6.1-9.1 (7.6)	6.3-9.8 (7.3)	6.2-7.7 (7.2)	6.6-8.0 (7.2)	5.6-8.2 (7.2)	6.4-8.3 (7.3)
Eye Length	4.6-9.7 (6.2)	5.4-9.0 (7.0)	5.7-8.3 (7.1)	6.2-8.2 (7.2)	5.5-8.2 (7.0)	6.0-7.2 (6.6)	6.0-7.2 (6.5)	6.4-8.1 (7.2)	5.8-7.7 (7.0)	5-5-7.8 (6.6)
Candal Peduncle Length	5.8-9.4 (7.8)	7.1-9.4 (8.2)	7.6-9.4 (8.3)	7.3-10.0 (8.6)	5.6-10.0 (8.4)	7.2-9.6 (8.7)	8.2-9.9 (8.8)	7.8-10.1 (8.9)	8.7-10.5 (9.5)	7.8-11.6 (9.0)

[a]Ranges are given for each measurement followed by mean enclosed in parentheses. For explanation of area numbers, refer to Table 2, page 13.

resolved. Allele frequencies for the 12 variable loci are given in Table 15, and estimates of genetic variability for all populations are listed in Table 16. For relative mobilities of all allelic products of variable loci, see Figure 5.

G-tests for goodness of fit revealed three significant deviations from expected Hardy-Weinberg frequencies at the 0.05 level. These were: PGM-1 in the Coharie Creek population; GOT-1 in the Waccamaw River R-4 population; PEP-2 in the Cayuga Inlet population. No consistent pattern of heterozygote excess or deficiency could be detected at polymorphic loci from any population.

Several enzyme systems examined during this study deserve special comments. Accounts of these follow. General Protein: four loci, three migrating anodally and one migrating cathodally, were resolved for this system and designated GP-1, GP-2, GP-3 and GP-4 (Figure 6). Buth (1982) also resolved four loci in a study of five darter species for a general protein stain. He determined the more slowly migrating anodal locus (analogous to my GP-3 locus) to represent the enzyme creatine kinase and designated this locus CK-A. In addition, he postulated that the two faster migrating anodal loci were acid calcium binding proteins (Cbp-1 and Cbp-2). These are analogous to my GP-1 and GP-2 loci, respectively. The cathodal component could not be identified and was designated GP-1 by Buth (1982). This locus I have labeled GP-4.

The loci GP-1, GP-2, and GP-4 were monomorphic for all populations examined. Two alleles $GP\text{-}3^b$ and $GP\text{-}3^c$ were resolved for GP-3

PO leles			IDH Alleles			PEP-1 Alleles		PEP-2 Alleles			PGM-1 Alleles				PGM-2 Alleles			
c	e	f	b	c	d	b	c	b	c	d	a	b	c	d	a	b	c	e
-	-	1.00	.584	-	.417	1.00	-	1.00	-	-	-	-	1.00	-	-	.959	-	.042
-	-	1.00	.650	-	.350	1.00	-	1.00	-	-	-	-	1.00	-	-	.925	-	.075
-	-	1.00	.291	.065	.645	1.00	-	1.00	-	-	-	-	1.00	-	.016	.967	-	.016
-	-	1.00	.276	.034	.690	1.00	-	1.00	-	-	-	-	1.00	-	.034	.965	-	-
-	-	1.00	.083	.116	.800	1.00	-	1.00	-	-	-	-	.933	.067	-	1.00	-	-
-	-	1.00	.043	.109	.848	1.00	-	1.00	-	-	-	-	1.00	-	-	1.00	-	-
-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-	.063	.937	-	-	.969	.032	-
-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-	.056	.945	-	.111	.889	-	-
.063	-	.938	-	-	1.00	1.00	-	1.00	-	-	-	.772	.227	-	.045	.954	-	-
-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-	.844	.156	-	-	1.00	-	-
-	-	.060	-	-	1.00	1.00	-	.920	.080	-	1.00	-	-	-	-	.842	.079	.079
-	-	.358	-	-	1.00	-	1.00	.125	.875	-	-	1.00	-	-	.040	.960	-	-
-	1.00	-	-	-	1.00	1.00	-	1.00	-	-	1.00	-	-	-	-	1.00	-	-
-	1.00	-	-	-	1.00	-	1.00	.067	.100	.833	.200	.800	-	-	-	1.00	-	-

TABLE 16. Estimates of Genetic Variability in 15 Populations of *Boleosoma*, Based on a Survey of 18 Gene Loci[1]

Population	$\bar{H}$[2]	P[3]	Number of loci Polymorphic	(+D; -D)[4]
Lake Waccamaw, North Shore	0.045	0.111	7	(1;4)
Lake Waccamaw, South Shore	0.067	0.167	4	(3;1)
Waccamaw R., R-1	0.050	0.111	8	(5;2)
Waccamaw R., R-2	0.063	0.167	6	(3;3)
Waccamaw R., R-3	0.052	0.222	6	(4;2)
Waccamaw R., R-4	0.039	0.167	6	(1;4)
Drowning Cr. (Pee Dee Dr.)	0.010	0.111	2	(1;0)
Mallard Cr. (Pee Dee Dr.)	0.031	0.167	7	(4;1)
Livingston Cr. (Cape Fear Dr.)	0.045	0.278	6	(1;2)
Coharie Cr. (Cape Fear Dr.)	0.063	0.278	6	(2;4)
Hinton Dr. (Santee Dr.)	0.070	0.167	3	(2;1)
Little R. (Neuse Dr.)	0.024	0.167	5	(3;2)
Cayuga Inl. (Oswego Dr.)	0.077	0.222	6	(1;3)
Etheostoma podostemone	0	0	0	-
Etheostoma longimanum	0.015	0.111	3	(0;2)

[1]Waccaman River localities are listed under materials.

[2]Observed average heterozygosity per individual in a population.

[3]Proportion of polymorphic loci (using 0.95 criterion).

[4]Selander's (1970) D-statistic. Numbers in parentheses represent the number of loci having an excess or deficiency of heterozygotes, respectively.

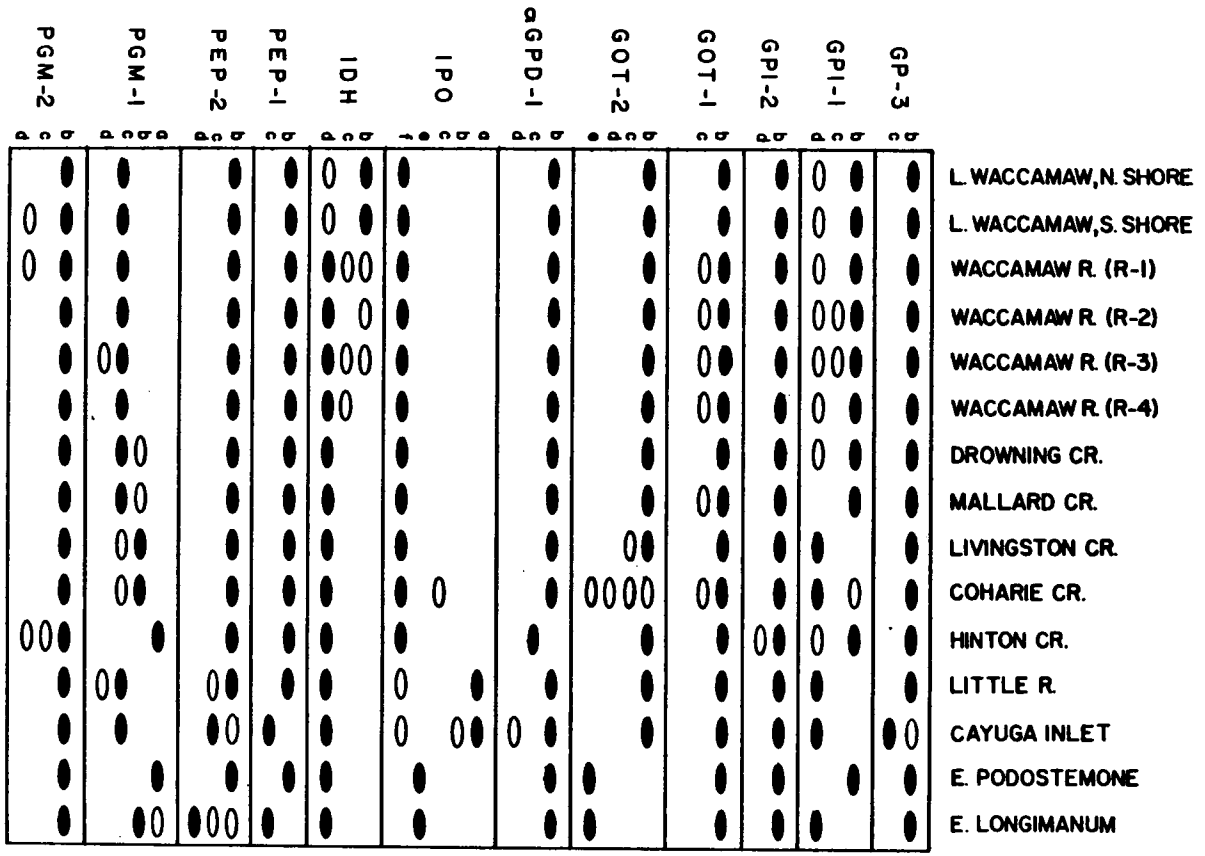

FIGURE 5. Electrophoretic mobilities of alleles in populations of Boleosoma studied. Diagrams represent relative mobilities of electromorphs and do not include heterozygotes or heterodimers. Alleles occurring at frequencies greater than 0.50 are represented by solid symbols. Those occurring at frequencies less than 0.50 but greater than 0.05 are represented by hollow symbols. Rare alleles occurring at frequencies less than 0.05 are not included. Waccamaw River localities are listed under materials.

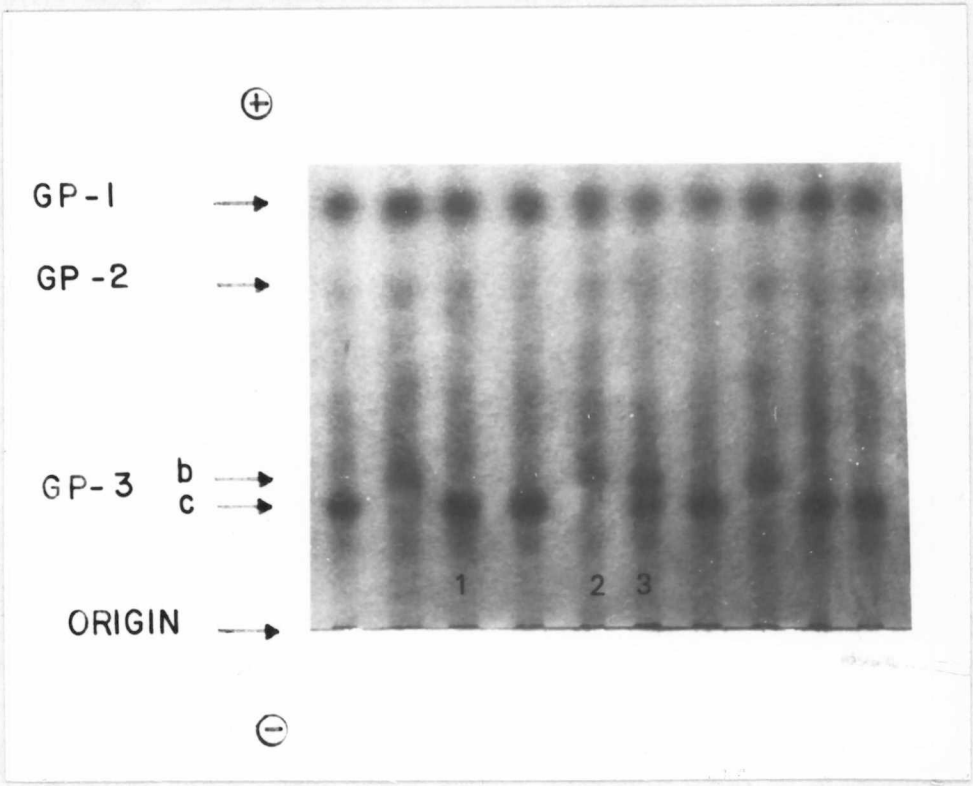

FIGURE 6. Gel stained for general protein. Three loci GP-1, GP-2 and GP-3 are illustrated. A fourth locus GP-4 migrates cathodally and is not shown. Genotypes of the variable GP-3 (numbered individuals) are: (1) $GP\text{-}3^{c}/GP\text{-}3^{c}$; (2) $GP\text{-}3^{b}/GP\text{-}3^{b}$; (3) $GP\text{-}3^{b}/GP\text{-}3^{c}$.

in the Cayuga Inlet (Oswego) population. All other populations were fixed for the allele GP-3^b.

Isocitrate dehydrogenase: a single locus, IDH, was scored for this enzyme system (Figure 7). An additional faster, less intense locus was observed, but not scored (see discussion for PGM-1 locus). Three IDH alleles were resolved, two of which (IDH^b and IDH^c) occurred only in Waccamaw drainage populations. All other populations were fixed for the IDH^d allele, which also occurred in all Waccamaw populations examined. The IDH^c allele was not present in Lake Waccamaw itself, and occurred only in low frequencies throughout the Waccamaw River. The second allele unique to the Waccamaw drainage (IDH^b), occurred as the dominant allele in Lake Waccamaw, but was gradually replaced further down the Waccamaw river by the IDH^d allele. This downriver replacement was somewhat clinal (see Figure 8).

Octanol dehydrogenase: two loci were apparent at this enzyme system, ODH-1 and ODH-2. ODH-1 was monomorphic for all populations examined. ODH-2 appeared to be slightly variable, but because of scoring inconsistancies, this locus was not considered in the analysis. Etheostoma podostemone appeared to be somewhat polymorphic at the ODH-2 locus, the only locus showing any variability for this species.

Phosphoglucomutase: two presumptive loci were scored for this system (Figure 9). The slower migrating, more intense locus (PGM-2) apparently corresponds to the single PGM locus described by Avise and Selander (1972) as occurring in fishes. The faster migrating

FIGURE 7. Gel stained for isocitrate dehydrogenase. A single IDH locus is illustrated. Genotypes of numbered individuals are: (1) IDH^b/IDH^c; (2) IDH^d/IDH^d; (3) IDH^b/IDH^b; (4) IDH^b/IDH^d.

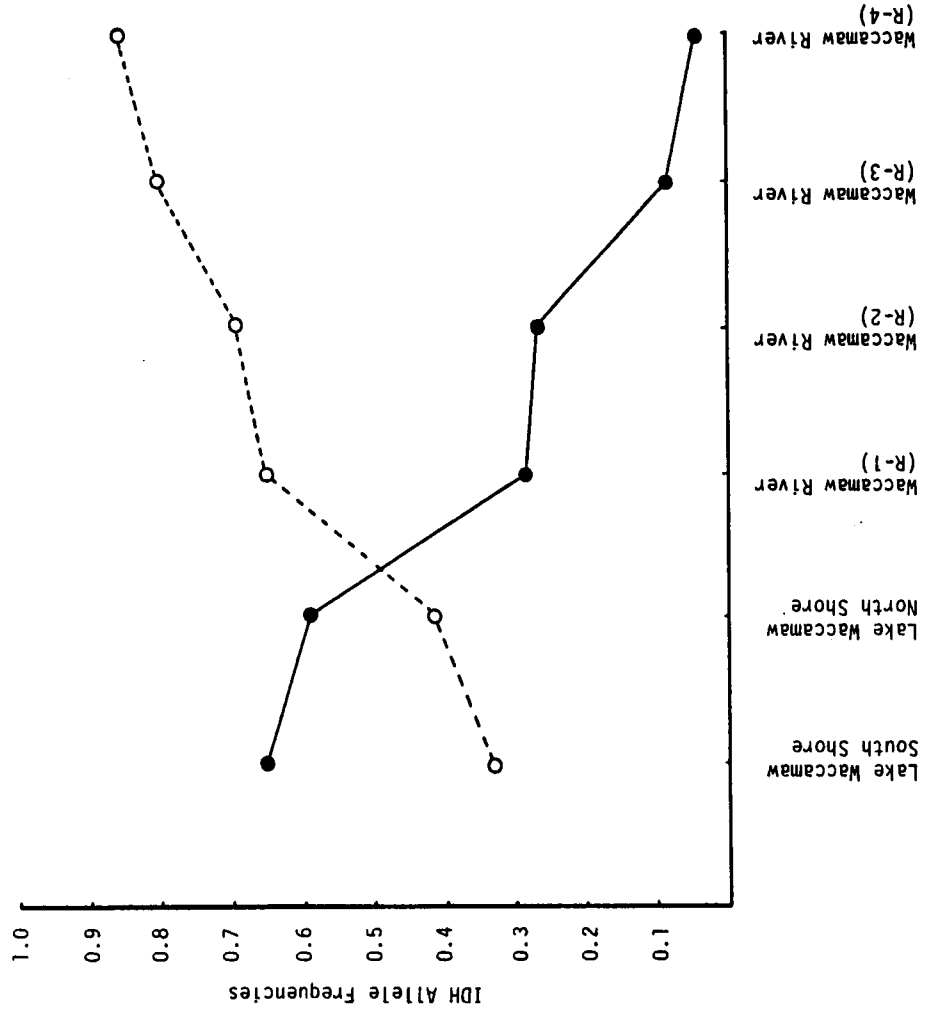

FIGURE 8. Relative frequencies of the IDH^b (solid line) and IDH^d (dashed line) alleles in Boleosoma of the Waccamaw drainage. Refer to Materials Section for Waccamaw River localities.

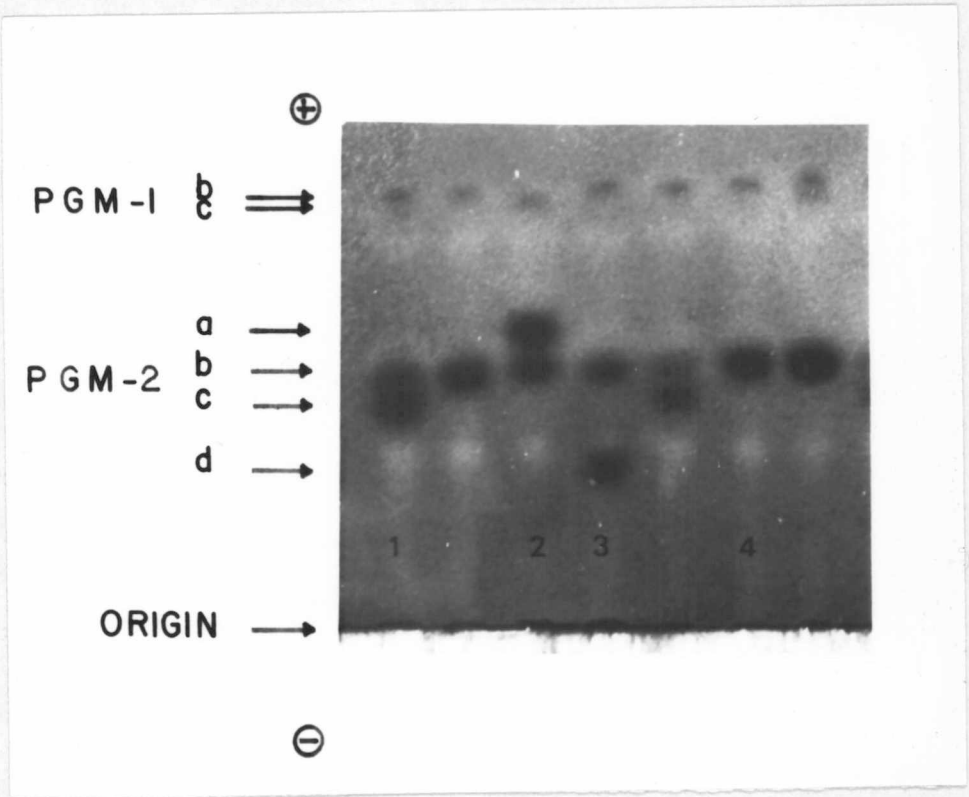

FIGURE 9. Gel stained for phosphoglucomutase. Two loci, PGM-1 and PGM-2 are illustrated. Genotypes for numbered individuals are: (1) PGM-1^b/PGM-1^b, PGM-2^b/PGM-2^c; (2) PGM-1^c/PGM-1^c, PGM-2^a/PGM-2^b; (3) PGM-1^b/PGM-1^c, PGM-2^b/PGM-2^d; (4) PGM-1^b/PGM-1^b/PGM-1^b, PGM-2^b/PGM-1^b.

locus, designated PGM-1, was much less intense, although consistently scoreable. This locus appears to be identical to the faster locus observed on gels stained for isocitrate dehydrogenase. In addition, this locus was sometimes apparent on gels stained for glucose-6-phosphate dehydrogenase and malate dehydrogenase (not used in this study). This unidentified locus was most consistantly scoreable (i.e., more intense) on gels stained for phosphoglucomutase, thus was tentatively designated PGM-1 for convenience.

An esterase system was also employed for most of the populations examined during this study with two polymorphic loci evident. Neither of these loci could be confidently scored for all populations, and goodness of fit G-tests revealed significant deviations from Hardy-Weinberg probabilities in several of the populations. Because these deviations were generally a deficiency of heterozygates, the presence of a "null" allele cannot be discounted. For these reasons, neither of the esterase loci were considered in this analysis. It was evident, however, that the upper esterase locus varied in a fashion similar to that of IDH within the Waccamaw drainage. Even though exact allele frequencies could not be calculated, it was obvious that an allele common in Lake Waccamaw was gradually replaced by another allele in populations farther down the Waccamaw River. As with the IDH locus, this replacement appeared to occur clinally.

Genetic similarity coefficients are presented in Table 17. All populations from within the Waccamaw drainage were genetically very similar (including those from Lake Waccamaw), with an average

TABLE 17. Nei's (1972) Genetic Identity Coefficients (I) (Above Diagonal) and Genetic Distance Coefficients (D) (Below Diagonal), Calculated from 18 Loci[a]

	Lake Waccamaw North Shore 1	Lake Waccamaw South Shore 2	Waccamaw R. R-1 3	Waccamaw R. R-2 4	Waccamaw R. R-3 5	Waccamaw R. R-4 6	Drowning Cr. (Pee Dee) 7	Mallard Cr. (Pee Dee) 8	Livingston Cr. (Cape Fear) 9	Conarie Cr. (Cape Fear) 10	Hinton Cr. (Santee) 11	Little R. (Neuse) 12	Cayuga I. (Oswego) 13	E. podostemone 14	E. longimanum 15
1		.998	.995	.994	.987	.983	.980	.978	.900	.881	.854	.887	.755	.808	.683
2	.002		.990	.991	.982	.976	.972	.970	.901	.880	.851	.888	.757	.800	.685
3	.005	.010		.998	.997	.994	.993	.993	.906	.891	.865	.893	.761	.823	.690
4	.006	.009	.002		.997	.995	.992	.991	.924	.903	.871	.910	.778	.819	.706
5	.013	.018	.003	.003		.998	.997	.996	.918	.902	.874	.902	.771	.829	.703
6	.018	.024	.006	.005	.002		.995	.995	.916	.900	.869	.902	.770	.823	.699
7	.020	.029	.007	.008	.003	.005		.999	.917	.903	.880	.899	.770	.835	.706
8	.022	.030	.007	.009	.004	.005	.001		.911	.897	.876	.893	.763	.833	.698
9	.106	.104	.098	.080	.085	.088	.086	.093		.975	.917	.914	.779	.791	.803
10	.126	.127	.116	.102	.104	.106	.102	.108	.025		.898	.878	.739	.847	.838
11	.157	.161	.145	.138	.134	.140	.128	.133	.087	.108		.820	.703	.756	.714
12	.120	.119	.114	.094	.103	.104	.107	.113	.090	.130	.198		.842	.781	.761
13	.281	.279	.273	.250	.260	.261	.261	.271	.249	.303	.352	.172		.629	.772
14	.213	.224	.195	.200	.187	.194	.181	.183	.234	.166	.280	.248	.464		.822
15	.381	.379	.371	.348	.352	.358	.348	.360	.219	.177	.337	.273	.258	.196	

[a]Waccamaw River localities are listed under materials.

value of I = 0.992 (ranging from 0.976 to 0.998). The Waccamaw populations were most similar to the two Pee Dee drainage populations with I values ranging from 0.972 to 0.997 (mean I = 0.988) between Waccamaw drainage populations and Drowning Creek, and 0.970 to 0.996 (mean I = 0.987) between Waccamaw drainage populations and Mallard Creek. The two Pee Dee populations were essentially identical to one another (I = 0.999). The Waccamaw populations were somewhat more divergent from the two Cape Fear populations (mean I = 0.902, range 0.880 to 0.924), the Santee population (mean I = 0.864, range 0.851 to 0.874), and the Neuse population (mean I = 0.897, range 0.887 to 0.910). All southern E. olmstedi populations (including ones from the Waccamaw drainage) clustered phenetically (Figure 10) with an average genetic identity of I = 0.889.

The greatest degree of genetic divergence between the Waccamaw populations and any other E. olmstedi involved the Oswego drainage (Cayuga Inlet) population (mean I = 0.765, range 0.755 to 0.778). This population clustered apart from all others (Figure 10). Mean values for I between the two outgroup species, E. podostemone and E. longimanum and the Waccamaw populations were 0.817 (0.800 to 0.829) and 0.694 (0.683 to 0.706), respectively. These two species clustered with one another in the phenogram (Figure 10).

Heterogeneity among darter populations within the Waccamaw drainage was tested using the two most highly polymorphic loci, GPI-1 (Figure 11) and IDH. Significant heterogeneity (P=0.05) for all heterogeneity tests) was detected when all Waccamaw populations

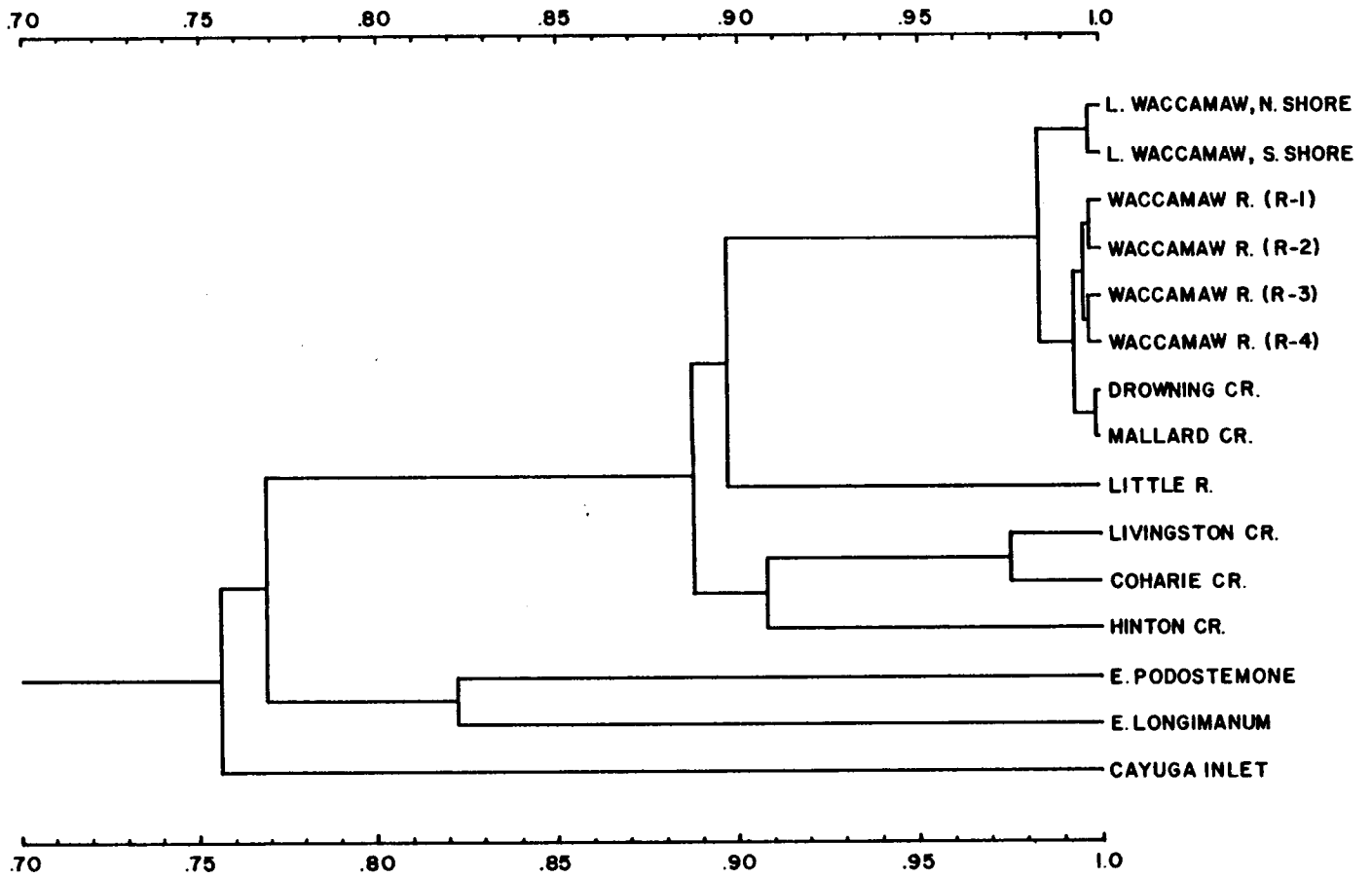

FIGURE 10. Phenogram, clustered using the unweighted pair-group method with arithmatic means (UPGMA) from Nei's (1972) coefficients of genetic similarities (I).

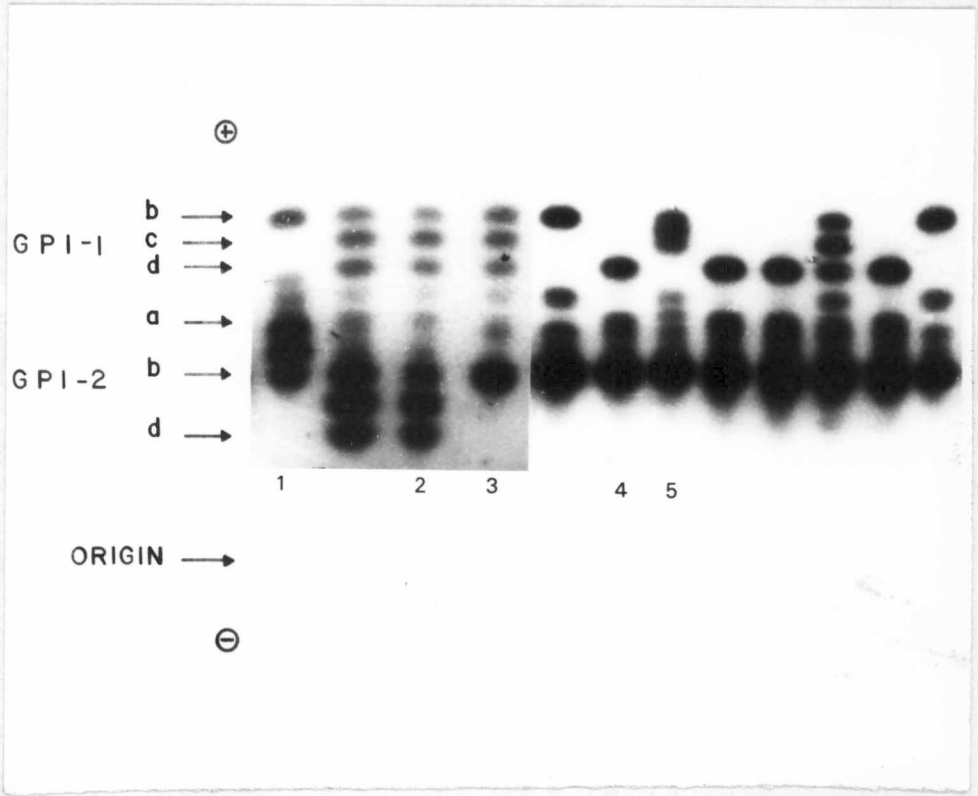

FIGURE 11. Gel stained for glucosephosphate isomerase. Two loci, GPI-1 and GPI-2 are illustrated. Genotypes for numbered individuals are: (1) GPI-1^b/GPI-1^b, GPI-2^a/GPI-2^b; (2) GPI-1^b/GPI-1^d, GPI-2^b/GPI-2^d; (3) GPI-1^b/GPI-1^d, GPI-2^b/GPI-2^b; (4) GPI-1^d/GPI-1^d, GPI-2^b/GPI-2^b; (5) GPI-1^b/GPI-1^c, GPI-2^b/GPI-2^b.

were considered together for both GPI-1 (G_H = 24.224; df = 10) and IDH (G_H = 34.453; df = 10). Subsequently, subsets of these populations were tested. For the IDH locus, no significant heterogeneity was present between the two Lake Waccamaw samples (G_H = 0.482; df = 2), although for the same two populations significant heterogeneity was detected for the GPI-1 locus (G_H = 12.536; df = 2).

Conversely, tests using the IDH locus revealed significant heterogeneity (G_H = 22.230; df = 6) between the four Waccamaw River populations, where none was detected using GPI-1 (G_H = 11.005; df = 6). With the four Waccamaw River populations subdivided where the two upper stations were compared and the two lower stations compared, no significant heterogeneity was detected at the IDH locus (G_H = 0.665; df = 2 and G_H = 0.741; df = 2, respectively).

CHAPTER V

DISCUSSION

Genetic Variation

Estimates of genetic variation observed in populations of Etheostoma olmstedi (including the Lake Waccamaw populations of "perlongum") are comparable to those obtained in other electrophoretic studies on fishes (see, for example: Avise and Selander, 1972; Avise and Smith, 1974; Avise et al., 1977; Buth and Burr, 1978; Merritt et al., 1978; Buth et al., 1980; and Falls, 1982). In addition, while mean heterozygosity ($\bar{H}$) values (Table 16) vary greatly from one population to another, the average value over all olmstedi populations of 0.049 is reasonably close to the estimate of 0.051 obtained by Falls (1982) for E. olmstedi in the James River drainage of Virginia.

The single population of E. podostemone examined showed no variability in the 18 loci scored and reported in this paper. An apparent reduction of genetic variability is also evident in E. longimanum ($\bar{H}$ = 0.015). Etheostoma podostemone and E. longimanum are closely related, though well differentiated, and each is confined to a single drainage (the upper Roanoke and upper James drainages, respectively). Despite the limited distributions of these species, both are relatively common in their respective drainages. Since only one population of each was sampled, it is not known whether a general

reduction in variability is characteristic of these species, or if these populations are atypical of other populations of E. longimanum or E. podostemone.

Relationships Between Lake Waccamaw and Waccamaw River Populations

Both morphological and biochemical data support the hypothesis regarding the high degree of relatedness between the Boleosoma populations in Lake Waccamaw and those found throughout the Waccamaw River. Genetic similarity coefficients are high between all of the Waccamaw drainage populations (Table 17) but tend to decrease as populations further down river are compared with those in the lake.

Though very similar to one another, a reasonable amount of genetic heterogeneity exists among darter populations in the Waccamaw drainage. Heterogeneity G-tests revealed significant heterogeneity between Lake Waccamaw populations and populations in the Waccamaw River for both loci (IDH and GPI-1) tested. Significant heterogeneity was also present between the two upper and two lower Waccamaw River populations for the IDH locus. These results suggest that gene flow is at least somewhat reduced between lake and river populations, probably due to the presence of a low dam at the river's origin along the south shore of Lake Waccamaw. Although darters could possibly enter the lake from the river during periods of high water (this is normal during winter and early spring months), significant numbers apparently do not. This is evidenced in part by the apparent absence

of the IDH^C allele in Lake Waccamaw, and its presence in all four Waccamaw River populations.

At least two factors could account for the amount of heterogeneity present between the upper and lower Waccamaw River samples. The first is distance between samples. The upper two river samples are separated by a distance of as few as three river km, while the third sample is separated from the upper by roughly 15 river km. The fourth sample is located approximately 50 river km below the third. Even though this is a substantial distance, the homogeneity between the lower samples could probably be explained by the second factor, habitat disruption. Between the upper and lower stations, the Waccamaw River widens and deepens into an area known locally as "the fishponds." This section of the river presents an area much more swamp-like, with greatly reduced flow. This is in contrast to the generally shallow, sandy, current-swept stretches of river both upstream and downstream of "the fishponds." Suitable Boleosoma habitat is not available in "the fishponds," and no E. olmstedi are known from there (Shute et al., 1981). Therefore, "the fishponds" may tend to reduce gene flow between darter populations. The lower two populations, even though separated by greater distances, maintain homogeneity because of the continuity of favorable habitat.

Significant heterogeneity at the GPI-1 locus was also present between the two Lake Waccamaw populations. The samples were collected at opposite ends of the lake, and were separated by approximately 5 km. In a relatively homogeneous environment such as that present

in Lake Waccamaw, this small distance would not appear to be sufficient to prohibit panmixia. Darters are relatively sedentary fishes however, and at least one study (Lee and Ashton, 1981) has shown that tagged specimens of two darter species moved very little throughout the year.

Waccamaw darters are known to winter in offshore waters in the middle of the lake and move inshore during the early spring to establish territories and spawn (Lindquist et al., 1981). Adults die shortly thereafter (Shute et al., 1982b), and the young eventually return to offshore waters the following winter. Although there is no evidence for this, the darters spawned on a particular shore may return to the same general area the following spring, thus effectively subdividing the entire population of Waccamaw darters in the lake. This could account for the observed heterogeneity between the lake populations. If this hypothesis were true, the observed differences might be the result of genetic drift rather than differential selective pressures, which certainly would appear not to exist in Lake Waccamaw.

Morphological analyses suggest clinal variation existing between lake and river populations of Boleosoma (Figures 2-4, pp. 14, 18 and 20). At least one enzyme locus (IDH) also demonstrates distinct clinal variation of allele frequencies in the comparison of lake and river darter populations (Figure 2, page 14).

Clinal variation of allele frequencies has been reported for a number of other fish species (Koehn, 1970; Merritt, 1972; Avise and Smith, 1974; Johnson, 1975; Mitton and Koehn, 1975; Merritt

et al., 1978; Powers and Place, 1978; Cashon et al., 1981; and others). Most of these studies, however, reported clinal variation occurring over an extensive geographical range, usually (with the exception of that reported by Avise and Smith, 1974) along a north-south latitudinal gradient.

Powers and Place (1978) propose three possible mechanisms for the establishment and maintenance of clinal variation in the killifish, Fundulus heteroclitus. The first involves random genetic drift of selectively neutral genes, whereby isolation of the northern and southern populations, followed by migration and subsequent gene flow between these populations, established allelic clines.

The second theory is similar to the first in assuming subdivision of the original population into northern and southern subpopulations. Directional selection acting upon the isolates (possibly in regards to temperature extremes) would affect genetic divergence. Migration after the "removal of the isolating barriers" would again result in gene flow and potentially, clinal variation.

The third theory presented by Powers and Place involves no subdivision of the population. This mechanism implies that clines originate in response to selective pressures relevant to an environmental gradient (temperature is again implied). These theories were elaborated upon by Cashon et al. (1981).

Avise and Smith (1974) reported an east-west clinal variation of allele frequencies in populations of the bluegill, Lepomis macrochirus. They hypothesized that this variation arose as a result

of independent evolution of bluegill races (one on peninsular Florida and one on the mainland), followed by their subsequent reunion, a resumption of gene flow, and eventual intergradation. This mechanism is similar to that later hypothesized for clinal variation in Fundulus heteroclitus by Powers and Place (1978) discussed above. This is also similar to what may have happened with the Waccamaw darters.

If E. perlongum evolved in Lake Waccamaw in isolation from other Boleosoma stock (this possibility will be discussed elsewhere in this thesis), one or both of the following mechanisms could account for the genetic divergence of the lake populations:

1. Random genetic drift, primarily due to the founder principle. A tendency toward either fixation or deletion of rare alleles could occur as a result of this.

2. Natural selection. Differential selecting factors (i.e., primarily lacustrine verses riverine, but other factors also may be implicated) could favor unique characteristics in the Lake Waccamaw population of darters.

Available biochemical data is consistent with what is expected as a result of the founder principle. Several alleles present in Waccamaw River darter populations could have been eliminated, or nearly so, from the lake populations. These include: GPI-1^{c}; GOT-1^{c}; and IDH^{c} (see Figure 5, page 33). Although no unique alleles were detected in the lake, the IDH^{b} allele occurred at greater frequencies in lake populations than in river populations (Figure 5, page 33). This, of course, does not rule out differential selection.

Regardless of the mechanisms responsible for this divergence of characters, the extensive intergradation and clinal variation present where lake and river populations converge provides evidence suggesting an absence of reproductive isolating mechanisms between the two forms, resulting in a nearly random mixing of genes. The processes leading to possible speciation of the Waccamaw darter, if they occurred, were apparently interrupted when contact occurred between the lake and river forms of Etheostoma olmstedi, resulting in incomplete divergence of the two.

Interdrainage Relationships

A high degree of genetic similarity exists not only among Waccamaw Boleosoma populations, but also between Waccamaw populations and those of the Pee Dee drainage (Table 17, Figure 10). Presumably a considerable amount of gene flow occurred between the Waccamaw and Pee Dee populations of E. olmstedi, at least as recently as the last glacial advance and its associated lowered sea level. This allowed mixing of the forms from these drainages in the lower stretches of the Waccamaw and Pee Dee rivers where they converged on the extended coastal plain area of that period. Present gene flow would be prevented, or at least severely limited, due to high salinities in Winyah Bay where the Waccamaw River presently joins the Pee Dee (Shute et al., 1981).

Despite their genetic similarities, the E. olmstedi populations of the Pee Dee drainage are easily separable from those of Lake

Waccamaw and the upper Waccamaw River based on morphology. They differ primarily in number of lateral line scales (Table 2, page 13, Figure 2, page 14), and degree of nape and cheek squamation (Table 5, page 17, Figure 3, page 18, and Table 6, page 19, Figure 4, page 20, respectively), modal number of dorsal fin rays (Table 9, page 24) and modal number of anal fin rays (Table 11, page 26). Morphologically, the darters of the Pee Dee drainage resemble those of the Cape Fear and Santee drainage (Tables 2-14, pp.13,15,16,17,19,21,22,24,25,26,27,28,29).

Inter drainage genetic similarities are relatively consistent for the southern populations of Etheostoma olmstedi, with the exceptional case between the Waccamaw and Pee Dee drainages discussed above. Though the Waccamaw populations are biochemically less similar to the Cape Fear, Santee, and Neuse populations than they are to the Pee Dee populations (Table 17, page 40), these differences are no greater than differences between the Pee Dee, Cape Fear, Santee, and Neuse drainages. Generally, similarity (I) values range from 0.850 to 0.923 between any two of the above drainages (excluding comparisons between the Waccamaw and Pee Dee drainages).

The greatest divergence between any of the drainages discussed above occurs between the Santee and Neuse populations (I = 0.820). Two major drainages (Pee Dee and Cape Fear) separate these populations. None of the other populations of E. olmstedi surveyed in this study are separated by more than one drainage except those from Cayuga Inlet in New York.

Geographic separation alone could account for the increased genetic divergence of these two populations. Other studies involving fishes with extensive geographical ranges have demonstrated that this increased divergence between widely separated populations is probably the rule rather than the exception (see, for example: Johnson, 1975; Avise and Smith, 1974; Avise et al., 1977; Buth and Burr, 1978). Avise and Smith (1974) point out that this is probably due to the island-like effect created by independent river drainages, whereby fish populations are subdivided and are subject to the effects of genetic drift.

Interspecific Variation Within Boleosoma

Studies of genetic variation over a wide range of gene loci in darters are limited. Buth et al. (1980) and McKeown et al. (1984) present genetic identity values (Nei, 1972) for darters of the subgenus Microperca and for the Etheostoma variatum species complex, respectively. Both studies reported between drainage genetic identities within a species to be generally much higher than those detected in the present study: I = 0.99 to 1.00 between populations of E. proeliare and I = 0.94 to 1.00 between populations of E. microperca (Buth et al., 1980); I = 0.924 between two populations of E. tetrazonum and I = 0.998 between two populations (separate subspecies) of E. euzonum (McKeown et al., 1984). McKeown et al. (1984) also found interspecific genetic similarities to be very high, ranging from I = 0.846 between E. variatum and E. tetrazonum to I = 0.998 between E. tetrazonum

and E. euzonum. Buth et al. (1980) found much lower interspecific similarities between species of the subgenus Microperca. In their study, genetic identities ranged from 0.55 to 0.57 between E. fonticola and E. microperca, from 0.88 to 0.89 between E. fonticola and E. proeliare, and from 0.56 to 0.64 between E. microperca and E. proeliare.

Interspecific variation within the subgenus Boleosoma is at least somewhat comparable to that found by Buth et al. (1980) for the subgenus Microperca. Identities range from I = 0.755 to 0.846 between E. podostemone and the southern E. olmstedi populations (including those from Lake Waccamaw) and from I = 0.683 to 0.837 between E. longimanum and the southern E. olmstedi populations. The genetic identity between E. podostemone and E. longimanum I = 0.822) also falls within this range.

One population of E. olmstedi sampled in this study, Cayuga Inlet, is highly differentiated, genetically, from other olmstedi populations sampled. Cayuga Inlet (Oswego drainage) approaches the northern limit of E. olmstedi with populations to the north occurring only in scattered localities (Cole, 1967). This population is biochemically unique at a number of loci, most notably GP-3, IPO, PEP-1, and PEP-2 (Figure 5, page 33). Of all populations sampled, this was the only one to demonstrate variation at a general protein locus.

Darters from this general locality have been considered by Cole (1957, 1967) to represent populations intermediate between two subspecies, E. olmstedi atromaculatum and E. o. olmstedi. Cole (1967,

Table 2, page 13) presents character differences separating the recognized subspecies of E. olmstedi, but generally, E. o. atromaculatum is characterized by its fully scaled nape, cheek, breast and belly. The nape and breast of E. o. olmstedi are diagnosed as naked, with the cheek variously scaled and the belly moderately scaled. Cursory examination of frozen specimens used in the present study revealed well scaled nape, cheek, breast, and belly, characteristics of E. o. atromaculatum. Numerous problems have arisen involving the recognition of these two olmstedi subspecies and these will be discussed elsewhere in this thesis.

Several possible explanations exist regarding the degree of differentiation between the Cayuga population and those from North and South Carolina. Perhaps the simplest is the geographical distance between these populations. Relatively low genetic identities between drainages appear to be the rule in E. olmstedi, as discussed earlier. Each drainage constitutes a population (i.e., gene pool) that is more or less isolated from other such populations. The Cayuga population is separated from the other sampled populations by many major drainages, and the effects of genetic drift would amplify the amount of divergence between these drainages.

An additional theory is that E. olmstedi may also exibit north-south clinal variation in allele frequencies, resulting in highly divergent forms at either end of the cline. This cline would not have been detected in the present study because of the lack of electrophoretic data for the populations between Cayuga Inlet and North Carolina.

Finally, the gene pool of the Cauyga population may have been contaminated by possible contact with the closely related E. (Boleosoma) nigrum. Both Bruner (1980) and Page (1983, map 44) indicate the presence of E. nigrum in the general area of Cayuga Lake.

This possibility is supported by data presented by McAllister et al. (1972), demonstrating that gene exchange occurred between sympatric populations of E. nigrum and E. olmstedi in the Ottawa, Canada area. In their study, hybrids were detected using a simple character index of 10 morpholigical features and two electrophoretic (General Muscle Protein) characters. They found hybrids to constitute less than 7.6% of the population, with no conclusive evidence of backcrossing.

Clark (1978) reported morphological evidence for introgressive hybridization between E. olmstedi and E. nigrum in the James River drainage of Virginia. In a follow-up study, Falls (1982) employed electrophoretic techniques on specimens from the same localities studied by Clark (1978). He concluded that there was no evidence for hybridization between E. olmstedi and E. nigrum. The two were in fact separable based on differences at two phosphoglucose isomerase (= GPI of the present study) loci.

Despite Fall's (1982) evidence against hybridization between sympatric E. olmstedi and E. nigrum populations in the James drainage, the possibility exists that hybridization may be or may have been occurring between other sympatric populations, particularly those examined by McAllister et al. (1972). Echelle et al. (1975) concluded

that introgressive hybridization with E. radiosum seemed to constitute a major source of genetic divergence in certain populations of E. spectabile.

The Cayuga Inlet population demonstrated highest genetic affinity to the Little River population of the Neuse drainage (I = 0.842). In both populations the IPO^a allele occurred in frequencies above 50% (Figure 5, page 33) while all other olmstedi populations were fixed or nearly so for the IPO^f allele (Figure 5, page 33, Table 15, page 31). Still, the Little River population clusters with the rest of the North and South Carolina E. olmstedi populations where the Cayuga population does not (Figure 10, page 42). An electrophoretic study of populations all along the Atlantic coast could possibly provide answers to clarify the relationship of this Cayuga population to other E. olmstedi.

The highly polymorphic nature of the wide-ranging members of the subgenus Boleosoma (Etheostoma olmstedi and E. nigrum) has led to considerable confusion regarding their taxonomy. Numerous studies have questioned whether or not E. olmstedi should be recognized as a distinct species, or a subspecies of E. nigrum (Stone, 1947; Cole, 1957, 1965, and 1967; McAllister et al., 1972; Clark, 1978; Kott and Humphreys, 1978; Falls, 1982) and how to treat the variously named subspecies of either (Underhill, 1963; Zorach, 1971; Clark, 1978; Starnes and Starnes, 1979; Falls, 1982).

The ranges of the two species are largely separate with areas of sympatry occurring primarily in Canada's Maritime Provinces, Lake

Ontario drainage of New York, and possibly some of the Atlantic coastal streams of Virginia and North Carolina. Studies of these sympatric populations have revealed the apparent existence of hybrids, or integrades, between E. olmstedi and E. nigrum, but all appear in agreement that E. olmstedi deserves specific status.

Populations in the James, Chowan, and Roanoke drainages of Virginia are particularly problematic in that they are "variously intermediate or interjacent to typical populations (of E. olmstedi or E. nigrum) . . ." (Jenkins and Burkhead, manuscript). These authors noted that upstream populations were more nigrum-like and that downstream populations were more olmstedi-like.

In addition, there is great variation within each of the species and this had led to the description of various subspecies for both E. olmstedi and E. nigrum. Distinctions between these are based primarily on lateral-line scale numbers and degree of scalation on the nape, cheek, breast, and belly. Both species include subspecific forms which are well-scaled and those that are less scaled in these areas.

Intraspecific Variation Within Etheostoma nigrum

Etheostoma nigrum eulepis is the scaly johnny darter and was described as occurring primarily in glacial lakes of the upper midwest (Hubbs and Green, 1935). The range of the scaly johnny darter lies completely within that of E. n. nigrum, the less scaled form (Underhill, 1963), which occurs more often in rivers and streams.

Underhill (1963) studied the distribution of these two forms in Minnesota and determined that the range of variability was so great that to recognize subspecific distinction between the two seemed unwarranted.

Intraspecific Variation Within Etheostoma olmstedi

Four subspecies of E. olmstedi are currently recognized (Cole, 1967): E. o. olmstedi Storer; E. o. atromaculatum Girard; E. o. vexillare Jordan; E. o. maculaticeps Cope. Again these are distinguished primarily by the number of lateral-line scales and the degree of scalation of the nape, cheek, breast, and belly. In addition, E. o. maculaticeps usually has two anal spines where the others have only one (Cole, 1967). This is also the only subspecies of olmstedi totally allopatric to any of the other three.

Two of the subspecies, E. o. olmstedi and E. o. atromaculatum are distributed more or less continuously up and down the Atlantic slope. Etheostoma olmstedi olmstedi is normally found in the upstream portions of a drainage, where E. o. atromaculatum usually occupies the lower reaches. Both subspecies often occur in the same drainage and Cole (1967) noted several areas where populations appear to represent integrades between the two subspecies.

Zorach (1971) studied the distribution of these two subspecies of E. olmstedi in southeastern Virginia and encountered considerable problems in assigning subspecific names to many populations. Based on scalation patterns, Zorach considered most of the populations as

integrades between E. o. atromaculatum. Very few "pure" populations of either subspecies were encountered. In addition to morphological inconsistencies, Zorach (1971) noted that the distribution patterns of the two forms do not comply with the definition of subspecies suggested by Mayr (1969). For these reasons, Zorach suggested that E. o. atromaculatum should no longer be recognized as a valid subspecies.

Unpublished studies by Ronald Watson (personal communication) of E. olmstedi from the Potomac drainage of Virginia further exemplify the extreme variability exhibited by the species. Of 237 specimens examined by Watson, 42 (17.7%) had two anal spines. According to Cole (1967), E. o. maculaticeps is the only subspecies of E. olmstedi possessing two anal spines; all others were diagnosed as having only one. Specimens from the Potomac should, according to Cole (1967), be either typical E. o. atromaculatum, or integrades between E. o. atromaculatum, E. o. olmstedi. Etheostoma olmstedi maculaticeps ranges south from the Cape Fear drainage in North Carolina.

Watson also noted considerable variability in meristic values, including lateral-line scale numbers and number of predorsal scales. He implied, based on these data, that assigning subspecific names to populations within the Potomac serves no useful taxonomic purpose.

Falls (1982) further demonstrated that E. o. olmstedi and E. o. atromaculatum were indistinguishable, electrophoretically, in the James drainage of Virginia. Therefore, based upon the evidence presented by Falls and the morphological studies mentioned above, considerable doubt is cast on the validity of E. o. atromaculatum.

Apart from studies by Cole (1958, 1967), E. o. vexillare (endemic to the Rappahannock drainage of Virginia) and E. o. maculaticeps have received little systematic treatment. These two subspecies differ, however, from the two previously discussed in that their distributions comply to Mayr's (1969) description of that typical of subspecies.

Relationships Within Boleosoma

The Waccamaw darter, like typical E. o. maculaticeps, normally possess two anal spines. This presumably symplesiomorphic character tends to align the Waccamaw darters more closely with E. o. maculaticeps rather than any of the other (single-spined) subspecies of E. olmstedi. However, based on the limited number of populations sampled in this study, and with the exception of the Pee Dee drainage populations, the darters from the Waccamaw drainage appear no more closely related biochemically to E. o. maculaticeps from the Santee and Cape Fear drainages than to E. o. olmstedi from Little River in the Neuse drainage (Table 17, page 40). Genetic identity between the Waccamaw and Pee Dee drainages (typical E. o. maculaticeps) is, however, very high. These biochemical data neither support nor negate the hypothesis that the Waccamaw darters are closest to E. o. maculaticeps. They do, however, suggest that there is relatively low genetic divergence between subspecies of E. olmstedi.

Cole (1972) suggested that E. perlongum, E. longimanum and E. podostemone probably evolved from relict remnants of an earlier

Boleosoma stock. Cole (1971) also noted that E. o. maculaticeps was the least studied member of the olmstedi complex and that it may well represent the link between the olmstedi group and the perlongum-podostemone-longimanum group.

Hubbs and Raney (1946), in their description of E. perlongum and the other Waccamaw endemic fishes, suggested that these fishes evolved their unique characteristics in response to intense predation pressure. Their theory is based on the fact that all of these endemics are more streamlined in shape, and presumably quicker and better able to escape predators.

This theory was refuted by Frey (1951a), who postulated that the lake Waccamaw endemics evolved during a period when drier climatic conditions resulted in lower water levels in the lake, effectively isolating the fauna. A presumably small founding population could have led to accelerated genetic divergence, and ultimately to speciation of some forms.

Perhaps even a moderate drying of climatic conditions could have served to limit gene flow between lake and river darter populations. Before the construction of the dam early in this century, Lake Waccamaw was apparently subject to considerable water level fluctuation. Local residents, who lived around Lake Waccamaw before the construction of the dam, talk of being able to drive completely around the shoreline of the lake. During these times, the Waccamaw River was cut off from the lake and the upper stretches of the river became intermittent. Populations of Etheostoma olmstedi in the river would have also certainly been greatly reduced and disjunct.

Even if darter populations in Lake Waccamaw were not completely isolated from those in the river, a genetic bottleneck would be produced between the two populations. This, coupled with differential selection pressures in the two habitats, could have resulted in the unique ecomorph known as the Waccamaw darter.

Lake Waccamaw, as well as the rest of the Carolina Bays, is considered as a relatively young geological formation. Cooke (in Hubbs and Raney, 1946) suggested that the present site of Lake Waccamaw was covered by the sea during the Sangamon interglacial. Therefore, Lake Waccamaw could not have been formed until the last quarter of the Pleistocene. Frey (1951b) conducted pollen studies on Singletary Lake (a nearby Carolina Bay) and concluded that the lowermost sediments were deposited between 100,000 and 40,000 years before present (late Sangamon to well into the Wisconsin). If these estimates are correct, the period of isolation and/or divergence of the Waccamaw darters could not have been great.

Colonization of Lake Waccamaw by primary freshwater fishes was probably not possible until much later. Presumably, early conditions in Lake Waccamaw were that of an estuarine environment, as the Pleistocene seas began to retreat. Both of the other described endemics are derived from estuarine forms, therefore these may have been isolated much longer than the darters.

In a biochemical study on the genus Menidia, Johnson (1975) determined M. extensa, the Waccamaw silverside, to be the most divergent member of the genus. This presumably reflects its more

prolonged period of isolation from congeners. Presently there is no zone of secondary contact between Menidia extensa and Fundulus waccamensis and either of their presumed ancestors which are restricted to coastal environments along the Atlantic Coast. This zone of contact does, however, exist between the darters in Lake Waccamaw and their presumptive ancestor, E. o. mactulaticeps. Where this contact exists, an apparent breakdown of reproductive isolation has occurred, resulting in a resumption of gene flow between the two forms.

CHAPTER VI

CONCLUSIONS

Based upon biochemical and morphological data collected during this study, I suggest that Etheostoma perlongum (Hubbs and Raney) be placed in synonomy with E. olmstedi Storer. The recognition of the Waccamaw populations as a subspecies does not seem warranted for several reasons. Even though morphological differences exist between the Waccamaw populations and other E. olmstedi, these characters (i.e., scale patterns and numbers, and even fin ray numbers) appear likely to be under the influence of environmental conditions. In addition, the Waccamaw darter populations (lake and river) are nearly identical, electrophoretically, to the darter populations of the Pee Dee drainage, and are no more divergent in relation to the Cape Fear, Neuse, and Santee populations than either of these are to one another or to the Pee Dee drainage populations. Although several unique alleles are present in the darter populations of Lake Waccamaw and the Waccamaw River, this condition is also evident in several of the Etheostoma olmstedi populations in various other drainages. There is, however, one unique characteristic separating the Waccamaw darter from all other Boleosoma thus far studied. It is an annual population (Shute et al., 1982b). This condition may well be a product of the unique environment in which they live. Perhaps the water chemistry, water temperature, and high biological productivity produced conditions which favored a

rapidly growing annual darter. That annualism in this population is genetically pre-determined is somewhat doubtful. Specimens have been kept for several years in the laboratory under relatively uniform conditions (personal observation).

Another darter reported to be short-lived (i.e., less than two years) is Etheostoma microperca (Winn, 1958; Burr and Page, 1979). These have also been maintained and bred in captivity with some breeding individuals exceeding five years of age (Nancy Garcia, personal communication).

Because both the Waccamaw darter and E. o. maculaticeps possess two anal spines, and because the Waccamaw darter lives entirely within the range of E. o. maculaticeps, I suggest that the Waccamaw darter population be recognized as a unique ecotypic race of this subspecies.

A new grouping of species within the subgenus can now be suggested. Etheostoma olmstedi (including former E. perlongum) and E. nigrum should be recognized as a group. These two species are evidently very close, as is suggested by the hybridization and/or intergradation that occurs where the two are sympatric. The close relationship of these two species and distance from the others in Boleosoma is also indicated by the fact that, although E. nigrum occurs sympatrically with both E. longimanum and E. podostemone, hybrids are unknown (R. E. Jenkins, personal communication). Therefore, Etheostoma podostemone and E. longimanum form a second natural group. Both share numerous morphological similarities (Jenkins and Burkhead, manuscript) and the sharing of a unique allele

at the relatively conservative IPO locus, and a nearly unique allele at the GOT-2 locus (see Figure 5, page 33) tend to denote recent common ancestry.

Many questions remain unanswered regarding the taxonomy of the subgenus Boleosoma. The complex relationship between the closely related E. olmstedi and E. nigrum deserves further study as do the relationships between the subspecies of each. Previous studies have seemingly placed too great an emphasis on characters that tend to be highly plastic in relation to environmental influences. An intense biochemical study of populations from problem areas and over a wide range of ecological conditions could probably answer many of these remaining questions.

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APPENDIX

THE FOLLOWING ARE STAINS AND RECIPES USED DURING THIS STUDY

General Protein
(stock solution, can be reused): 2.0 g Napthol Blue Black
100 ml 5:5:1 methanol, deionized H_2O, acetic acid

Glucose-6-phosphate dehydrogenase: 47 ml 0.05M Tris-HCl, pH 8.0
3 ml 0.1M $MgCl_2 \cdot 6H_2O$)
10 mg glucose-6-phosphate
0.5 ml NADP
0.5 ml NBT
0.2 ml PMS

Glucosephosphate isomerase: 25 ml 0.2M Tris-HCl, pH 8.0
500 mg Ionager (for agar overlay)
15 ml distilled H_2O
10 mg fructose-6-phosphate
0.3 ml 0.1M $MgCl_2 \cdot 6H_2O$
0.3 ml G-6-PDH
0.6 ml NADP
0.1 ml PMS
1.2 ml MTT

Glutamate-oxaloacetate transaminase: 0.5 mg pyridoxal-5'-phosphate
50 mg L-aspartic acid
100 mg α-ketoglutaric acid
100 mg fast blue RR salt
50 ml 0.2M Tris-HCl, pH 8.0

α-Glycerophosphate dehydrogenase: 50 ml 0.2M Tris-HCl, pH 8.0
1 ml 0.1M $MgCl_2 \cdot 6H_2O$
250 mg α-DL-glycerophosphate
2 ml NAD
1.3 ml NBT
0.5 ml PMS

Indophnyloxidase: This locus was present on gels stained for octanol dehydrogenase and incubated under lights. See below for recipe.

Isocitrate dehydrogenase: 25 ml 0.1M $MgCl_2.6H_2O$
25 ml 0.2M Tris-HCl, pH 8.0
1 ml 0.1M Isocitrate
0.5 ml NADP
0.5 ml PMS
0.5 ml MTT

Octanol dehydrogenase: 50 ml 0.2M Tris-HCl, pH 9.0
1 ml 1-octanol (caprylic alcohol; octyl alcohol, normal)
5 ml NAD
1.3 ml NBT
0.1 ml PMS

Peptidase (PL/LGG): 20 mg L-phenylalanyl-L-leucine
20 mg L-leucylglycyl-glycine
20 mg peroxidase
10 mg O-dianisidine
10 mg snake venom
50 ml 0.2M Tris-HCL, pH 8.0
0.5 ml 0.25M $MnCl_2$

Phosphoglucomutase*: 30 ml distilled H_2O
5 ml 0.2M Tris-HCl, pH 8.0
5 ml 0.1 M $MgCl_2 \cdot 6H_2O$
70 mg glucose-1-phosphate
4 ml G-6-PDH
0.5 ml NADP
0.5 ml PMS
1.0 ml MTT

*Initially, 5 ml of glucose-1,6-diphosphate were used in addition to the above ingredients for the PGM stain. This was, however, discontinued after experimentation proved that the stain worked equally well without glucose-1,6-diphosphate on the fishes studied. By not using this component of the stain, the price was considerably less.

Stock solutions used in some of the above stains follow. These were kept refrigerated after their initial preparation.

G-6-PDH: 100 units glucose-6-phosphate dehydrogenase
10 ml distilled H_2O

MTT: 1.0 g MTT tetrazolium
125 ml distilled H_2O

NAD: 1.0 g β-nicotinamide adenine dinucleotide
100 ml distilled H_2O

NADP: 100 g β-nicotanimide adenine dinucleotide phosphate
100 ml distilled H_2O

NBT: 1.0 g nitroblue tetrazolium
100 ml distilled H_2O

PMS: 1.0 g phenazine methosulphate
100 ml distilled H_2O

VITA

John Raymond Shute, IV was born in Monroe, North Carolina on May 2, 1955. He attended elementary schools in Monroe and graduated from high school in 1973 from Berry Academy, Rome, Georgia. His first two years of college were spent at Appalachian State University. He continued his undergraduate education and later received a Bachelor of Arts degree in Biology from the University of North Carolina at Wilmington in August 1977.

Soon after graduating, he spent approximately one year working for David S. Lee at the North Carolina State Museum of National History as a research technician compiling data for the Atlas of North American Freshwater Fishes. He returned to the University of North Carolina at Wilmington in 1979 to work as a research technician on a project studying Lake Waccamaw's endemic fishes under the direction of David G. Lindquist. He spent approximately three years on this job before moving to The University of Tennessee, Knoxville.

In August 1976, he married Peggy Wright, with whom he has worked on many projects studying freshwater fishes in the southeast.

Currently, John is a member of the American Society of Ichthyologists and Herpetologists, the Association of Southeastern Biologists, and the Southeastern Fishes Council.