Taxometric Comparison of the Atlantic Slope and Ohio River Populations of Etheostoma caeruleum Storer

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ABSTRACT: Morphometric and meristic data of *Etheostoma caeruleum* Storer from the Gauley, Kanawha, Monongahela and Potomac drainages were compared using discriminant analysis. Ten of 21 characters from the four drainages had group means which were significantly different ($P \leq .05$), and the group centroids were significantly different ($P \leq .05$). The discriminant analysis, which maximizes group differences, could correctly assign 67% of the specimens to the *a priori* group. Unexpected variation was observed in anal spine and pelvic ray counts from two drainages.

INTRODUCTION

Etheostoma caeruleum Storer, the rainbow darter, inhabits moderate-sized streams with gravel or rubble bottoms E of the Rocky Mountains. The darter is distributed throughout the Ohio and Mississippi river drainages and is found in all of the Great Lakes drainages except Lake Superior (Stauffer and Hocutt, 1980). Although widely distributed throughout the central Appalachians, it had not been reported from the New river drainage or from any Atlantic slope drainages until recently.

The darter was first reported above Kanawha Falls by Hocutt *et al.* (1973) from the East River, W. Va. Subsequent collections in the New River found it well distributed throughout the main stem and its tributaries (Hocutt *et al.*, 1979), excluding the Greenbrier River (Hocutt *et al.*, 1978).

The darter is absent from the Atlantic slope except for recent collections from two forks of the South Branch of the Potomac River (Stauffer *et al.*, 1978). The ichthyofauna of the Potomac River has been well studied in the past (Lee *et al.*, 1976, and references therein). The discovery of a previously unreported species as distinctively colored as *Etheostoma caeruleum* is, therefore, surprising.

Knapp (1964) examined the meristics and morphometrics of *Etheostoma caeruleum* from the Great Lakes, Mississippi and upper Ohio rivers. The purpose of this paper is to present meristic and morphometric data for specimens from the Potomac, Kanawha, Monongahela and New river drainages and compare the populations using discriminant analysis.

METHODS AND MATERIALS

The initial selection of characters to be examined in the analysis was based on Knapp's (1964) study. Other characters were included to aid in group separation.

Meristic characters examined were nape scalation, number of lateral bands, total lateral line scales, pored lateral line scales, unpored lateral line scales, scales above lateral line, scales below lateral line, least caudal peduncle scales, dorsal spines, dorsal rays, total dorsal fin elements, pectoral rays, pelvic spines, pelvic rays, anal rays, anal spines and caudal rays. Morphometric characters which were standardized by dividing by standard length included: body depth at dorsal origin, caudal peduncle depth, head length, snout length, orbit length, upper jaw length, snout to dorsal fin insertion, and snout to pelvic fin insertion. Additionally, various ratios of standardized characters were examined (*i.e.*, orbit length to snout length, orbit length to upper jaw length).

All counts and measurements were made following the method outlined by Hubbs and Lagler (1974) except number of lateral bands and snout tip to pelvic fin insertion. Number of lateral bands was determined by counting all bands whether complete or in-complete. Snout to pelvic fin insertion is the distance from the tip of the snout to the point of insertion of the pelvic fin spine. Morphometric measurements were made with a dial caliper to 0.1 mm.

A total of 189 fish from the Appalachian Environmental Laboratory Fish Museum (AEL), University of Maryland, Frostburg, were used in the analysis. All known specimens (39) from the Potomac River (AEL 421, 422, 604, 618, 623, 626) were examined, while 50 fish from each of the following drainages were examined: Gauley River (AEL 146, 149, 154, 158), Kanawha (AEL 279, 284, 313, 320, 321, 324, 325, 328) and Monongahela drainage (AEL 269, 270, 636, 640, 642, 691, 736).

Data were originally examined to generate means, standard deviations, maximum and minimum values, kurtosis and skewness. Characters with large kurtosis and skewness values were transformed to reduce these values. Two variables were thus transformed; body depth by taking the natural log, and head length by adding one, then taking the natural logarithm.

Data were then examined by Pearson's correlation (R) analysis to determine which variables had high degrees of intercorrelation, and thus not independent. Characters were selected so that no two of them had an R value larger than 0.7.

Linear discriminant function analysis was used to determine the maximum separation which exists among the groups. Discriminant analysis is considered to be the appropriate multivariate technique when dealing with *a priori* groups (Cooley and Lohnes, 1971) and when examining taxa at the subspecific and population levels (Sneath and Sokal, 1979). This analysis used the four drainage basins (Potomac, Monongahela, Gauley and Kanawha) as the designated *a priori* groups. Discriminant analysis also has the advantages of being structurally and mathematically related to multivariate analysis of variance, which allows for hypothesis testing about the differences among the centroids of the *a priori* groups (Neff and Marcus, 1980).

The Statistical Package for the Social Sciences (SPSS) discriminant procedure, Version 8 (Klecka, 1975; Hull and Nie, 1979), was used in the analysis. This procedure was chosen because it performs canonical variate analysis, and also a pairwise F-ratio test of the difference between the centroids of the groups. The discriminant procedure also calculates a univariate F-ratio test of the differences between group means of each character used in the analysis.

The SPSS stepwise procedure, using Mahalanobis' distance method, was modified to allow the use of all characters not previously rejected for reasons of normality and intercorrelation. This was accomplished by setting the entry and removal F-ratios to zero, which eliminates the problems that result from the use of a stepwise procedure (Pimentel and Fry, 1978; Green, 1979). The group probability memberships were calculated using the individual group covariance. The equality of the canonical variate function covariance matrices was tested using Box's M test and found to be not statistically different ($P > 0.05$).

The characters in the discriminant analysis were also examined using univariate means. A one-way ANOVA was performed on the characters meeting the assumptions of equality of variances as tested by Bartlett-Box F-test. Character means which did not have equal variances among the basins were tested using BMDP-1977 P-series Kruskal Wallis nonparametric one-way analysis of variance.

RESULTS AND DISCUSSION

Nape scalation was considered by Knapp (1964) to be an important characteristic to separate the races of *Etheostoma caeruleum.* All darters examined in the study had naked to slightly scaled napes indicating they belong to Knapp's (1964) upper Ohio River race of *E. caeruleum caeruleum.* Since nape scalation is not a strict quantitative measure, it was not included in the discriminant analysis.

Of the 25 original characters (including nape scalation) examined, 21 were included in the canonical variate analysis (Table 1). The standard length of all specimens

TABLE 1.—Comparison of means, standard deviations and ranges of 21 meristic and morphometric characters examined in *Etheostoma caeruleum* from Potomac, Gauley, Kanawha and Monongahela drainages and overall group means. Asterisks (*) indicate level of significant differences among drainages. Morphometric values expressed as proportion of standard length

Character	Drainages							
	Potomac				Gauley			
	$\bar{x}$	SD	Min.	Max.	$\bar{x}$	SD	Min.	Max.
Bands	11.97	1.01	11	15	11.06	1.02	9	13
Lateral line scales	44.39	2.21	41	51	44.20	2.31	40	49
Unpored lateral line scales	14.62	2.34	11	20	16.28	2.79	10	23
Scales above lateral line	4.08	0.35	3	5	4.02	0.14	4	5
Scales below lateral line	7.26	0.59	6	9	7.42	0.54	7	9
Least caudal peduncle scales	17.89	0.45	16	18	17.84	0.55	16	18
Pelvic ray count	5.00	0.00	5	5	5.00	0.00	5	5
Pectoral ray count	12.89	0.55	12	14	13.00	0.40	12	14
Dorsal spines	10.21	0.41	10	11	9.98	0.38	9	11
Dorsal rays	12.41	0.79	11	14	12.12	0.69	10	13
Anal spines	2.00	0.00	2	2	2.00	0.202	1	3
Anal rays	6.92	0.27	6	7	7.20	0.57	6	8
Caudal rays	17.21	0.52	16	19	17.04	0.53	16	18
Body depth	0.225	0.001	0.203	0.256	0.225	0.001	0.187	0.297
Upper jaw length	0.084	0.008	0.071	0.106	0.090	0.008	0.077	0.133
Snout length	0.082	0.006	0.067	0.093	0.079	0.006	0.070	0.110
Head length	0.279	0.001	0.112	0.309	0.278	0.001	0.253	0.420
Orbit length	0.073	0.010	0.060	0.117	0.075	0.008	0.063	0.113
Least caudal peduncle depth	0.114	0.006	0.102	0.130	0.110	0.008	0.093	0.150
Snout to dorsal fin length	0.373	0.049	0.231	0.577	0.377	0.037	0.298	0.556
Snout to pelvic fin length	0.329	0.030	0.243	0.369	0.332	0.040	0.208	0.513

* = Significance $P \leq 0.05$
** = Significance $P \leq 0.01$
*** = Significance $P \leq 0.001$

TABLE 1.(Cont'd)

Character	Drainages							
	Kanawha				Monongahela			
	$\bar{x}$	SD	Min.	Max.	$\bar{x}$	SD	Min.	Max.
Bands	10.76	1.02	9	14	11.36	1.17	9	14
Lateral line scales	45.10	2.74	40	51	44.10	3.20	39	49
Unpored lateral line scales	16.46	2.82	10	25	16.06	2.18	11	21
Scales above lateral line	4.10	0.30	4	5	4.00	0.202	3	5
Scales below lateral line	7.68	0.55	6	9	7.66	0.63	7	9
Least caudal peduncle scales	17.68	0.48	16	18	7.84	0.55	16	18
Pelvic ray count	5.06	0.24	5	6	5.00	0.00	5	5
Pectoral ray count	12.78	0.51	12	14	12.78	0.47	11	13
Dorsal spines	10.02	0.62	10	11	10.01	0.34	9	11
Dorsal rays	12.10	0.54	11	14	12.18	0.66	11	14
Anal spines	1.98	0.14	1	2	2.00	0.00	2	2
Anal rays	7.16	0.42	6	7	7.16	0.42	6	8
Caudal rays	16.56	0.91	16	19	16.52	0.84	14	18
Body depth	0.222	0.001	0.181	0.250	0.223	0.001	0.204	0.470
Upper jaw length	0.086	0.006	0.076	0.110	0.085	0.007	0.062	0.101
Snout length	0.081	0.006	0.069	0.094	0.081	0.006	0.068	0.097
Head length	0.283	0.001	0.297	0.319	0.279	0.001	0.248	0.324
Orbit length	0.075	0.007	0.060	0.090	0.071	0.006	0.057	0.082
Least caudal peduncle depth	0.105	0.006	0.094	0.125	0.111	0.004	0.097	0.118
Snout to dorsal fin length	0.368	0.039	0.246	0.516	0.368	0.017	0.339	0.401
Snout to pelvic fin length	0.338	0.027	0.301	0.486	0.337	0.017	0.297	0.382

examined ranged from 2.33-5.58 cm with an overall mean of 4.11 cm. The 21 characters used in the discriminant analysis were also examined using univariate methods. Seven characters (pelvic ray count, scales below lateral line, anal ray count, body depth, upper jaw length, caudal peduncle depth and orbit length) had variances which were not equal among the basins. The basin means for these characters were analyzed using a nonparametric Kruskal-Wallis ANOVA. All seven characters had basin means which were significantly different ($P<0.05$; df, 3). The 14 remaining characters were analyzed using a one-way ANOVA. Three characters (bands, unpored lateral line scales and caudal ray count) had means which were significantly different ($P<0.05$; df 3,185) among the stream basins.

Pored lateral line scales, total dorsal fin elements and all ratios of standardized body measurements to one another were excluded from the discriminant analysis because of intercorrelation with one or more other characters. Pelvic spine count was not included in the analysis because no variation was seen in any of the populations examined.

The multivariate technique of discriminant analysis, using canonical variate analysis, is useful to: (1) establish and maximize group differences; (2) discern which characters are important in separating the groups, and (3) provide a predictive method of classifying new subjects (Sparling and Williams, 1978). The procedure has been used previously by various workers. Stone (1947) and Cole (1965) reported that the morphologically similar darters *Etheostoma olmstedi* and *E. nigrum* could be separated with discriminant functions.

The discriminant analysis of our data generated three statistically significant ($P\leq0.05$) canonical discriminant functions. Tatsuoka's W^2 multi (Tatsuoka, 1970) value for the three functions was calculated to be 0.698; thus, 69.8% of the total variability of the canonical discriminant functions is attributed to real group differences. Function 1 (Func 1) accounted for 56.8% of the discriminant power while Function 2 and Function 3 (Func 2 and Func 3) accounted for 28.1 and 15.1%, respectively. Eigenvalues, percentage of variance explained by the canonical functions, and the canonical correlation coefficients are shown in Table 2.

TABLE 1.(Cont'd)

Character	Drainage Overall Group				
	$\bar{x}$	SD	Min.	Max.	
Bands	11.25	1.17	9	15	***
Lateral line scales	44.45	2.62	39	51	
Unpored lateral line scales	15.93	2.62	10	25	**
Scales above lateral line	4.05	0.26	3	5	
Scales below lateral line	7.52	0.59	6	9	***
Least caudal peduncle scales	17.86	0.51	16	18	
Pelvic ray count	5.02	0.13	5	6	*
Pectoral ray count	12.86	0.49	11	14	
Dorsal spines	10.06	0.46	9	12	***
Dorsal rays	12.19	0.67	10	14	
Anal spines	1.99	0.13	1	3	
Anal rays	7.12	0.45	6	9	*
Caudal rays	16.81	0.78	13	19	
Body depth	0.226	0.001	0.181	0.470	
Upper jaw length	0.086	0.008	0.062	0.133	
Snout length	0.081	0.006	0.067	0.110	
Head length	0.280	0.001	0.112	0.420	
Orbit length	0.073	0.008	0.057	0.118	
Least caudal peduncle depth	0.110	0.007	0.093	0.150	
Snout to dorsal fin length	0.371	0.036	0.231	0.577	
Snout to pelvic fin length	0.334	0.030	0.208	0.513	

Standardized canonical variate coefficients were calculated by multiplying canonical vector coefficients by the pooled within-groups standard deviations. This causes the pooled within-group covariance matrix to be an identity. The resulting standardized coefficients, which are unit-free, are a true measure of the relative contribution of each character to the discrimination. The standardized coefficients are shown in Table 3. The most discriminating variables in Func 1 were caudal peduncle depth, snout length and upper jaw length. Func 2's major contribution was upper jaw length, while Func 3 had major contributions of unpored lateral line scales and caudal peduncle depth.

Group centroids were significantly different at an overall level of $P \leq 0.05$ (Table 4). The overall significance level was set by using the Bonferroni method. Each pairwise comparison was thus significant at $P \leq .001$ (df, 21,165). The pairwise F-ratio and pairwise levels of significance are shown in Table 5.

The canonical variate analysis could correctly assign 67.2% of the fish examined to the correct groups based on the discriminant functions. A completely random classification of cases into four groups would correctly assign only 25% of the cases into the correct group (Table 6).

Knapp (1964) states there is relatively little geographic variation on a racial or subspecific level in all but the southwestern population of *Etheostoma caeruleum*. Our

TABLE 2.—Eigenvalues, percentage of variance explained and canonical correlation coefficients for the three functions generated in the discrimination analysis of *E. caeruleum* from four central Appalachian drainages

Function	Eigenvalue	Percentage of variance	Canonical correlation
1	.892	56.83	.687
2	.441	28.08	.553
3	.237	15.09	.438

TABLE 3.—Standardized canonical variate coefficients generated by the discrimination analysis for four populations of *Etheostoma caeruleum* from the central Appalachians

Character	Function I	Function II	Function III
Bands	−0.404	0.002	0.039
Lateral line scales	0.206	−0.002	−0.375
Unpored lateral line scales	0.173	−0.003	0.514
Scales above lateral line	0.121	−0.108	−0.429
Scales below lateral line	0.210	−0.292	0.190
Least caudal peduncle scales	−0.005	−0.023	−0.169
Pelvic ray count	0.217	0.253	0.136
Pectoral ray count	0.228	−0.235	−0.155
Dorsal spines	−0.051	−0.346	−0.078
Dorsal rays	−0.301	−0.189	−0.183
Anal spines	0.052	0.131	0.105
Anal rays	0.232	0.074	0.271
Caudal rays	−0.298	0.452	−0.314
Body depth	−0.001	−0.382	0.209
Upper jaw length	−0.507	0.760	0.207
Snout length	−0.530	−0.413	−0.089
Head length	0.126	−0.163	−0.345
Orbit length	0.287	0.200	−0.401
Least caudal peduncle depth	−0.658	0.230	0.489
Snout to dorsal fin length	−0.092	0.192	−0.061
Snout to pelvic fin length	0.076	−0.394	0.071

data, analyzed with both univariate and multivariate methods, do not support this contention for the darters from the central Appalachians. Ten out of 21 characters from the four drainages had group means which were significantly different. Additionally, each group centroid calculated by the discriminant analysis was significantly different.

Discriminant analysis could correctly assign 67% of the specimens to the *a priori* group. The fact that discriminant analysis found significant differences among closely related populations of the same subspecies indicates there is a large amount of intrapopulation variation.

A question exists as to whether *Etheostoma caeruleum* in the Potomac River is a native population or an introduced form. The discriminant analysis shows the centroid of the Potomac group to be distinct from the other three groups. If the darter were a bait bucket introduction, it most likely was introduced from one of the three other drainages due to their geographic proximity. The centroid of the introduced Potomac population would, therefore, be expected to be very close to the centroid of the parental population. This is not the case as the Potomac population shows no close affinities to the other populations. The Gauley and Monongahela populations are similar to the Kanawha populations along the first two canonical variate axes (Axis 1 and 2), respectively. The Potomac is similar only to the Kanawha along the relatively unimportant (Eigenvalue-.237) Axis 3. This evidence, along with Stauffer *et al.*'s (1978) evidence of stream capture in the area, and restricted range in two forks of the Potomac, lends credence to the assertion that the population is native.

On the other hand, one could argue that the centroid of the Potomac population may be different from other populations due to the founder effect. While this cannot be totally discounted, one would expect a smaller within-population variance if the population originated from a recent bait bucket introduction. An examination of the data from the Potomac population suggests that this does not occur.

TABLE 4.—Centroids of the three canonical variate functions of four populations of *E. caeruleum* from the central Appalachians

	Function 1	Function 2	Function 3
Potomac	−1.54	0.08	−0.51
Gauley	0.39	1.01	0.23
Kanawha	1.10	−0.44	−0.47
Monongahela	−0.29	−0.64	0.63

TABLE 5.—Comparison of Potomac, Gauley, Kanawha and Monongahela drainage centroids by pairwise F-ratios for 21 and 165 degrees of freedom. Significance denoted by asterisk

Group	Potomac	Gauley	Kanawha
Gauley	4.77***		
Kanawha	6.75***	3.29***	
Monongahela	3.15***	3.56***	3.39***

***Significance $P \leq 0.001$

TABLE 6.—Percentages of specimens of *E. caeruleum* examined correctly classified by the analysis

	Predicted membership			
Actual	Potomac	Gauley	Kanawha	Monongahela
Potomac	74.4%	12.8%	0%	12.8%
Gauley	12.0%	80.0%	10.0%	8.0%
Kanawha	2.8%	16.0%	64.0%	18.8%
Monongahela	12.0%	8.0%	18.0%	62.0%
Overall correct classification: 67.20%				

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