

1990

Stock Identification of Weakfish, *Cynoscion regalis*, by Discriminant Function Analysis of Morphometric Characters

Daniel R. Scoles

College of William and Mary - Virginia Institute of Marine Science

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Stock Identification of Weakfish, Cynoscion regalis, by Discriminant
Function Analysis of Morphometric Characters

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

by

Daniel R. Scoles

1990

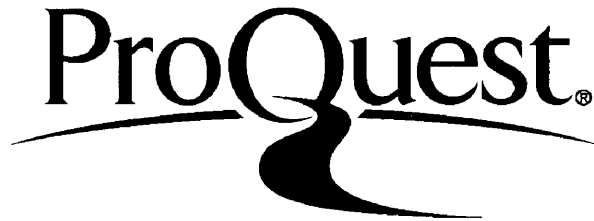
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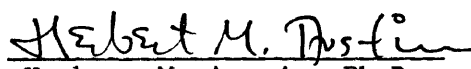
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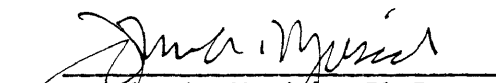
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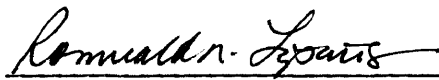

Daniel R. Scoles

Approved: November 1990


Herbert M. Austin, Ph.D.
Committee Chairman/Advisor


John E. Graves, Ph.D.


John A. Musick, Ph.D.


Romuald N. Lipcius, Ph.D.

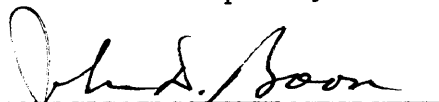

John D. Boon, Ph.D.

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ABSTRACT

The weakfish (*Cynoscion regalis*) is a migratory species of the family Sciaenidae that inhabits near-shore and estuarine waters of the western Atlantic from Florida to Nova Scotia. Weakfish are among the most economically important finfishes harvested from northwest Atlantic coastal waters, but large interannual fluctuations have been observed in both historical and recent commercial and recreational landings. To understand the causes and consequences of fluctuations in weakfish abundance, as well as to effectively manage the weakfish fishery, it is essential to obtain a better understanding of weakfish stock structure and migratory behavior.

In an effort to elucidate weakfish stock structure, discriminant function analysis was conducted on 658 adult and juvenile weakfish collected from South Carolina to New York in 1988. These samples were classified to one of two reference samples from the extreme ends of the sampled range. The objectives of this analysis were to determine if samples of weakfish differ significantly in morphometric variables using multivariate analysis of covariance, to demonstrate an effective method of classifying weakfish using discriminant function analysis, and to utilize this approach to develop a hypothesis of movements of weakfish and possible stock composition. The results suggested the following: 1) At least two morphological types of weakfish occur based on significant differences found between reference samples. 2) The two reference morphological types are nearly equally represented among large weakfish sampled from Long Island Sound and Delaware Bay in spring. 3) Medium weakfish sampled from Delaware Bay and Chesapeake Bay in spring are not similar in morphology, in contrast to the results presented in a previous study of weakfish morphometrics. 4) Medium weakfish sampled from Delaware Bay and Chesapeake Bay in fall classify mostly with the northern reference morphological type. 5) Juvenile weakfish of the northern part of the range apparently undergo extensive southern migrations.

This study demonstrated that significant morphological variation occurred among samples of weakfish which were subsequently classified to two reference samples using discriminant function analysis. The results suggested that at the time of sampling a cline of morphometric characters or substantial mixing among the morphological types occurred intermediate in the range for weakfish. Recent genetic analyses of weakfish indicated that Atlantic coast weakfish share a common gene pool suggesting that morphometric differences which were found may be a result of phenotypic plasticity. Whether or not the observed morphological character variation is genetically or ecophenotypically based, these differences provide fisheries managers with a means to investigate weakfish stock composition and migratory habits.

Stock Identification of Weakfish,
Cynoscion regalis, by Discriminant Function
Analysis of Morphometric Characters

INTRODUCTION

The weakfish, Cynoscion regalis (Bloch and Schneider), is a migratory species of the family Sciaenidae that inhabits near-shore and estuarine waters of the western Atlantic from Nova Scotia to Florida (Johnson, 1978). Weakfish are commercially harvested throughout most of their range and they make up an important recreational fishery (Hildebrand and Schroeder, 1928; Bigelow and Schroeder, 1953; Stagg, 1986). The stability of the weakfish population has been in question because of large interannual fluctuations of commercial landings (Wilk, 1979; Stagg, 1986). Because of the economic importance of weakfish both commercially and recreationally, maintaining stability of weakfish stocks is a management priority.

In the 1930's and 1940's the commercial landings of weakfish varied over a wide range, up to 20 million pounds annually (Wilk, 1979; Stagg, 1986). Between 1941 and 1943 landings fell to about 9 million pounds annually, a change attributed by some to a decline in fishing effort with the onset of World War II (Merriner, 1973; McHugh, 1980). Following a period of low harvests, a steady increase in landings occurred between 1970 and 1980. In 1980 commercial landings peaked at 35.9 million pounds (Mercer, 1983). But harvests again began to decline in 1981 to 20.5 million pounds in 1988 (NMFS, 1989).

Annual harvests by recreational fisheries have closely followed the trends of the commercial harvest (Wilk, 1981). In some years the recreational catch surpassed the commercial catch (Deuel, 1973). Since the early 1960's recreational fishermen have been landing increasing numbers of increasing size fish. Mercer (1983) reported that from 1960 to 1970 the recreational catch per unit effort per angler doubled. Seagraves (1981) reported that the average size of prize fish taken in the Delaware Sport Fishing Tournament more than doubled from 1968 to 1979.

The cause of the fluctuations in the weakfish landings is unknown; however, the apparent parallel trends of commercial and recreational harvests (Wilk, 1981) suggest these fluctuations are likely reflections in actual abundance and not changes in fishing effort alone. Merriner (1973) and Austin (1981) both suggested that the cause of these fluctuations is related to the periodicity of successful year classes. Declines in abundances have been attributed to a number of causes including overfishing (Joseph, 1972; Merriner, 1973), pollution related mortality (Joseph, 1972; Merriner, 1973), increase in fishing skill (Stagg, 1986), and capture of young as bycatch of shrimp fisheries of southern states (Perra et al., 1988). Excellent reviews of the recreational and commercial fisheries are provided by Merriner (1973), Wilk (1979), McHugh (1980), Seagraves (1981), Mercer (1983), Mercer (1985), Stagg (1986), Perra et al. (1988) and Hawkins (1988).

Weakfish undergo extensive migration in spring from their winter grounds off North Carolina to spawn in near-shore and estuarine zones from North Carolina north. Mercer (1983) concluded from an extensive

literature review that spawning, hatching and larval development occurs from March to October with peak production from April through June. In more recent analyses gonadal somatic indices plotted over time indicated that peak spawning occurs from the last week in May to the end of the first week of June in Delaware Bay (Villoso, 1989) and in the third week of June in Long Island Sound (DiTommaso, 1990). During the spawning peak the presumed stocks are believed to be distributed on separate spawning grounds. A study by Welsh and Breder (1923) provided evidence that two spawning peaks may occur. They identified a major spawning run into the Delaware and Chesapeake Bays from April to May, followed by minor spawning activity in September.

The movements of weakfish appear to differ with age. Tagging results and size composition data of the weakfish suggest that during the spawning season large weakfish are found primarily in the northern part of the range (Nesbit, 1954). It was hypothesized by Wilk (1979) that as weakfish get older and larger they move farther north during the spring migration. With winter cooling the large weakfish appear to move south and offshore of North Carolina, and the smaller fish to inshore waters of the South Atlantic Bight (Wilk, 1979). With increasing spring temperatures migration to spawning grounds occurs again (Wilk, 1979). If weakfish move as described, then a large portion of the large weakfish found in the north are apparently derived from the spawning grounds to the south.

Despite these hypotheses of Wilk (1979), the source of large weakfish found in the north has yet to be empirically determined. Locating the source of these weakfish has been a major management issue

since adult weakfish apparently overwinter off North Carolina where they are heavily fished. In 1988 the total commercial landings for weakfish in North Carolina was 15.1 million pounds which represented 73.5 percent of the total weakfish landings for all states combined (Table 1).

A study by Hawkins (1988) provided evidence suggesting that the weakfish that overwinter offshore of North Carolina have more similar growth parameters to weakfish of the northern part of the range than to those of the southern part of the range. Before a revised management plan for the North Carolina winter fishery can be implemented, more definitive evidence is needed to show that a large proportion of the weakfish that overwinter off North Carolina are derived from northern spawning grounds.

Although there have been a number of studies designed specifically to determine the weakfish stock structure, it remains unclear whether one, two or three stocks exist. Welsh and Breder (1923) found two size classes of weakfish egg diameters between Cape May and Chesapeake Bay suggestive of the occurrence of two sympatric stocks in this region. Nesbit (1954) reviewed studies of age and growth, developed a hypothesis and tested it using methods of mark-recapture and comparisons of scale morphology. One of his conclusions was that weakfish of a group distributed from Pamlico Sound to Chesapeake Bay have a distinct migratory pattern from a group distributed from Exmore, Virginia to New York. Perlmutter et al. (1956) examined meristics, scale morphology and growth of young-of-year and adult weakfish and suggested that weakfish of New York are unlike those of Chesapeake Bay, a conclusion in support of Nesbit's work. Seguin (1960) examined morphometrics and meristics

Table 1. Summary of the 1988 Atlantic coast weakfish commercial landings. (Source: NMFS, 1989)

<u>State</u>	<u>Commercial Landings Pounds</u>	<u>Percent of Total Catch</u>	<u>Dollar Value</u>
Massachusetts	9,000	0.04%	\$8,000
Connecticut	10,000	0.05%	\$12,000
Rhode Island	20,000	0.10%	\$27,000
New York	124,000	0.60%	\$160,000
New Jersey	2,331,000	11.35%	\$882,000
Delaware	525,000	2.56%	\$341,000
Maryland	821,000	4.00%	\$315,000
Virginia	1,474,000	7.18%	\$913,000
North Carolina	15,091,000	73.49%	\$5,220,000
South Carolina	0	0.00%	\$0
Georgia	0	0.00%	\$0
Florida	0	0.00%	\$0
	-----		-----
Total	20,533,000	Total	\$7,948,000

with univariate analysis of covariance and concluded that weakfish of New York and North Carolina comprise northern and southern stocks respectively, separated by an intermediate stock located in waters of Delaware and Virginia. Examination of growth parameters led to the conclusion that weakfish between Ocean City, MD and Virginia Beach, VA are intermediate in these parameters relative to weakfish of regions north and south (Shepherd, 1982; Shepherd and Grimes, 1983). More recently it was suggested that weakfish of the Middle Atlantic region make up a single stock based on starch gel electrophoresis of allozymes (Crawford, 1984; Crawford et al., 1988) and restriction fragment length polymorphism analysis of mitochondrial DNA of the same weakfish used in this morphometric study (McDowell et al., 1990).

The objectives of this study were to determine if samples of weakfish differ significantly in morphometric variables using multivariate analysis of covariance, to demonstrate an effective method of classifying weakfish using discriminant function analysis and to utilize this approach to develop a hypothesis of the movements of weakfish and possible stock composition. Although Seguin (1960) already examined weakfish morphometrics statistically, she used a univariate method of analysis. The analysis of morphometric variables is a multivariate problem and is best examined with a multivariate technique (Pimental, 1979). Discriminant function analysis was selected for use in this study primarily because it is a multivariate technique which classifies observations to one or more reference samples known to differ in measurable characters, and secondarily because it has become an

accepted and widely-used technique for identification of fish stocks (Hill, 1959; Fukuhara et al., 1962; Amos et al., 1963; Pearson, 1964; Anas and Maria, 1969; Parsons, 1972; Messieh, 1975; Cook and Lord, 1978; Berggren and Lieberman, 1978; Wilk et al., 1980; Shaklee and Tamaru, 1981; Humphries et al., 1981; Saila et al., 1983; Misra and Ni, 1983; Winans, 1984; Misra, 1985; Reist, 1985; Fabrizio, 1987; Henault and Fortin, 1989; Schaefer, 1989).

MATERIALS AND METHODS

Collection of Samples

During 1988, 417 adult and 241 juvenile weakfish were collected between New York and South Carolina. Samples of adult weakfish were purchased from fishermen who caught them by hook and line and gill net in New York and Delaware, and pound net in Virginia and North Carolina. Adult weakfish were collected from Long Island Sound, NY; Brown Shoal, Broadkill Beach and Slaughter Beach, DE; Rappahannock River and York River, VA and Pamlico Sound, NC. Juvenile weakfish (standard length less than 200mm) were collected by otter trawl from Peconic Bay, NY, Charleston Harbor, SC and offshore North Carolina in two hauls of a trawl net (from NOAA R/V Ferrel cruise #FE8803) that were pooled, one at latitude $34^{\circ} 1.3'$, longitude $76^{\circ} 26.0'$, the other at latitude $35^{\circ} 1.3'$, longitude $75^{\circ} 59.7'$. All samples were processed immediately after they were obtained or were placed in a freezer for later processing. All adult weakfish collected in spring were in the gravid stage of development. Weakfish of the Rappahannock River and Slaughter Beach samples, which were collected in the fall, had already spawned. Determination of sex and stage of development was conducted on all weakfish except the juveniles. Adult samples fell into two size classes: medium fish of 200 to 460mm standard length, and large fish of 560 to 750mm standard length. A summary of sample sites, dates, mean standard lengths and sample sizes is provided in Table 2.

Table 2. Summary of sample sites, dates, mean standard lengths (STL) and sample sizes of A) adult weakfish and B) juvenile weakfish.

A. Adult weakfish.

<u>Sample Site</u>	<u>Date</u>	<u>Mean STL(mm)</u>	<u>Sample Size</u>
Long Island, NY (med)*	May 4 - June 8, 1988	329.7	54
Long Island, NY (lg)*	May 4 - June 8, 1988	667.0	30
Brown Shoal, DE	May 27, 1988	639.7	22
Broadkill Beach, DE	May 26, 1988	305.4	36
Slaughter Beach, DE	Sept 15, 1988	315.7	87
Rappahannock River, VA	Oct 3, 1988	329.4	48
York River, VA	May 4 - June 13, 1988	299.9	63
Pamlico Sound, NC	June 7, 1988	272.8	77

			N = 417

B. Juvenile weakfish.

<u>Sample Site</u>	<u>Date</u>	<u>Mean STL(mm)</u>	<u>Sample Size</u>
New York	Oct 18, 1988	112.9	85
North Carolina	Nov 7, 1988	158.1	80
South Carolina	Dec 5, 1988	118.9	76

			N = 241

* med - medium weakfish (200 to 460mm STL)
 lg - large weakfish (560 to 750mm STL)

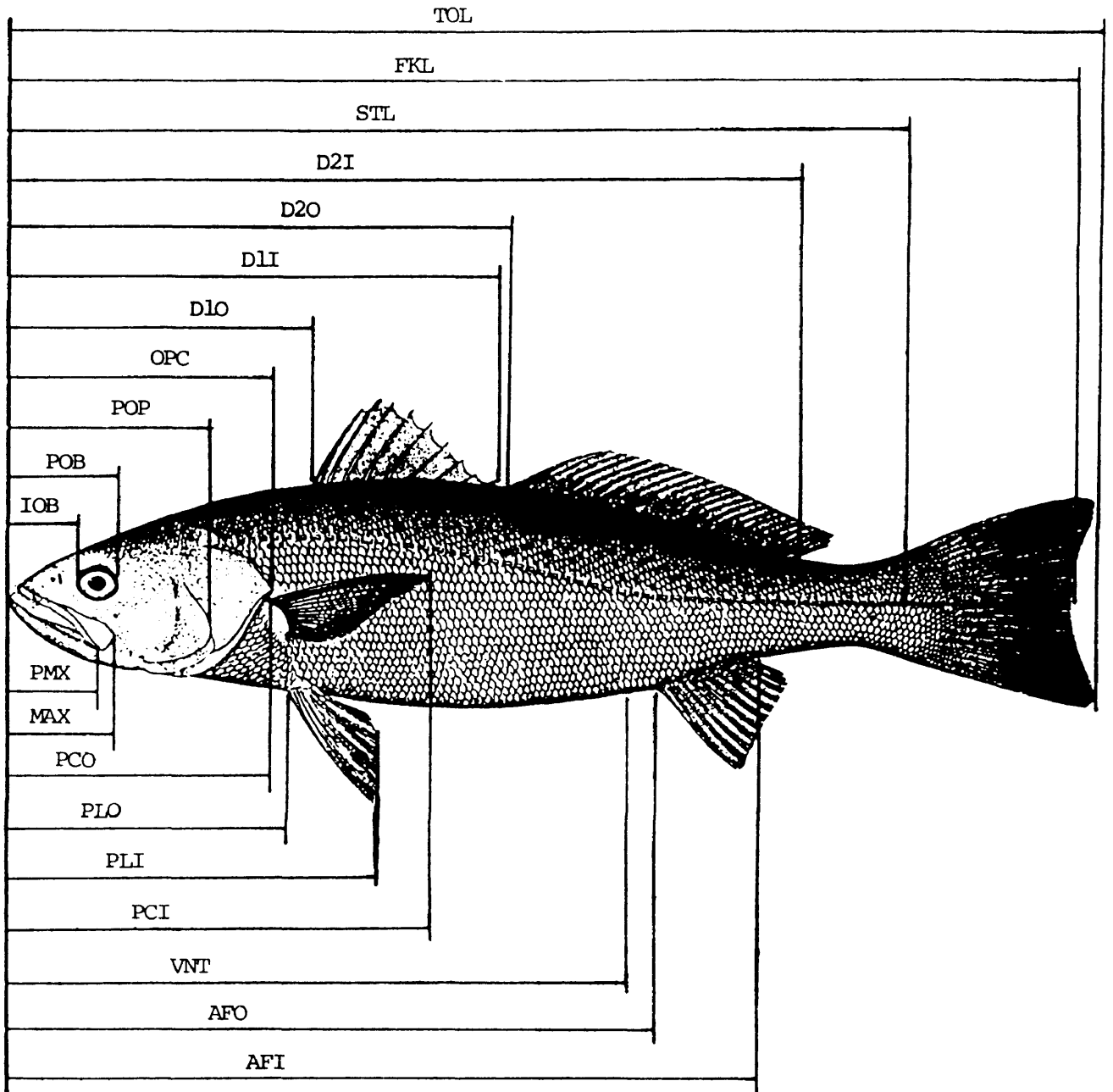
Morphometric Measurements

Twenty-two measurements, modified after those described in Hubbs and Lagler (1958) and Wilk et al. (1980), were recorded from each fish. Head depth was measured with a caliper at the pectoral fin origin, and girth was obtained by measuring the length of a string placed around the fish at the pectoral fin origin. All other measurements were recorded as a linear distance parallel to the body axis from the tip of the snout to the character of interest with a meter stick to the nearest millimeter as diagrammed in Figure 1. Abbreviations for these variables used in the following text, tables and figures are defined in Table 3. To be consistent that all variables be less than STL (used as a standard measure of size) TOL and FKL were omitted from all analyses.

Statistical Analysis

The data were examined using simple plotting techniques. For each sample, each variable was regressed against standard length to determine the degree of linearity of regression. The significance of regression for each was determined. Regressions of residuals against predicted values from these plots (Draper and Smith, 1981), frequency histograms and normal probability plots of each variable for each sample were developed. These plots were used to obtain a graphic indication of normality and homogeneity of variance.

Figure 1. Illustration of the weakfish, Cynoscion regalis, with morphometric measurements diagrammed (illustration by H. L. Todd from Goode, 1884. Modified jaw and pelvic fin.)



Additional Measurements: IIDP Head depth at PCO
 GTII Girth at PCO

Table 3. Abbreviations of morphometric measurements used in text, tables and figures.

PMX	Premaxilla
MAX	Maxilla
IOB	Interorbital
POB	Postorbital
POP	Preoperculum
OPC	Operculum
HDP	Head Depth
PCO	Pectoral Fin Origin
PCI	Pectoral Fin Insertion
PLO	Pelvic Fin Origin
PLI	Pelvic Fin Insertion
VNT	Vent
AFO	Anal Fin Origin
AFI	Anal Fin Insertion
D1O	First Dorsal Fin Origin
D1I	First Dorsal Fin Insertion
D2O	Second Dorsal Fin Origin
D2I	Second Dorsal Fin Insertion
GTH	Girth
TOL	Total Length
FKL	Fork Length
STL	Standard Length

One-way analysis of variance was conducted between all variables of males and females with the null hypothesis that there is no difference between the means of the variables. The null hypothesis was accepted in each test ($P > 0.05$), suggesting that sexual dimorphism for the characters examined was minimal or absent. Males and females were pooled for all subsequent analyses. The Cochran's C test for homogeneity of variance was nonsignificant ($P > 0.05$) in each of these tests as well.

Pimental (1979) suggested that before conducting discriminant analysis it is useful to examine differences between reference samples from which the discriminant function is calculated with a multivariate hypothesis test. Treating adults and juveniles separately, multivariate analysis of covariance (MANCOVA) of selected variables were conducted between reference samples using Wilks' criterion. Wilks' lambda is the ratio of the within-groups sum of squares to the total sum of squares which ranges from 0 to 1 (Norusis, 1986). Values of Wilks' lambda that are small are associated with high among-groups variability and low within-groups variability (Norusis, 1986). To test the null hypothesis that there is no difference between the means of two groups, Wilks' lambda is calculated, then converted to a variable which approximates the F distribution to which it is compared (Norusis, 1986). When Wilks' lambda is 1, among-groups variability does not exist, thus classification with discriminant function analysis is impossible (Norusis, 1986).

The assumptions of multivariate analysis of covariance are equal variance-covariance matrices and multivariate normality (Norusis, 1986).

An indication of multivariate normality is obtained (but not guaranteed) when homogeneity of variance and normality of distributions are found for each dependent variable (Berggren and Lieberman, 1978; Norusis, 1986). To increase the level of homogeneity of variance between samples of adult fish, data were transformed by the natural logarithm. Variables recorded from juvenile weakfish did not require transformation. The Box's M test (SPSS^X Inc.) was used to test for equality of variance-covariance matrices for both comparisons. An additional assumption when a covariate is used is that no significant area by covariate interactions can occur. A nonsignificant area by covariate interaction suggests that slopes between samples for a particular variable regressed on the covariate are equal. To determine if parallel slopes occurred between samples, area by standard length (the covariate) interactions of univariate analyses of covariance for each variable of each comparison were examined. Variables with poor homogeneity of variance [determined by Cochran's C ($P > 0.05$)], visibly poor regressions or significant area by standard length interactions ($P > 0.05$) were eliminated from MANCOVA comparisons.

Discriminant function analysis, first introduced by Fisher (1936), is a procedure by which linear or quadratic equations of measured variables are developed which maximize Mahalanobis' distance between reference samples (Davis, 1986; Saila et al., 1983; Norusis, 1985; SAS Institute, 1985). Reference samples (sometimes called learning groups, calibration groups or morphotypes) are samples of individuals with similar measured variables within a sample, which are known to differ between samples. It is assumed that these reference samples consist of

individuals which do not belong to other such samples (Davis, 1986; Pimental, 1979). Thus, reference samples in a fisheries problem are assumed to be of pure stock (Amos et al., 1963; Pearson, 1964; Saila et al., 1983; Fabrizio, 1987). Mahalanobis' distance is a generalized measure of difference between reference samples means, known as centroids (Davis, 1986). Mahalanobis' distance is directly proportional to the difference of measured characteristics between reference samples. The equation of the discriminant function is constructed such that variables which do not provide information between reference samples are omitted, and that proper coefficients of variables are selected so that Mahalanobis' distance is maximized between reference samples while also minimizing variance within samples. A linear discriminant function is developed if the variance-covariance matrices between reference samples are equal (i.e. can be pooled), otherwise quadratic terms will occur in the discriminant function (Williams, 1983; Misra, 1985).

Once the discriminant function is developed, it is used to classify samples in which observations are thought to be mixed. This is done by determining the values of the discriminant functions (known as discriminant scores) of each observation of each reference sample, and discriminant scores of each observation of other samples (unknowns). The mean discriminant score between the reference samples is used as a criterion for classification. Unknowns with discriminant scores greater than the mean score are classified to one reference sample, and the remaining unknowns are classified to the other. Well-written descriptions of the underlying mathematics of this technique can be found in Klecka (1980) and Davis (1986) for the linear discriminant

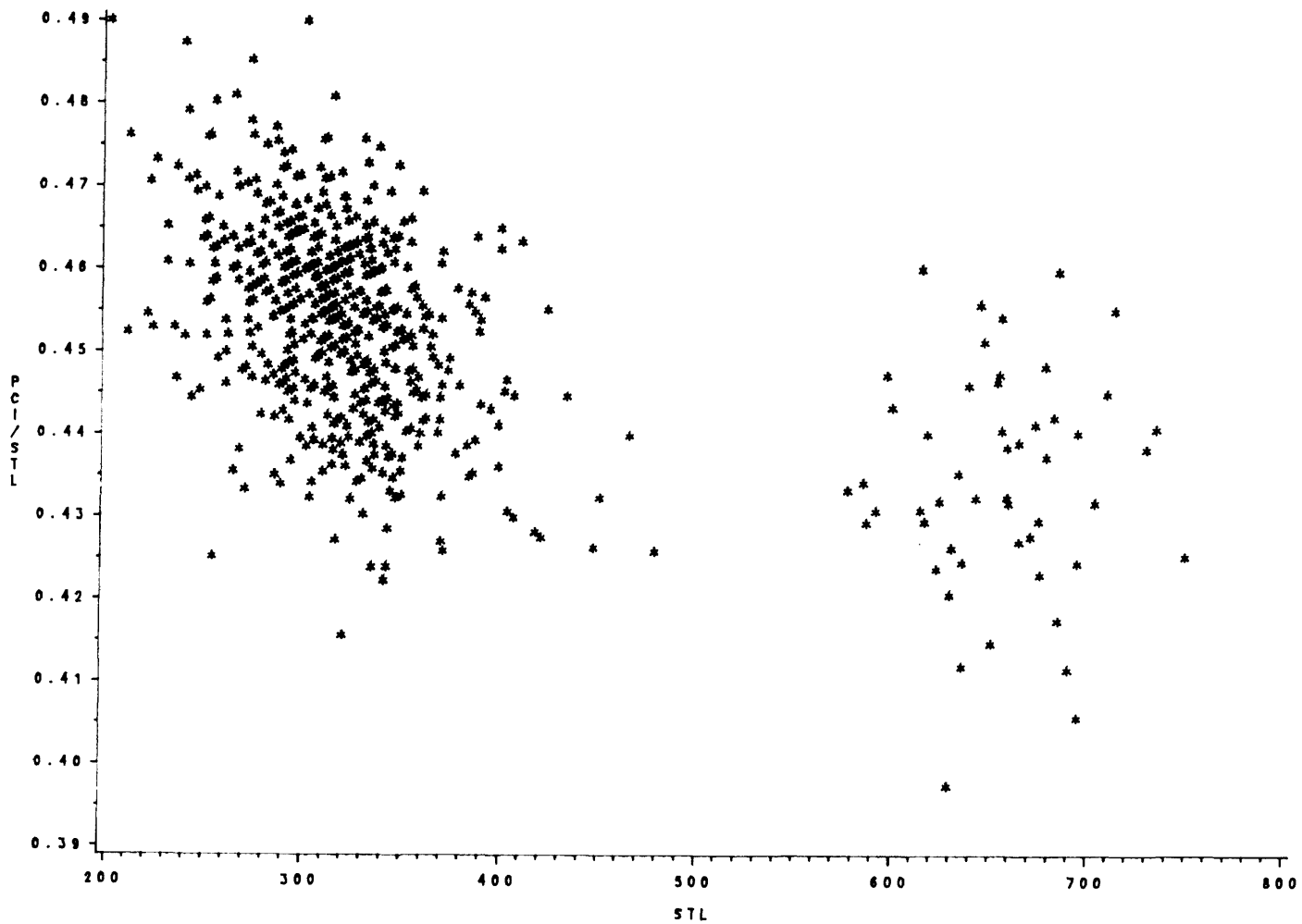
function, and Misra (1985) and Rao (1973) for the quadratic discriminant function.

The assumptions of discriminant function analysis, listed here, are taken from Klecka (1980) unless otherwise noted: 1) Two or more samples are required with at least two individuals per sample. 2) Any number of discriminating variables can be used providing that it is less than the number of measured samples minus two. 3) Discriminating variables must be measured at the interval level. 4) Discriminating variables should not be a linear combination of other such variables, because the variable defined by a combination of other variables does not provide additional information. 5) Each reference sample must be drawn from a population with a multivariate normal distribution. Variables recorded from individuals of classified samples need not be normally distributed. 6) For the linear discriminant function, variance-covariance matrices for each reference sample must be approximately equal, otherwise quadratic discriminant function analysis should be used (Williams, 1983; Misra, 1985). 7) When analyzing morphometric measurements, data should be transformed to remove the effect of size (Thorpe, 1975; Reist, 1985). 8) Individuals of reference samples to which individuals of other samples will be classified are themselves never misclassified (Davis, 1986). The first four assumptions are satisfied by the design of this study. All other assumptions are discussed below.

Weakfish were shown to grow allometrically by plotting the ratios of measured variables divided by STL against STL. These plots were patterned and had a highly negative slope (Fig. 2). To reduce the effects of size, an allometric growth transformation was conducted.

Figure 2. Plot of the ratio PCI/STL against STL for all adult weakfish.

PLOT OF PCI/STL vs STL
ADULT WRAKFISH



This transformation adjusts the variables to the values they would possess if they were recorded from individuals of the mean body size (Thorpe, 1975) and provides a data set which approximates multivariate homogeneity of variance (Schaefer, 1990). The result is a shape variate from which size effects have been reduced.

Each variable was transformed using the following equations taken from Thorpe (1975):

$$\hat{Y}_i = 10^{\hat{Y}_i} \quad (1)$$

$$\hat{Y}_i = \log_{10} Y_i - b (\log_{10} X_i - \log_{10} \bar{X}) \quad (2)$$

where \hat{Y}_i is the adjusted measurement of the i th specimen, Y_i is the measurement to transform of the i th specimen, b is the allometric coefficient (obtained as the slope of $\log_{10} Y$ plotted against $\log_{10} X$), X_i is a standard measure of size of the i th specimen for which standard length was used, and \bar{X} is the grand mean of standard lengths. Equation 3 results upon combining equations 1 and 2 and simplifying:

$$\text{Log}_{10} [Y_i / \hat{Y}_i] = b \text{Log}_{10} [X_i / \bar{X}] \quad (3)$$

It is seen that the relationship between the ratios X_i / \bar{X} and Y_i / \hat{Y}_i is dependant on an estimate of the allometric coefficient b , and that the

adjusted measurement \hat{Y}_i is an estimate of the average Y_i for an individual of standard length X_i .

The transformed variables were regressed against standard length. This regression should reveal a random pattern with a slope of zero when the effects of size have been satisfactorily reduced.

The DISCRIMINANT procedure of SPSS^x was used (with default variable entry parameters) using Mahalanobis' criterion to select variables in a stepwise manner which displayed the greatest difference between reference samples. The stepwise analysis selected variables that maximized Mahalanobis' distance between the two reference samples. Variables which were not used in discriminant analyses because of poor regressions of reference samples were PMX and MAX for the adults, and PMX, MAX, IOB and POB for the juveniles. Additionally, D20 was not used in either analysis because of its similarity to D11.

Two discriminant functions were developed with the PROC DISCRIM procedure of SAS, one of adult weakfish with reference samples Pamlico Sound and Long Island (med) and the other of juvenile weakfish with reference samples New York and South Carolina. This procedure provided a method of analysis whereby variance-covariance matrices were tested for equality [by the method of Kendall and Stuart (1961)] to determine whether subsequent analyses would be based on quadratic or linear discriminant functions. The default alpha of 0.10 was used in this test. Discriminant functions were subsequently used to classify weakfish of other samples.

RESULTS

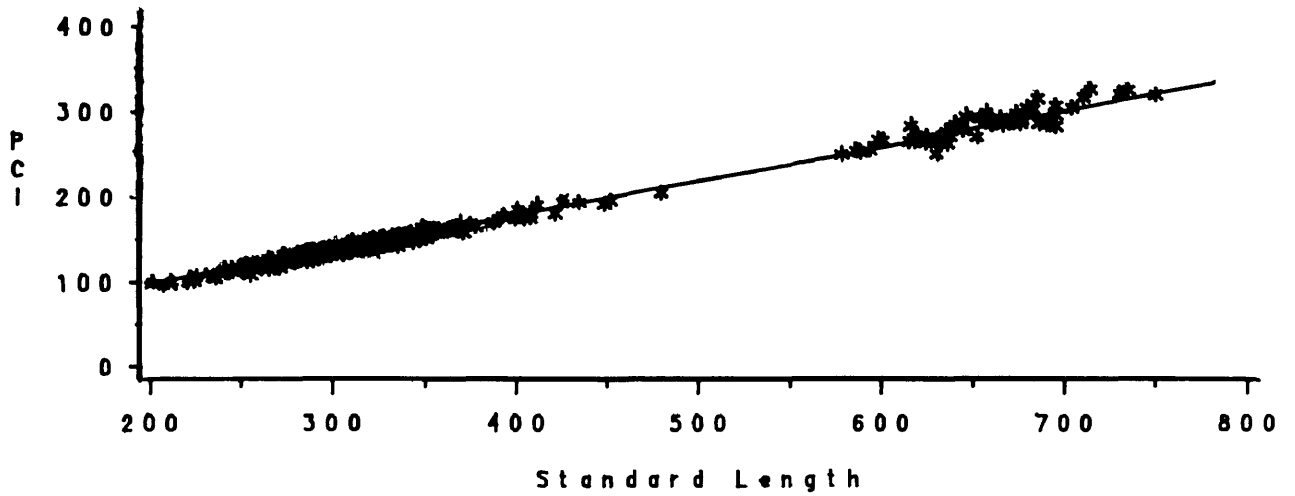
Frequency histograms and normal probability plots suggested that the data did not deviate from normality. The significance of regression for all plots of variables on STL were high ($P < 0.001$).

The variables PCI, VNT, AFO, AFI, D10 and D2I were selected for MANCOVA comparisons of reference samples of adult weakfish. Heteroscedasticity was revealed in plots of variables against STL and corresponding plots of residuals vs predicted values (Fig. 3). The natural log transformation decreased the level of heteroscedasticity considerably (Fig. 4). The results of Cochran's C tests indicated that the assumption of homogeneity of variance was satisfied for all variables of this comparison ($P \geq 0.05$) except for the covariate STL ($P = 0.043$). The assumption of equal variance-covariance matrices was violated ($P < 0.01$). MANCOVA results between reference samples of adult weakfish demonstrated that the null hypothesis that means are equal between the reference samples of adult weakfish was rejected (Table 4).

Two variables, POP and D10, were selected for MANCOVA of reference samples of juvenile weakfish. Heteroscedasticity was not revealed in plots of variables against STL and corresponding plots of residuals vs predicted values (Figs. 5 and 6). The natural log transformation of these data served to decrease homogeneity of variance (as determined by Cochran's C) and to increase the number of area by STL interactions,

Figure 3. Plot of PCI against standard length of adult weakfish and corresponding plot of residuals against predicted values.

PCI vs Standard Length



Plot of Residual vs Predicted

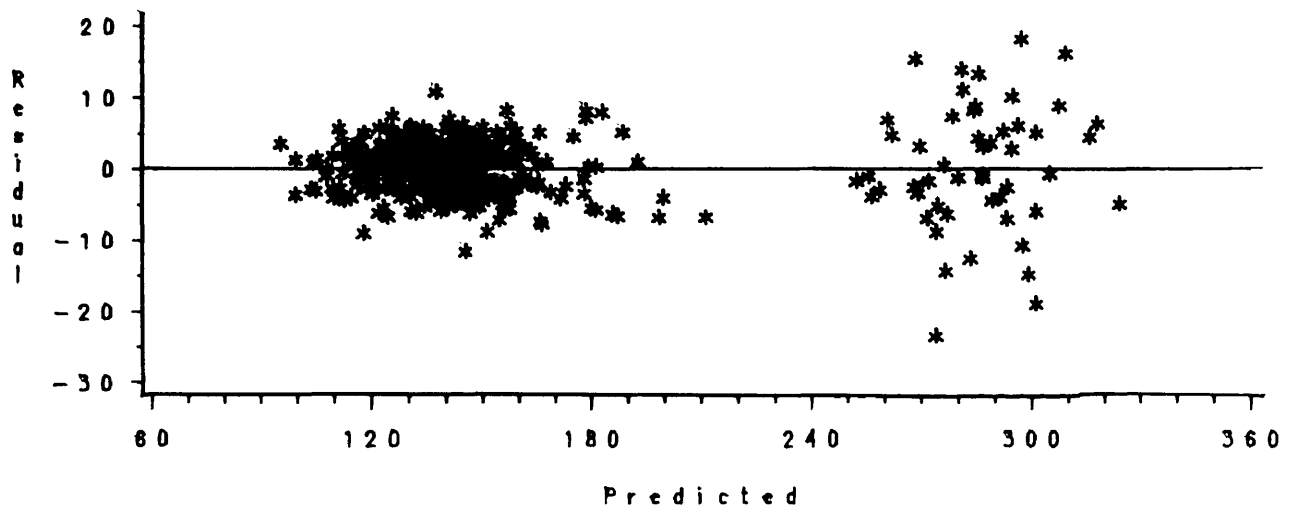
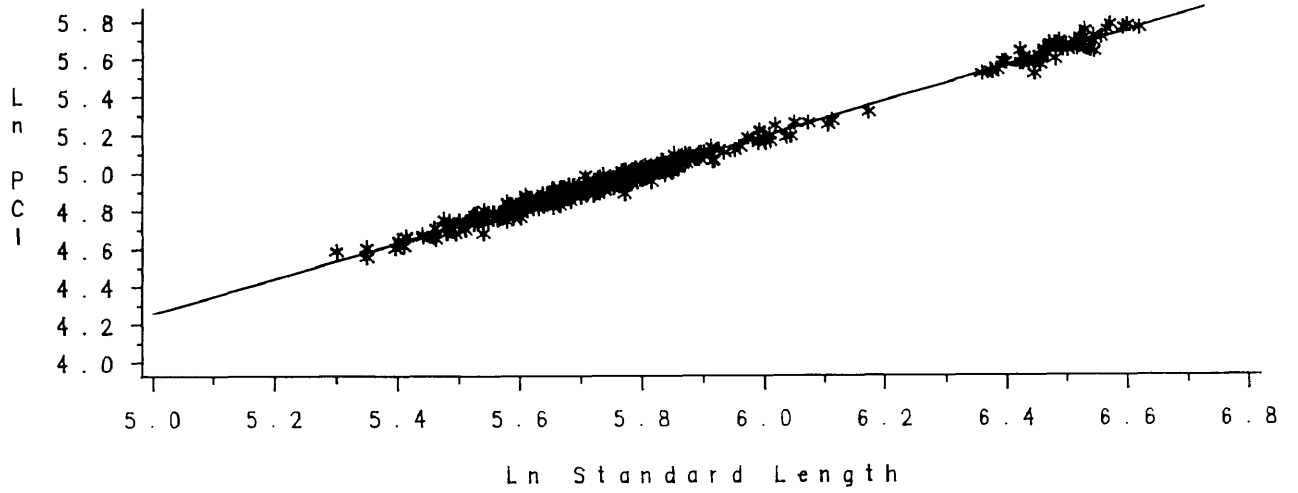


Figure 4. Plot of $\ln(\text{PCI})$ against $\ln(\text{STL})$ of adult weakfish and corresponding plot of residuals against predicted values.

PCI vs Standard Length

Ln Transformed



Plot of Residual vs Predicted

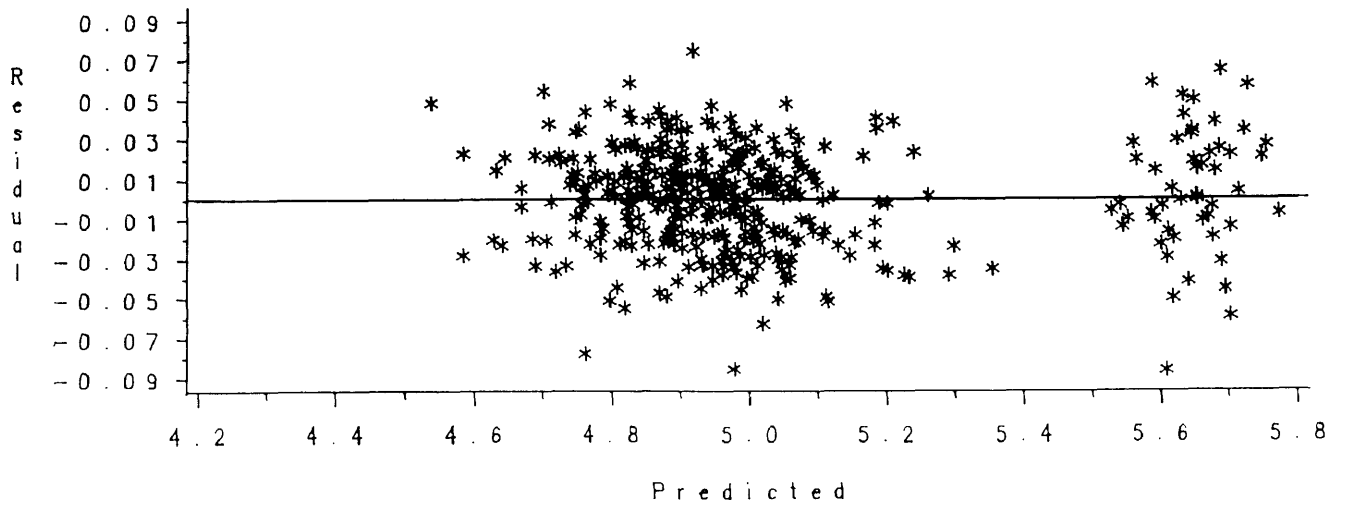


Table 4. Results of multivariate analysis of covariance between the Long Island (med) and Pamlico Sound reference samples of adult weakfish.

Variable	Probabilities of Significance		
	Cochrans' C	Univariate F	Area x STL
PCI	0.186	0.388	0.081
VNT	0.052	0.339	0.719
AFO	0.056	0.831	0.483
AFI	0.050	0.625	0.666
D10	0.073	<0.001**	0.420
D2I	0.056	0.034*	0.206
STL (covariate)	0.043*	---	---

Test of equality of variance-covariance matrices:

Box's M = 53.83236

Chi-Square at 28 df = 50.62000

P = 0.006**

Test of equality of adjusted means:

Wilks' Lambda = 0.81977

F(6, 123) = 4.50693

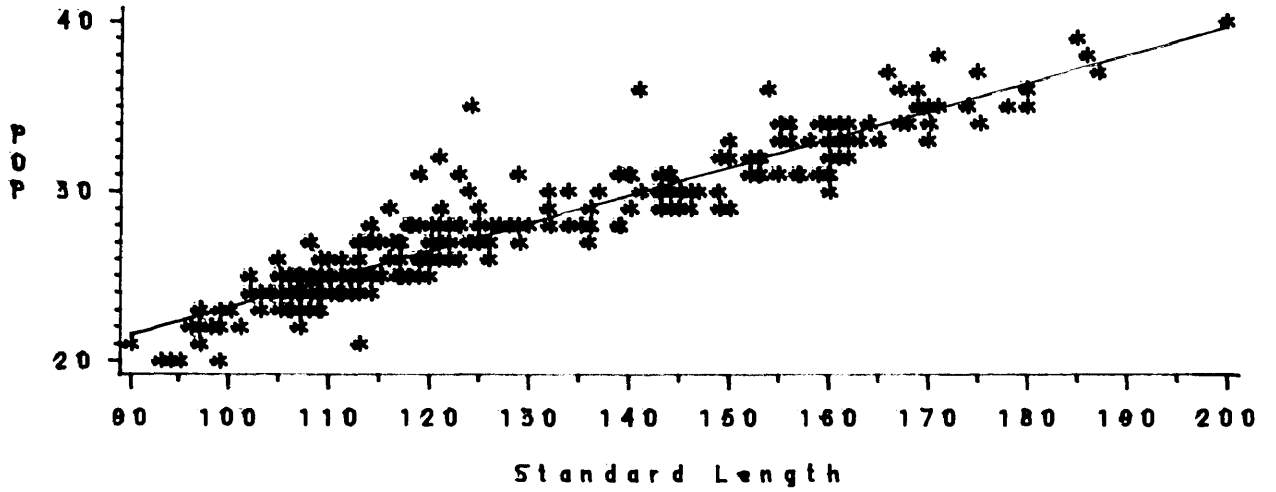
P < 0.001**

* Denotes significant difference at the 0.05 alpha level.

** Denotes significant difference at the 0.01 alpha level.

Figure 5. Plot of POP against standard length of juvenile weakfish and corresponding plot of residuals against predicted values.

POP vs Standard Length



Plot of Residual vs Predicted

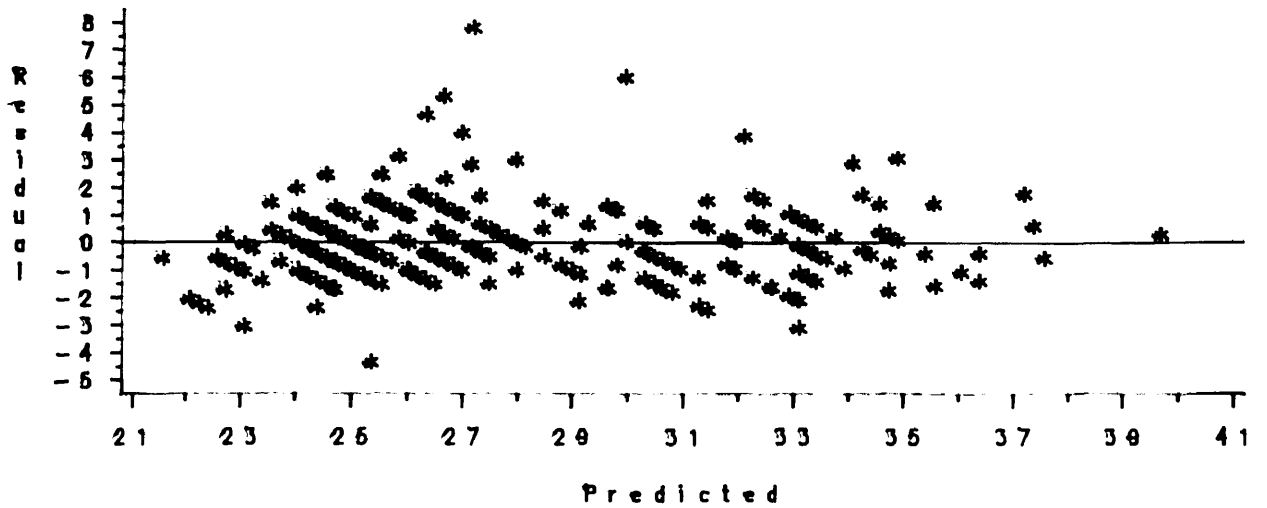
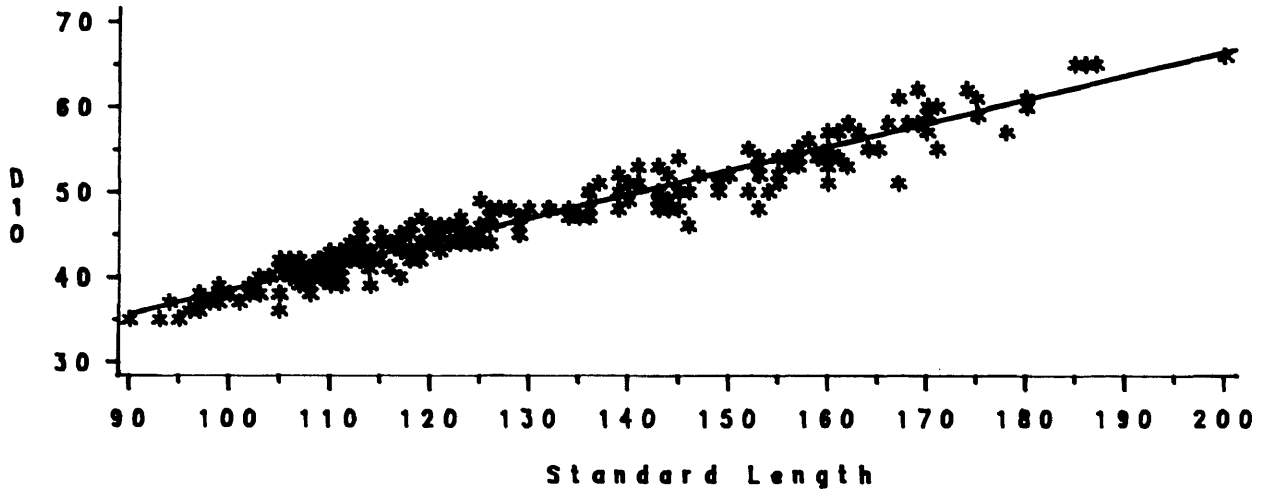
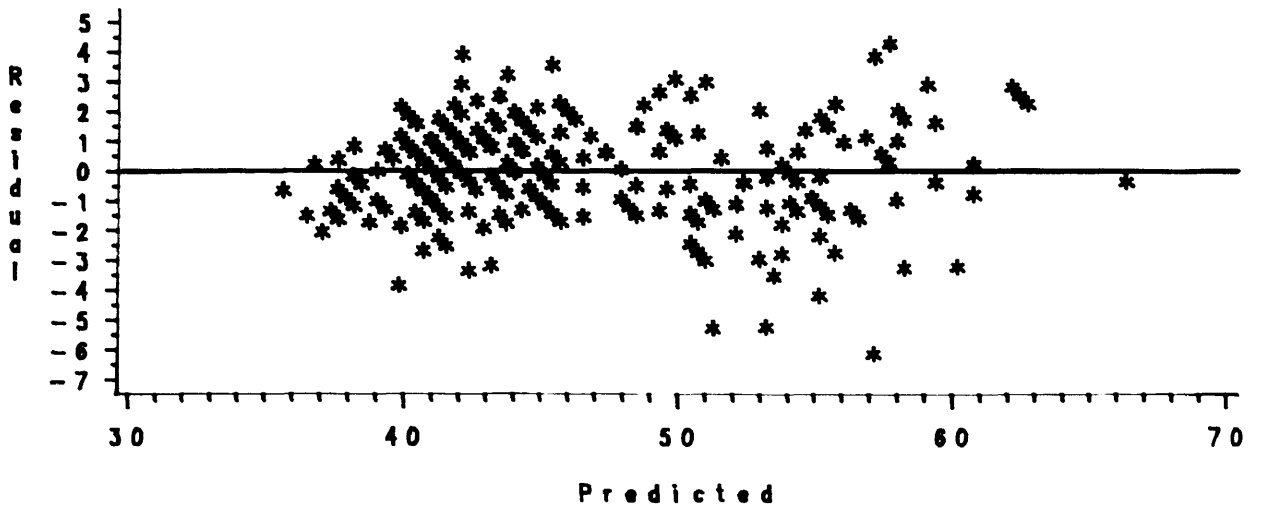


Figure 6. Plot of D10 against standard length of juvenile weakfish and corresponding plot of residuals against predicted values.

D10 vs Standard Length



Plot of Residual vs Predicted



thus the data were not transformed. The assumption of homogeneity of variance was satisfied for all variables ($P > 0.05$). The assumption of equal variance-covariance matrices was also satisfied ($P > 0.05$). MANCOVA results between reference samples of juvenile weakfish demonstrated that the null hypothesis that means are equal between the reference samples of juvenile weakfish was rejected (Table 5).

Variables selected for the discriminant analysis of adult weakfish were IOB, PCO, VNT, D1I, PCI, GTH, OPC, D2I and POB. For discriminant analysis of juvenile weakfish the variables PCI, D1O, AFO, AFI and D1I were selected. Table 6 lists each variable and change in Mahalanobis' distance and Wilks' lambda in the order they were selected by stepwise linear discriminant analysis. Plots of these variables transformed by the allometric growth transformation of STL were nonpatterned and with a slope of zero indicating that the effects of size were satisfactorily reduced (Fig. 7).

The tests of the assumption of equal variance-covariance matrices revealed that these matrices were not equal between reference samples in both discriminant analyses at the default alpha level of 0.10 (Tables 7A and 7B). Therefore, all classifications were conducted using the quadratic discriminant function with within-group variance-covariance matrices.

Classification matrices that summarize the results of each of the discriminant analyses are provided in Table 8. The results of the discriminant function analysis of adult weakfish (Table 8A) are illustrated in Figure 8. The results of discriminant function analysis of juvenile weakfish (Table 8B) are illustrated in Figure 9.

Table 5. Results of multivariate analysis of covariance between the New York and South Carolina reference samples of juvenile weakfish.

Variable	Probabilities of Significance		
	Cochrans' C	Univariate F	Area x STL
POP	0.323	<0.001**	0.612
D10	0.427	<0.001**	0.068
STL (covariate)	0.751	---	---

Test of equality of variance-covariance matrices:

Box's M = 10.27105

Chi-Square at 6 df = 10.06600

P = 0.122

Test of equality of adjusted means:

Wilks' Lambda = 0.79405

$F_{(6, 161)} = 20.87941$

P < 0.001**

** Denotes significant difference at the 0.01 alpha level.

Table 6. Variable selection for discriminant analyses: Mahalanobis' distance and Wilks' lambda by variable added by stepwise linear discriminant analysis.

A. Variable selection for discriminant analysis of adult weakfish using the Pamlico Sound and Long Island (med) reference samples.

Step	Variable Added	Mahalanobis' Distance	Wilks' Lambda
1	IOB	2.30841	0.63776
2	PCO	3.25582	0.55522
3	VNT	3.93370	0.50816
4	D1I	4.76072	0.46054
5	PCI	5.02466	0.44716
6	GTH	5.29661	0.43417
7	OPC	5.71150	0.41575
8	D2I	5.89757	0.40798
9	POB	6.07485	0.40085

B. Variable selection for discriminant analysis of juvenile weakfish using the South Carolina and New York reference samples.

Step	Variable Added	Mahalanobis' Distance	Wilks' Lambda
1	PCI	2.24077	0.63879
2	D1O	2.64017	0.60015
3	AFO	2.96304	0.57217
4	AFI	3.20054	0.55320
5	D1I	3.39673	0.53845

Figure 7. Plot of PCI transformed by the allometric growth transformation against STL for all adult weakfish.

PLOT OF TRANSFORMED PCI vs STL

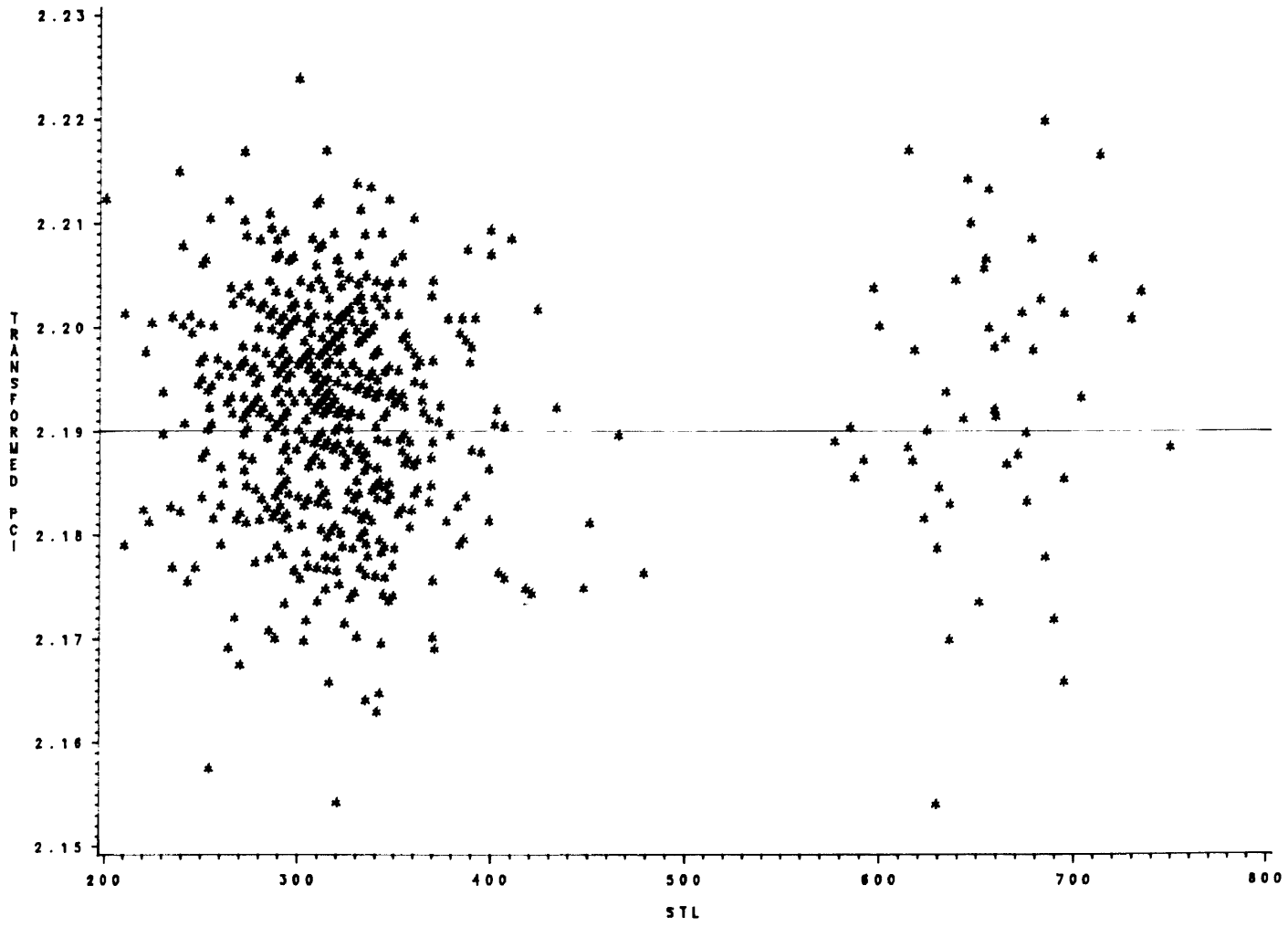


Table 7. Results of tests of equality of variance-covariance matrices between reference samples used in discriminant analyses [by the method of Kendall and Stuart, (1961)].

A. Discriminant analysis of adult weakfish.

Chi Square	96.1396
df	45
Probability	<0.001**

B. Discriminant analysis of juvenile weakfish.

Chi Square	34.7671
df	15
Probability	0.0027**

** Denotes significant difference at the 0.01 alpha level.

Table 8. Classification matrices developed from discriminant function analyses between indicated reference samples of A) adult and B) juvenile weakfish. Tabular values are percent classified followed by the number of observations in parentheses. Note that in all analyses reference samples are reclassified.

A. Results of discriminant analysis of adult weakfish based on the Pamlico Sound and Long Island (med) reference samples. Variables allowed into the analysis are IOB, PCO, VNT, DII, PCI, GTH, OPC, D2I and POB.

<u>Classified Samples</u>	<u>Reference samples</u>	
	<u>Pamlico Sound</u>	<u>Long Island (med)</u>
Long Island (med)	5.56 (3)	94.44 (51)
Long Island (lg)	40.00 (12)	60.00 (18)
Brown Shoal	50.00 (11)	50.00 (11)
Broadkill Beach	36.11 (13)	63.89 (23)
Slaughter Beach	17.24 (15)	82.76 (72)
Rappahannock River	6.25 (3)	93.75 (45)
York River	66.67 (42)	33.33 (21)
Pamlico Sound	92.21 (71)	7.79 (6)

B. Results of discriminant analysis of juvenile weakfish based on the South Carolina and New York reference samples. Variables allowed into the analysis are PCI, D10, AFO, AFI and DII.

<u>Classified Samples</u>	<u>Reference samples</u>	
	<u>South Carolina</u>	<u>New York</u>
New York	7.06 (6)	92.94 (79)
North Carolina	32.50 (26)	67.50 (54)
South Carolina	81.58 (62)	18.42 (14)

Figure 8. Histogram of results of discriminant analysis of adult weakfish.

Classification Results

Adult Weakfish

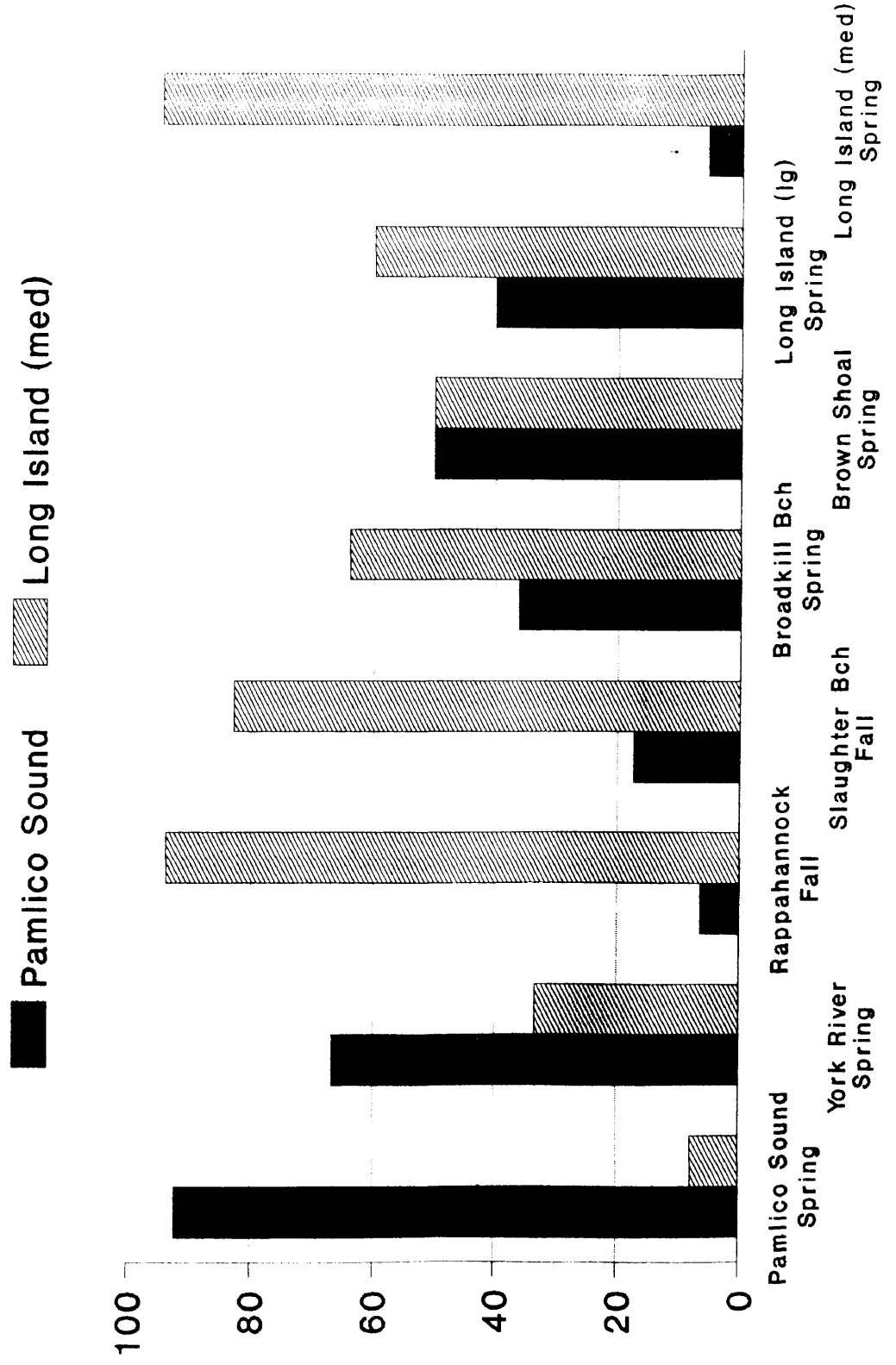
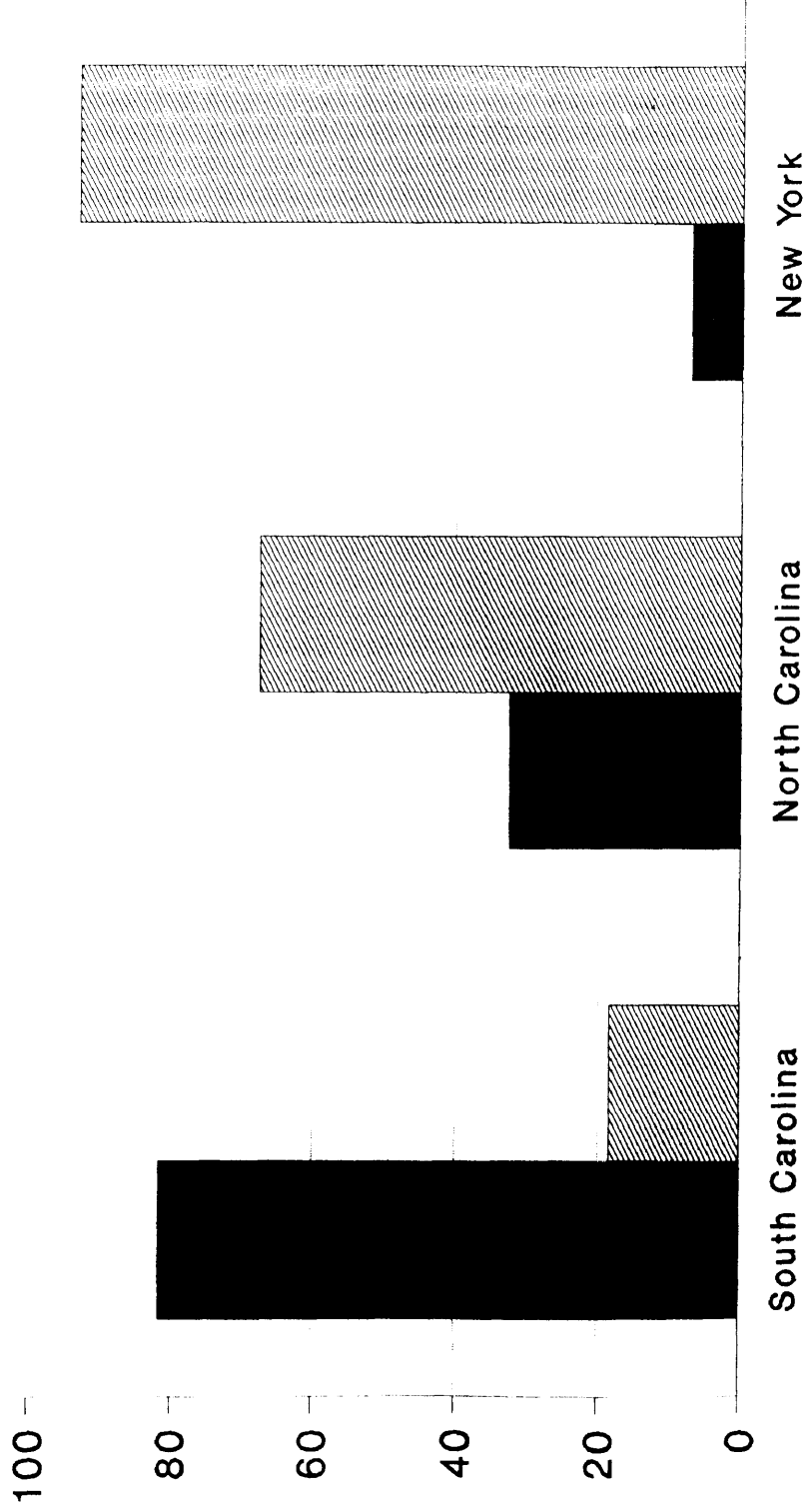


Figure 9. Histogram of results of discriminant analysis of juvenile weakfish.

Classification Results

Juvenile Weakfish

■ South Carolina ▨ New York



DISCUSSION

Examination of variables between reference samples of adult weakfish by multivariate analysis of covariance suggested that measured variables of sampled weakfish differed significantly. However, the assumption of homogeneity of variance for the covariate STL and the assumption of equal variance-covariance matrices were not satisfied. The comparison between reference samples of juvenile weakfish, for which all assumptions were satisfied, also suggested that reference samples differed in the measured variables examined. Although it is important to be aware that violation of assumptions may produce erroneous results, Pimental (1979) suggested that these tests are robust, and that the violation of some assumptions may not necessarily nullify the results. Significant morphological differences were found which warranted the use of discriminant analyses.

Discriminant analyses were conducted using different variable sets than those used in multivariate analyses of covariance. An examination of the same sets of variables would have been favorable, but some of the variables selected by the stepwise selection procedure for discriminant analyses demonstrated significant area by standard length interactions (a violation of an assumption of MANCOVA). If the variables used in both MANCOVA and discriminant analysis were entirely different, it would not have been reasonable to suggest significant morphological

differences occurred which warrant the use of discriminant function analysis. However, some of the variables used in both MANCOVA and discriminant function analysis were the same. In both of these analyses of adult weakfish the variables VNT, PCI and D2I were used. Only one variable, D10, was used in both of these analyses of juvenile weakfish (see Tables 4 - 6).

Regarding the transformation of variables for discriminant analysis, there is a some controversy over the method to use to remove the effect of size. Atchley et al. (1976) advised strongly against the use of ratios because of induction of spurious correlations. However, Hill (1978), Dodson (1978) and Albrecht (1978) published in reply and suggested that the conclusions of Atchley et al. (1976) may be misleading. Ratios are not a choice method of transformation if the organism of interest grows allometrically to any degree. When I tried the transformation using ratios of variables divided by STL, classification results with major size interactions resulted because of allometric growth. Despite the potential problems associated with the use of ratios to reduce size effects, others have found them useful (Mosimann and James, 1979; Shaklee and Tamaru, 1981; Wilk et al., 1980).

Reist (1985) and Claytor and MacCrimmon (1986) evaluated a number of transformations that remove the effect of size and correct for allometric growth. They suggested that residuals provide information free from size effects and correlations. This transformation was also suggested by Atchley et al. (1976). With use of the residual transformation, I found that the reclassification of individuals of reference samples was about 20 percent below that which was obtained

when I used the allometric growth transformation recommended by Thorpe (1975).

The results of the discriminant function analysis of adult weakfish suggested that weakfish with characteristics found in the north in spring were not as abundant in the southern part of the range in spring. Medium weakfish sampled from Broadkill Beach in spring classified mostly with the Long Island (med) reference sample while those sampled from the York River in spring classified mostly with the Pamlico Sound reference sample. These classification results suggested that at the time of sampling in spring there was either a cline in morphometric characters of weakfish along the Atlantic coast, or that there were separate morphological types of weakfish that did not segregate in spring, but appeared to mix where they were sampled. Furthermore, these results do not support the hypothesis of Seguin (1960) that weakfish of Virginia and Delaware are similar in morphometric variables. While it is possible that a third morphologically distinct group of weakfish occurs, detecting it with discriminant function analysis would be difficult without more extensive temporal and spatial replication in sampling. Such an analysis might reveal several samples with similar proportions of classification suggestive of a third group.

In the Chesapeake and Delaware Bays two peaks of spawning were identified by Welsh and Breder (1923), one occurring from April to May, and the other in September. Results of discriminant analyses suggested that the weakfish collected in September and October from these regions were most similar to weakfish of the northern reference sample. The Slaughter Beach and Rappahannock samples classified primarily to the

Long Island (med) reference sample. All of the weakfish of these two samples had already spawned. It may be that these fish moved into Chesapeake and Delaware Bays to spawn a second time, and that spawning was completed by the time they were sampled. It is also possible that these fish spawned in Long Island Sound during the spring and moved into Chesapeake and Delaware Bays to feed.

Both reference morphological types were represented in nearly equal proportions among large weakfish sampled from Long Island Sound and Brown Shoal. As mentioned in the introduction, Wilk (1979) suggested that as weakfish get older and larger, they move farther north. Wilk's hypothesis is supported by the classification results of large weakfish. This conclusion assumes that weakfish of the northern part of the range remain in the area as they get older and larger, and were mixed with southern weakfish in samples of large sized individuals.

The results of the discriminant analysis of juvenile weakfish, collected in late fall when inshore waters begin to decrease in temperature, suggested that the weakfish sampled off North Carolina were more similar in measured variables to those in Long Island Sound than to those in Charleston Harbor. It appears that the juvenile weakfish from North Carolina to Long Island were similar in morphology at the time of sampling. The majority of the North Carolina sample may have classified to the New York reference sample because the juvenile weakfish of the northern part of the range moved south as the temperature of the water decreased. Nesbit (1954) also suggested that juvenile weakfish undergo extensive migrations based on tag return results.

Although it is desirable to understand how weakfish differ in their measured variables so that an individual can be classified based on a few simple measurements, the similarities between northern and southern weakfish are great enough that such classification is difficult. To illustrate this, I plotted the first two variables selected by the stepwise variable selection procedure (those that differ the most between the reference samples) against each other by area. For the adult weakfish IOB was plotted against PCO for both the Long Island (med) and Pamlico Sound reference samples (Fig. 10). The results revealed two parallel lines of regression which demonstrated that on average for any PCO measurement, Pamlico Sound weakfish sampled in spring had an IOB measurement about 2mm smaller than that of Long Island weakfish sampled in spring. For the juvenile weakfish PCI was plotted against D10 for both New York and South Carolina reference samples (Fig. 11). Similarly, parallel lines of regression were revealed, suggesting that on average for any D10 measurement, New York juveniles had a PCI measurement about 3.5mm smaller than that of South Carolina juvenile weakfish. These plots illustrate general differences between weakfish of reference samples, but as is seen from Figures 10 and 11, the overlap of data points is too great to determine the group an individual belongs to based on the measurement of two variables. The differences that were revealed between weakfish of the northern part of the range and those of the southern part were slight enough that in order to classify individuals to one of these groups, it was necessary that many variables be examined simultaneously.

Figure 10. Plot of IOB against PCO of adult weakfish of both the Long Island (med) and Pamlico Sound reference samples.

PLOT OF IOB vs PCO

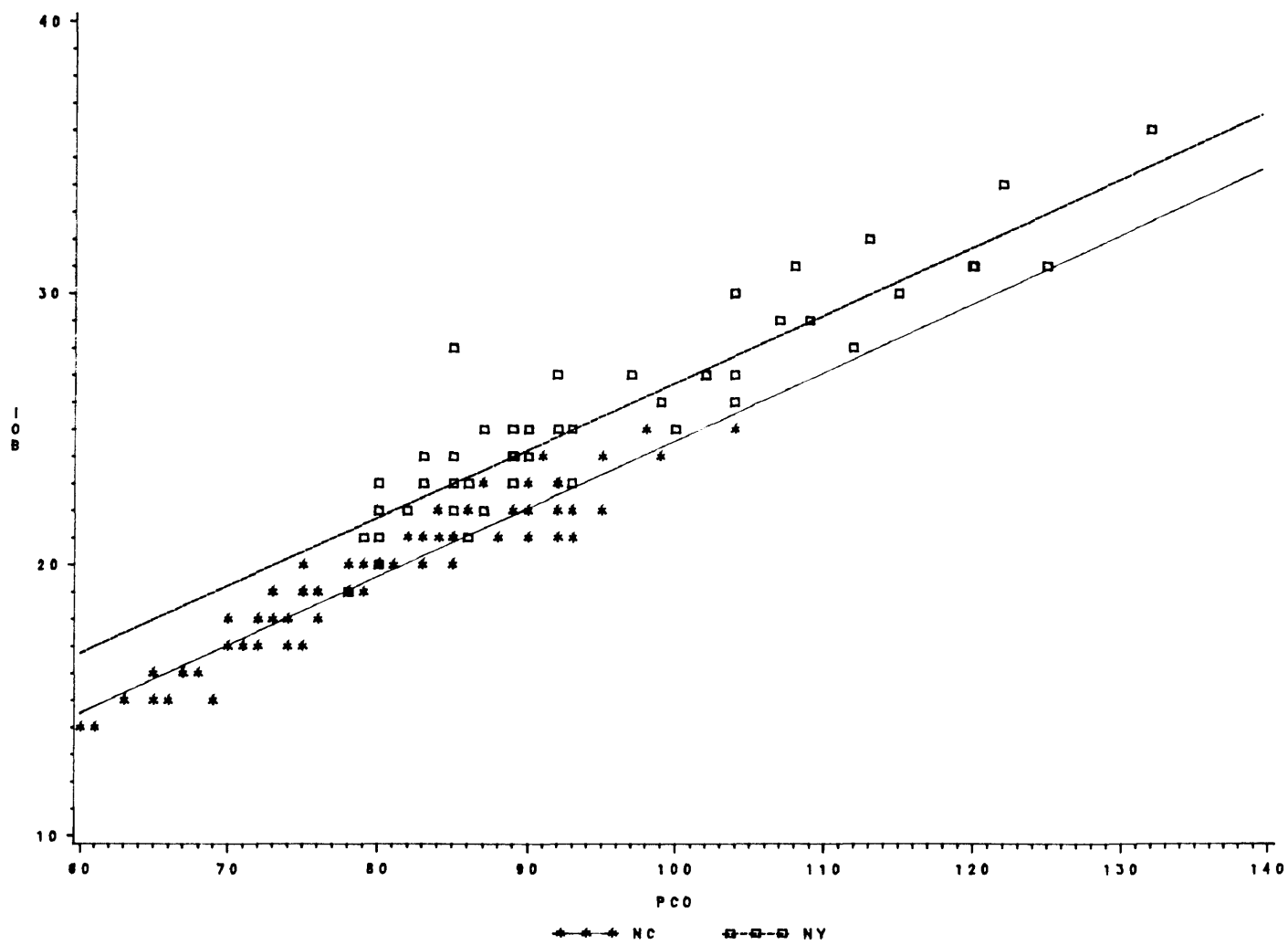
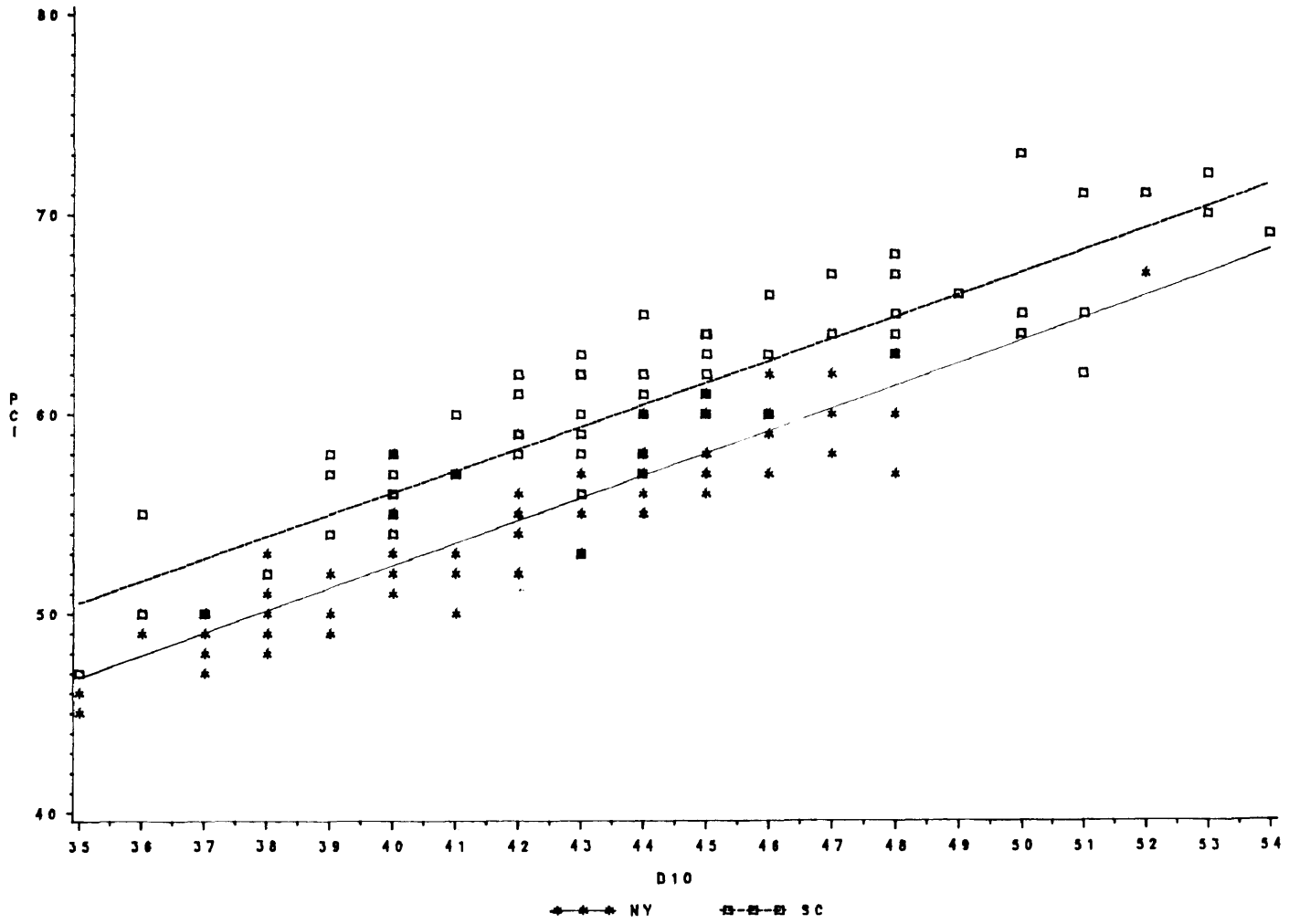


Figure 11. Plot of PCI against D10 of juvenile weakfish of both the New York and South Carolina reference samples.

PLOT OF PCI vs D10
JUVENILE WRAKFISH



The validity of the results of discriminant analysis depends on how well the assumptions are satisfied. In this study there are two assumptions of discriminant analysis in question. It is not certain whether the assumption of multivariate normality is satisfied, but as mentioned previously, the allometric growth transformation aids in its approximation. The assumption that reference samples did not include weakfish from other reference samples is also in question. Weakfish of both Long Island (med) and Pamlico Sound reference samples reclassified to their sample of origin above 92 percent. It was assumed the reference samples were 'pure' enough that valid and useful conclusions can be drawn from the classifications.

Some possible complicating factors of the design of this study are related to sample size and sampling times. It is desirable that samples be as large as possible. This applies especially to reference samples which provide the data to develop discriminant functions. The sample sizes obtained in this study were small in comparisons to other studies (see pg. 8). The Long Island (med) and Pamlico Sound reference samples of adult weakfish consisted of 54 and 77 individuals, respectively. The smallest sample of adult weakfish included 22 individuals, and the largest 87. Samples of juvenile weakfish, which were more uniform, ranged from 76 to 85 individuals.

Sampling times are important because samples taken at different times at any one location may be of different stock composition. Temporal replication was conducted between spring and fall, but not within either of these seasons. In an effort to minimize variation within, while maximizing variation among spring samples, collections

were made near the peak of the spring spawn, because it is assumed that if stocks segregate, it must at least be at the time of spawning.

All of the studies which provide evidence of weakfish stock structure, including this study, suggest that differences in measured parameters exist. However, the genetic analyses by Crawford et al. (1988) and McDowell et al. (1990) concluded that weakfish along the mid-Atlantic coast share a common gene pool. Since it is apparent that there is little genetic variation among weakfish, the observed morphological character variation is probably a result of phenotypic plasticity (environmentally induced phenotypic variation). Such variation is not uncommon (Stearns, 1989). Meyer (1987), Kornfield et al. (1982) and Sage and Selander (1975) documented extensive phenotypic plasticity in neotropical cichlids of the genus, Cichlasoma. Morphometric and meristic variables have been shown to be variable depending on temperature, salinity, light exposure and diet (Taning, 1950; Lindsey, 1954; Lindsey, 1958; Barlow, 1971; Meyer (1987).

This study demonstrates that significant morphological variation occurred among samples of weakfish which were subsequently classified to two reference samples using discriminant function analysis. The results suggested that at the time of sampling a cline of morphometric characters or substantial mixing among the morphological types occurred intermediate in the range for weakfish. Whether or not the observed morphological character variation is genetically or ecophenotypically based, these differences provide fisheries managers with a means to investigate weakfish stock composition and migratory habits.

EPILOGUE

On January 16, 1990 a sample of 177 weakfish was collected from the winter fishery off North Carolina from Wimble Shoal. These fish were classified by the discriminant function developed in this study from reference samples Long Island (med) and Pamlico Sound. The results revealed that 140 individuals (79.1 percent of the sample) classified to the Long Island (med) reference sample and 37 individuals (20.9%) classified to the Pamlico Sound reference sample. Though these results are strictly preliminary, they suggest that a significant proportion of weakfish that overwinter off North Carolina are similar in morphology to those found in Long Island Sound in spring. As indicated by the results of this thesis, it appears likely that a majority of these fish are found in Delaware Bay and north in spring, and Chesapeake Bay and north in fall. A report of these results (Scoles, 1990) can be obtained from the Atlantic States Marine Fisheries Commission.

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VITA

Daniel Roy Scoles

Born in Fontana, California, December 27, 1962. Graduated from Rubidoux High School, Rubidoux, California, 1981. Earned B. S. in Biochemistry from the University of California at Riverside in 1987. Entered the Master of Arts program of the College of William and Mary in 1987.