Northeast Fisheries Science Center Reference Document 16-07
doi:10.7289/V50Z71B9

Geomorphometric Analysis Indicates Overlap in Body Shape between Sexes of Black Sea Bass *(Centropristis striata)*

by Bradley Keigwin, Gary R Shepherd, and Mark J Wuenschel

Northeast Fisheries Science Center Reference Document 16-07
doi:10.7289/V50Z71B9

Geomorphometric Analysis Indicates Overlap in Body Shape between Sexes of Black Sea Bass *(Centropristis striata)*

by Bradley Keigwin[1], Gary R Shepherd[2], and Mark J Wuenschel[2]

[1]Department of Mathematics and Statistics
Lederle Graduate Research Tower, 1623D
University of Massachusetts Amherst
710 N. Pleasant Street
Amherst, MA 01003-9305

[2]NOAA National Marine Fisheries Service
Northeast Fisheries Science Center
166 Water Street
Woods Hole, MA 02543

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts
August 2016

Northeast Fisheries Science Center Reference Documents

This series is a secondary scientific series designed to assure the long-term documentation and to enable the timely transmission of research results by Center and/or non-Center researchers, where such results bear upon the research mission of the Center (see the outside back cover for the mission statement). These documents receive internal scientific review, and most receive copy editing. The National Marine Fisheries Service does not endorse any proprietary material, process, or product mentioned in these documents.

All documents issued in this series since April 2001, and several documents issued prior to that date, have been copublished in both paper and electronic versions. To access the electronic version of a document in this series, go to *http://www.nefsc.noaa.gov/nefsc/publications/*. The electronic version is available in PDF format to permit printing of a paper copy directly from the Internet. If you do not have Internet access, or if a desired document is one of the pre-April 2001 documents available only in the paper version, you can obtain a paper copy by contacting the senior Center author of the desired document. Refer to the title page of the document for the senior Center author's name and mailing address. If there is no Center author, or if there is corporate (*i.e.*, non-individualized) authorship, then contact the Center's Woods Hole Laboratory Library (166 Water St., Woods Hole, MA 02543-1026).

Information Quality Act Compliance: In accordance with section 515 of Public Law 106-554, the Northeast Fisheries Science Center completed both technical and policy reviews for this report. These predissemination reviews are on file at the NEFSC Editorial Office.

This document may be cited as:

Keigwin B, Shepherd GR, Wuenschel MJ. 2016. Geomorphometric analysis indicates overlap in body shape between sexes of black sea bass (*Centropristis striata*). US Dept Commer, Northeast Fish Sci Cent Ref Doc. 16-07; 26 p. Available from: National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026, or online at http://www.nefsc.noaa.gov/publications/

TABLE OF CONTENTS

INTRODUCTION

Black sea bass (*Centropristis striata)* is a protogynous hermaphrodite and member of the family Serranidae (Steimle et al. 1999). The species is considered a temperate reef fish (Steimle et al. 1999), which is distributed from the Gulf of Mexico to Maine with 3 distinct and separate populations partitioned at Cape Hatteras, North Carolina, and the Florida Keys (Bowen and Avise 1990; Roy et al. 2012; McCartney et al. 2013). Black sea bass are routinely targeted by commercial and recreational fisheries and are regulated primarily by quota, seasons, and minimum sizes (Shepherd and Terceiro 1994).

As a protogynous hermaphrodite, black sea bass begins life as female but may switch to male either pre- or post- maturity (Lavenda 1949). In the male stage the phenotypic characteristics may differ if an individual takes on a role as a dominant male. Dominant males display aggressive behavior during spawning season and either control a group of females (a harem) or control a territory while fending off other males (a lek). The secondary sexual characteristics in black sea bass are dimorphic, with dominant males generally larger than females. Dominant males develop a nuchal hump, a prominent fat deposit on their nape that becomes iridescent blue during mating season, as well as white highlights on their fins and face. In contrast, other, perhaps secondary males, look very similar to females that lack the nuchal hump and bright coloration. Determining the sex of fishes is generally accomplished through internal examination of gonad tissue, unless there are definitive external characteristics unique to each sex (e.g., the blue nuchal hump in black sea bass). In this study, we analyzed the shape of males and females to quantitatively evaluate differences between sexes. By using shape analysis, sexes were compared to determine the reliability of sex determination from external features.

Geomorphometrics (geometric morphometrics) is commonly used to analyze and characterize the physical appearance of shapes (Adams et al. 2003). The morphological comparisons may be shapes of interest within species or between different species or populations. The methods have also been used for other purposes such as facial recognition software or as diagnostic tools in examining medical images (Bookstein 1982). Geomorphometrics use landmarks to analyze shape (Bookstein 1982; Rohlf 1990). Landmarks are discrete locations (anatomical features) that can be identified consistently on each specimen but are not dynamic features; the relative location of the landmark must be constant and independent of size (Smith 2002). The objective of geomorphometrics is to identify landmarks that can be recorded in Cartesian coordinates, so the landmarks and the interlandmark distances can be used as a representation of shape.

The shape coordinates can be analyzed by using several methods (Cadrin 2000), but one of the more common approaches is Principal Component Analysis (PCA). PCA creates linear combinations of orthonormal eigenvectors to describe the variance within a data set. The eigenvectors are the Principal Components (PC), which are linear combinations of the distances presented as a correlation matrix or covariance matrix. In the correlation matrix, the weight of each element in the matrix ranges between -1 and 1, whereas, in a covariance matrix, the values would not be bounded by -1 and 1. The PCs make up a vector space, and each PC axis is used to describe variance components. Higher weighted variables are the more important ones for a particular principal component. The first PC (PC1) accounts for the most variance, and the second (PC2) describes less, and so on. Since the PCs are orthogonal, variables that are weighted high in PC1 will not likely be weighted high in PC2, etc. By using PCA we can reduce the

dimension of the data to determine which landmark distances account for the most variance and consequently could best be used to describe shape.

METHODS

Black sea bass used for analysis were caught during the Massachusetts Division of Marine Fisheries survey cruises in May and June 2014. The fish were briefly frozen and then thawed before they were photographed, dissected, and their shape information was evaluated. Landmark locations were identified with dissecting pins on individual fish and positioned for photographs (Figure 1). Landmarks were chosen based on a similar morphometric evaluation of scup by Love and Chase (2009). Sea bass were photographed by using an adjustable camera stand with a Nikon[1] digital camera positioned approximately 80cm above the fish. Total length (mm), weight (g), maturity stage, gonad weight (g), and sex (based on macroscopic evaluation of the gonad) were then determined.

Interlandmark distance measurements were obtained from the digital images using the software tpsUtil 1.53 (Rohlf 2004[2])). Each image included a ruler which was used to rescale image measurements from pixels to millimeters. The resulting x, y coordinates created in tpsUtil, from 77 fish were imported into Microsoft Excel and SAS (2000). (Figure 1, Table 1). The distances between every landmark (n=22) combination (252 distances) were calculated with the equation:

$$dist_i = sqrt((x_i - x_j)^2 + (y_i - y_j)^2) \qquad (1)$$

where $dist_i$ is the distance from landmark i to j measured in pixels. Distance coordinates (Table 1, Figure 1) for each fish ($dist_i$) were converted from pixels to millimeters and standardized by dividing by total fish length. Total length accounted for obvious variation among fish and, since we are interested in the sex-specific differences in shape among fish, dividing by length removed length as an influencing factor of shape.

Principal components can be calculated by using either a correlation matrix or a covariance matrix. We used the correlation matrix since the correlation matrix can give accurate results for 2 variables of different units. All the measurements were initially standardized to the mean.

$$\text{Standardized } dist_i = (dist_i - \text{mean}(dist_i)) / (\text{standard deviation}(dist_i)) \qquad (2)$$

Since the correlation matrix is symmetric and semipositive definite, we can make use of the Spectral Theorem and Schur's Lemma to diagonalize the matrix with orthonormal eigenvectors (Strang 2006). The eigenvalues are arranged from highest to lowest across the diagonal, and their corresponding eigenvectors are arranged accordingly. Only the highest eigenvalues that explain the majority of the variance in the data are important. The correlation matrix could be recreated by matrix multiplication of eigenpairs. The lower valued eigenpairs could be discarded, and most

[1] All specific brand names found in this document are used for descriptive purposes only and do not constitute endorsement of any product, service, organization, or company.
[2] available at http://life.bio.sunysb.edu/ee/rohlf/software.html

of the data would still be explained since the low valued eigenpairs have little influence in the correlation matrix.

The standardization process using all possible landmark measurement combinations produced a 77 x 252 matrix. There were 22 zeroes in the data set along the diagonal, since the distance from landmark i to i is 0, and those zeroes were removed. Two of the 4 head bump landmarks were considered redundant, so distances originating from them were removed. Also removed were any distances originating at the tip of the pelvic fin since it was evident that location of the end of the fin was not constant in each photo. Removing these values resulted in 210 possible distances from the 19 remaining landmarks.

The 210 distances were evaluated for normality by using a Shapiro-Wilk's test (SAS Proc Univariate). Data should be normally distributed in order to perform PCA, since it requires linear relations (Zar 1974). We identified distances that have Wilk's value <0.05, implying a departure from a normal distribution. Therefore a $\log_e$ transformation (m_distX = log(m_distX +1)) was done to normalize the distance measurements.

Principal component analysis (PCA) was conducted on the 210 distance measurements (SAS Proc Princomp; Table 2). However, a sample size of 77 fish presented a problem since there can only be as many principal components (in this case 210) as there are samples in the dataset. Principal components 77 through 210 had values of zero and were removed, as were distances that had low weights on each of the principal components. Higher weighted components correspond to a larger variance, which imply features of interest, whereas lower valued components correspond to noise within the data set. In addition, if distances were highly correlated with each other, one was removed to prevent redundancy. Reducing the data based on these criteria resulted in a final set of 11 distance measurements that accounted for the greatest degree of separation among sexes (Table 3). An alternative approach was evaluated which replaced some landmark combinations with ones expected to have biological significance among sexes, particularly head, body, and tail measurements. Final principal components were calculated (Table 5) for each fish by using the standardized distance multiplied by each coefficient (eigenvalue) in each eigenvector (Table 4). For example, for each fish:

Principal component 1 = 0.304*m_dist4 + 0.317*m_dist17 + 0.323*m_dist15 + 0.311*m_dist77 + 0.337*m_dist72 + 0.249*m_dist107 + 0.259*m_dist112 + 0.389*m_dist9 + 0.186*m_dist216 - 0.377*m_dist135 + 0.187*m_dist223

with each m_dist standardized to total length prior to use.

In the PCA plots of principal component 1 vs. principal component 2 for the reduced data set (115 distance measurements), 2 fish were identified as outliers (Figure 2). Further examination of the photos showed that both fish had abnormal body shapes because of either a distended stomach (full of squid) or an eroded/damaged tail (Figure 3). These fish were excluded from further analysis, leaving a total sample size of 75 fish.

Centroid size was calculated for each fish to compare body shape among sexes. The photo of each fish was partitioned into 9 triangles by using 11 landmarks (Figure 4). The centroid position of each local triangle was calculated by adding the 3 vertex coordinates of the local triangle and dividing by 3. The area of each local triangle was determined by using the law of cosines and the area formula: area = height* (base/2). The fishes "main centroid" was calculated by multiplying coordinates of local centroids by its corresponding area. The main

centroid position of each fish was generally slightly above the midpoint of the pectoral fin. The centroid size is the square root of the sum of distances from each landmark to the main centroid.

RESULTS

The number of distance measurements used in the PCA were reduced from the original 210 distance measures, and each reduction increased separation between the clusters of males and females (Figures 5-10). We removed the highly correlated distances with low weight in PC1 and PC2, but if a distance was low in PC1 but high in PC2, it was not removed. The selection process identified 11 distance measures that had the most explanatory power. PC1 explained 46% of the total variance while PC2 explained 16% (Figure 10). The first 5 principal components explained almost 90% of the variance as shown in a scree plot (Figure 11). However since the first 2 PCs only explained 62% of the variance (Table 3), this suggests that the 2 sexes cannot be distinguished accurately from external morphometric characteristics.

Principal component 1 (PC1) accounts for the greatest source of variation among fish morphometric characteristics, which is generally various length measurements. Consequently the highest weights are related to distances 9 and 135, which are measures of fish length (Table 4; Figure 1). Principal component 2 (PC2) appears to be influenced by head measurements; it has highest weights on distances 216 and 223 (Table 4; Figure 1). In the graph of PC2 vs total length, there is almost no correlation, implying that PC2 is not influenced by length (Figure 12).

The separate clusters of males and females (Figure 10) show that males are different from females based on the final suite of 11 landmark distance measurements; however, some overlap between the sexes is apparent (Figures 13-14). Overlapping males (i.e., males with form similar to females) have larger body length measurements (Figure 14b) and shorter tails than average males (Figure 14i). Conversely, overlapping females (i.e., females with form similar to males) have smaller tails than average females (Figure 14i). Additionally, the overlapping males lacked a noticeable nuchal hump, as seen in their low values of distance 223 (tip of operculum to head bump #2; Figure 14k), and may be considered secondary males. They also have larger than average distance 4 (mouth to start of dorsal fin; Figure 14a), distance 15 (mouth to tip of operculum; Figure 14c), and distance 17 (mouth to start of pelvic fin; Figure 14d) measurements, which imply they have longer heads than typical males; this is a characteristic of females. Compared to other females, the overlapping females have lower than average distance 4, distance 15, distance 17, and distance 77 (start of dorsal fin to pelvic fin; Figure 14f) and distance 107 (start of anal fin to end of dorsal fin; Figure 14g) measurements, meaning that they are more slender and have smaller heads than average females, which are characteristics of a secondary male. Within the cluster of males there are many males without humps, implying that there are features that distinguish sex other than the presence of a nuchal hump. Several males that are mixed within the female cluster do not have nuchal humps, which is often a characteristic of secondary males that practice sneak copulation (sneakers). However, there are also similar shaped males within the male cluster. Several females are interspersed within the male cluster, which could be females prior to transitioning to males, although they were not categorized macroscopically as transitioning.

The centroid size measurement was not as good at reducing the influence of shape on PC1 as was the measurement of length as (Figures 15A-C). The centroid size does not account for differences in shape (circular, or varying degrees of stretched ovals that may or may not be aligned with the long axis). An oval aligned with the long axis would represent the centroid of a

longer fish, while a more circular centroid would indicate a wider fish, but the different centroid shapes could have the same size (area). Thus, 2 fish with different shapes could have the same centroid size, limiting the explanatory power of this metric.

DISCUSSION

We expected clearly separated PC clusters by sex, but instead the PCA does not support the conclusion that there are a suite of morphological features that distinguish between males and females in all cases. The fish used for measurements were in spawning or postspawning condition with the exception of 3 immature females. During the spawning season males typically develop prominent secondary sex characteristics such as a colorful nuchal hump. Given that these secondary characteristics occur during the spawning season, we expected to see the maximum degree of separation among sexes at the time when the samples were collected. We did not encounter any "transitional" (females going through the process of becoming males), nor did we expect to because black sea bass do not transition during the spawning season when these samples were taken. Similar to other Serranidae, they usually change sex following the spawning season, in this case during the fall to winter. Similar morphometric analysis of transitional fish from the nonspawning period may reveal distinguishing characteristics.

Black sea bass exhibit wide variation in external appearance during the breeding season, with distinguishable differences between most males and females. The secondary sexual characteristics were most evident in males that were presumed to be "dominant," typified by large a nuchal hump, long feathery tail, and dark blue coloration (with a lighter face mask). Females are generally lighter in color and lacked the prominent nuchal hump. However, many individuals of both sexes were "indistinguishable," possessing morphometric characteristics that clustered with the opposite sex. It is unknown whether these morphological differences are indicative of different behavioral roles (i.e. dominant, subordinate, sneaker).

The complex life history of black sea bass probably contributes to the wide variation in morphological characteristics. Although the species is hermaphroditic, individual life histories vary. Some individuals change sex before maturing as a female (prematurational sex change), evidenced by the presence of small and young males. Some individuals never change sex, evidenced by large and old females in the population. Between these 2 functionally gonochoristic conditions, individuals may first reproduce as female for 1 or more years before changing sex and reproducing as male for 1 or more years. Therefore larger and older males may arise from different individual trajectories, with accompanying morphometric characteristics. For example, it is reasonable to expect that the morphometric characteristics of a 5 year old male that transitioned at age 4 could be different from that of a 5 year old male that underwent prematurational sex change at age 1 and has only functioned as a male. Further complicating matters are the multiple roles that males can play in spawning. Though observations are limited, it appears that large "dominant" males with prominent secondary sexual characteristics initiate the majority of spawning bouts, keeping "subordinate" (or secondary) males to the periphery (J. Rosendale, NOAA-NEFSC, 74 Magruder Rd., Sandy Hook, NJ, pers. comm., unpublished data). The subordinate males typically lack secondary sexual characteristics, and take on a more "female" appearance, consistent with a "sneaker" male strategy. The multiple paths leading to functional reproductive males, and multiple roles of males in the mating system, probably leads to variation in the appearance of males. Similar variation in female morphometrics may result if the transition to male is preceded by physiological changes (e.g., accumulation of fat, energy)

that may alter the external shape/appearance of soon-to-transition females. Experimental studies have shown that black sea bass injected with hormones displayed secondary male sexual characteristics within 2 weeks (Benton and Berlinsky 2006). Therefore the external appearance and morphometric characteristics may be more labile than the production of functional gametes. The gonadosomatic index of black sea bass decreases to very low levels in the nonspawning period (NEFSC 2012), as do some of the secondary sexual characteristics. It is unknown if the degree of separation between sexes based on morphometrics increases or decreases outside the spawning period.

The result of the analysis of black sea bass morphometric landmarks using Principal Component Analysis demonstrates that external features alone cannot be used to determine sex with 100% accuracy. Head shape is commonly used to identify males, but these results suggest it may only provide correct sex identification if the secondary characteristics (e.g. prominent nuchal hump and blue coloration) are present. Ultimately the most effective method to determine the sex of sea bass is internal macroscopic examination of the gonads.

REFERENCES CITED

Adams D, Rohlf JF, Slice DE. 2003. Geometric morphometrics: Ten years of progress following the 'Revolution'. Italian Journal of Zoology 71:5-16.

Benton CB, Berlinsky DL. 2006. Induced sex change in black sea bass. Journal of Fish Biology 69:1491-1503.

Bookstein FL. Foundations of morphometrics. 1982. Annual Review Ecological Systems 13:451-70.

Bowen BW, Avise JC. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: Influence of zoogeographic factors and life-history patterns. Marine Biology 107:371-381.

Cadrin SX. 2000. Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries 10:91-112

Lavenda N. 1949. Sexual differences and normal protogynous hermaphroditism in the Atlantic sea bass, *Centropristes striata*. Copeia 1949 (3): 185-194.

Love JW, Chase PD. 2009. Geometric morphological differences distinguish populations of scup in the Northwestern Atlantic Ocean. Marine and Coastal Fisheries: Dynamics, Management and Ecosystem Science I:22-28, 2009.

McCartney MA, Burton ML, Lima TG. 2013. Mitochondrial DNA differentiation between populations of black sea bass (*Centropristis striata*) across Cape Hatteras, North Carolina (USA). Journal of Biogeography 40:1386-1398.

Northeast Fisheries Science Center. 2012. 53rd Northeast Regional Stock Assessment Workshop (53rd SAW) Assessment Report. US Dept Commer, Northeast Fish Sci Cent Ref Doc. 12-05; 559 p. Available from: http://nefsc.noaa.gov/publications/

Rohlf FJ. 1990. Morphometrics. Annual Review Ecological Systems 21:229-316.

Rohlf FJ. 2004. TpsDig. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.

Roy EM, Quattro JM, GreigTW. 2012. Genetic management of black sea bass: Influence of biogeographic barriers on population structure. Marine Coastal Fisheries 4(1):391-402.

SAS Institute Inc. 2000. Statistical Analysis System, version 8. Cary, NC.

Shepherd GR, Terceiro M. 1994. The summer flounder, scup, and black sea bass fishery of the Middle Atlantic Bight and southern New England waters. NOAA Technical Report NMFS 122. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Smith LI. 2002. A tutorial on principal components analysis. http://nyx-www.informatik.uni-bremen.de/664/1/smith_tr_02.pdf

Steimle FW, Zetlin CA, Berrien PL, Chang S. 1999. Essential fish habitat source document: Black sea bass, *Centropristis striata*, life history and habitat characteristics. NOAA TM NMFS-NE-143. 50 pp.

Strang G. 2006. Linear algebra and its applications. 4th edition. Pacific Grove,CA. Thomson & Brooks/Cole.

Zar JH. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Table 1. All 252 calculated interlandmark standardized mean distances (m_dist)) were labeled with a number. The 11 distances used in the final Principal Component Analysis (PCA) are listed with a description of measurements' location.

Distance Number	Measurement
m_dist4	Mouth to Start of Dorsal Fin
m_dist 9	Mouth to End of Lateral Line
m_dist 15	Mouth to Tip of Operculum
m_dist 17	Mouth to Start of Pelvic Fin
m_dist 72	Start of Dorsal Fin to Start of Anal Fin
m_dist 77	Start of Dorsal Fin to Pelvic Fin
m_dist 107	Start of Anal Fin to End of Dorsal Fin
m_dist 112	Start of Pelvic Fin to End of Dorsal Fin
m_dist 135	End of Lateral Line to End of Tail
m_dist 216	Start of Lateral Line to Head Bump #3
m_dist 223	Tip of Operculum to Head Bump #2

Table 2. First Principal Component Analysis (PCA) output using 210 distances from 77 fish. Principal Component (PC) numbers, corresponding eigenvalues, amount of variance contributed by each PC, and cumulative percent of variance.

PC number	Eigenvalue	Percent Variance	Total Percent
1	80.438	38.30%	38.30%
2	29.692	14.14%	52.44%
3	19.478	9.28%	61.72%
4	14.701	7.00%	68.72%
5	10.759	5.12%	73.84%
6	8.429	4.01%	77.86%
7	5.681	2.71%	80.56%
8	5.218	2.48%	83.05%
9	5.079	2.42%	85.46%
10	3.943	1.88%	87.34%
11	3.818	1.82%	89.16%
12	3.503	1.67%	90.83%
13	2.583	1.23%	92.06%
14	2.404	1.14%	93.20%
15	2.024	0.96%	94.17%
16	1.932	0.92%	95.09%
17	1.517	0.72%	95.81%
18	1.210	0.58%	96.39%
19	1.079	0.51%	96.90%

Table 3. Final Principal Component Analysis (PCA) output using the 11 chosen distances described in Table 1.

PC number	Eigenvalue	Percent Variance	Total Percent
1	5.110	46.45%	46.45%
2	1.756	15.97%	62.42%
3	1.383	12.58%	74.99%
4	0.854	7.76%	82.75%
5	0.668	6.07%	88.83%

Table 4. Summary of weights for the first 2 Principal Components (Prin1 and Prin2) for each of the final distances (see Table 1 for description of each numbered distance). The highest weighted distances are highlighted.

	Prin1	Prin2
m_dist 4	0.304	-0.245
m_dist17	0.317	-0.167
m_dist15	0.323	-0.259
m_dist77	0.311	0.293
m_dist72	0.337	0.102
m_dist107	0.249	0.034
m_dist112	0.259	0.150
m_dist9	**0.389**	-0.221
m_dist216	0.186	**0.523**
m_dist135	**-0.377**	0.223
m_dist223	0.187	**0.594**

Table 5. Summary of the first 2 Principal Component values (Prin1 and Prin2) for each of the 75 observed fish (obs). 1 = male, 2 = female

Obs	Prin1	Prin2	sex
1	-2.094	0.978	1
2	1.046	-0.302	2
3	-1.189	0.271	1
4	0.058	-1.793	2
5	2.515	0.006	2
6	-3.030	0.498	1
7	-1.188	-0.399	2
8	4.273	2.672	1
9	0.576	1.873	2
10	1.194	0.089	2
11	-0.278	0.127	2
12	1.134	-3.557	2
13	0.639	-0.655	2
14	1.307	-2.350	2
15	-2.584	-2.459	2
16	2.707	0.013	2
17	2.480	0.603	2
18	0.942	-1.303	2
19	1.076	-0.948	2
20	0.849	0.347	2
21	2.982	1.376	2
22	2.066	-0.309	2
23	2.032	-1.291	2
24	-1.911	-1.502	2
25	1.081	-0.941	2
26	0.791	0.499	2
27	1.924	-1.174	2
28	1.796	0.694	2
29	1.946	1.348	1
30	-1.197	0.063	2
31	1.703	-0.114	2
32	-0.871	-1.088	1
33	-3.400	-0.526	1
34	-2.832	-1.806	2
35	-4.350	0.470	1
36	-2.158	1.223	1
37	-0.209	-0.716	1
38	-0.686	0.559	1
39	-2.637	-1.611	1
40	-1.169	2.609	1

Table 5, continued. Summary of the first 2 Principal Component values (Prin1 and Prin2) for each of the 75 observed fish (obs). 1 = male, 2 = female.

Obs	Prin1	Prin2	sex
41	-1.795	-0.896	1
42	-2.408	0.204	1
43	-1.110	1.347	1
44	-0.465	1.503	1
45	-3.110	-0.531	1
46	-4.874	-0.274	1
47	-2.770	1.059	1
48	-2.509	2.270	1
49	-0.197	-0.652	2
50	2.455	2.365	2
51	-2.712	1.801	1
52	-3.850	1.462	1
53	-1.104	-1.576	1
54	2.684	0.616	2
55	0.965	-0.011	1
56	-1.149	-0.208	1
57	-0.278	1.889	1
58	-1.450	-0.699	2
59	-1.146	-0.708	2
60	0.527	0.626	2
61	2.791	1.599	2
62	1.444	0.272	2
63	-0.376	-1.447	2
64	-0.979	-1.910	2
65	3.086	-0.797	2
66	4.393	0.276	2
67	-3.046	-0.793	1
68	1.511	0.365	2
69	4.129	0.503	2
70	2.930	0.252	2
71	2.067	0.465	2
72	-1.509	1.543	1
73	3.596	-3.390	2
74	-3.678	2.088	1
75	2.607	-0.090	2

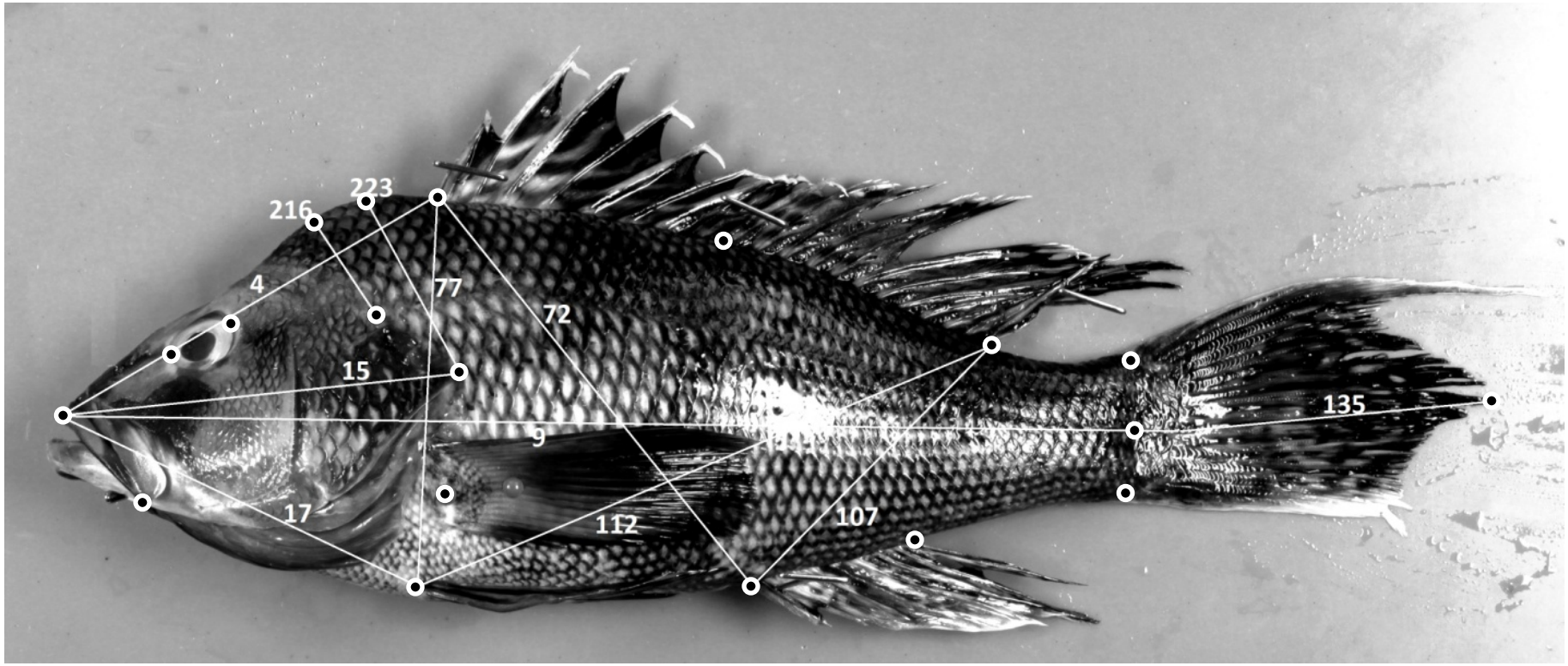


Figure 1. Locations for the 11 selected (of 252 calculated) interlandmark distances (lines) from the 19 landmarks (circles) used in the final analysis. See Table 1 for a description of each distance (labeled by number).

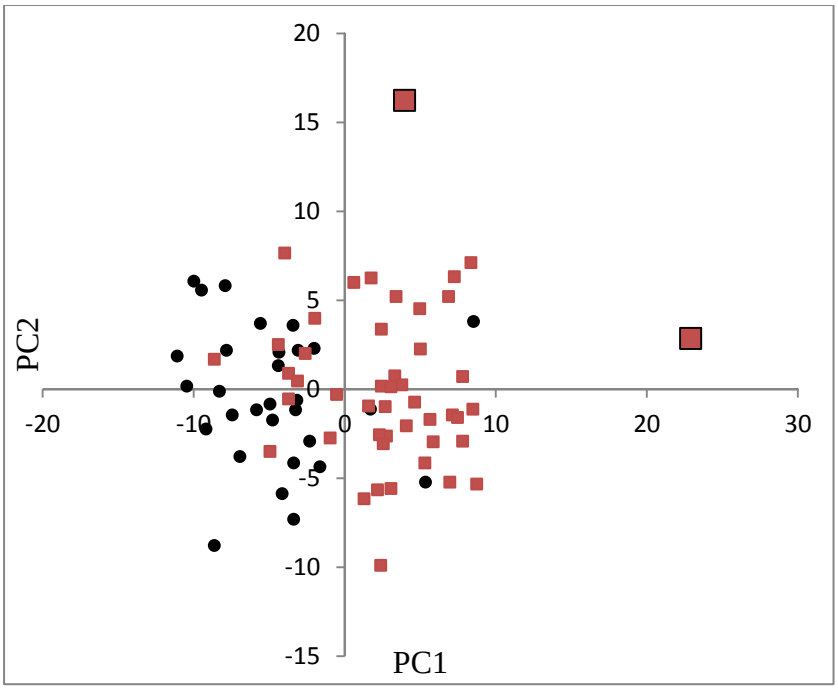


Figure 2. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) for reduced data set of 115 distances. Irregular fish from Figure 3 are red squares outlined in black.

A.

B.

Figure 3. Fish removed from data set because of deformed shapes. The tail of fish A was stunted and in bad condition, influencing distance measurements. Fish B had a distended stomach from large squid which created irregular shape measurements.

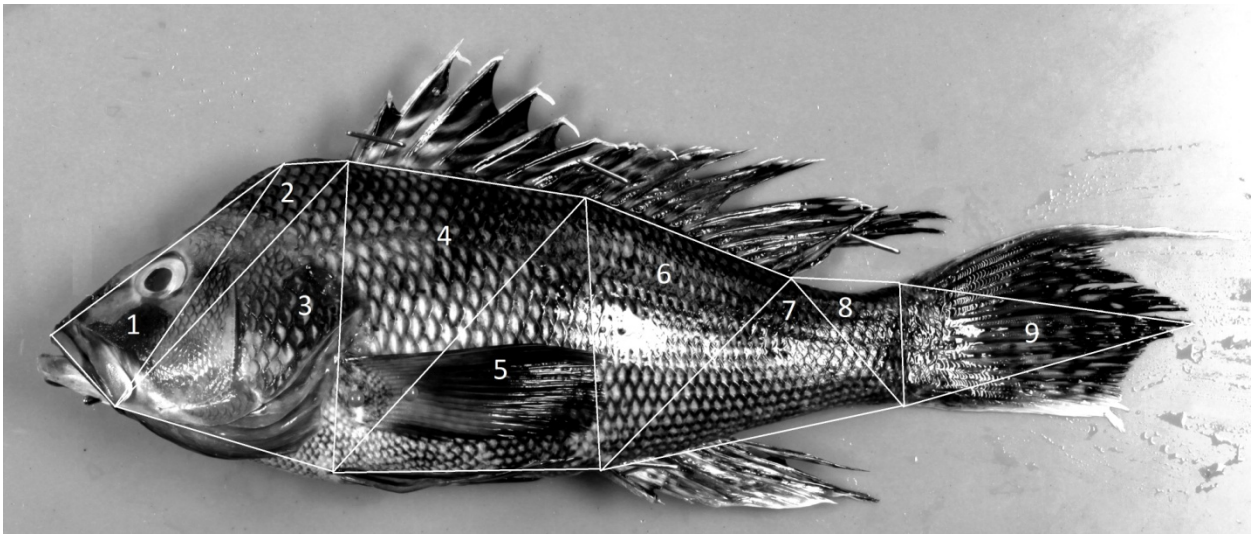


Figure 4. Black sea bass (Centropristis striata) separated into 9 local triangles by using 11 landmarks for calculating centroid size (CS).

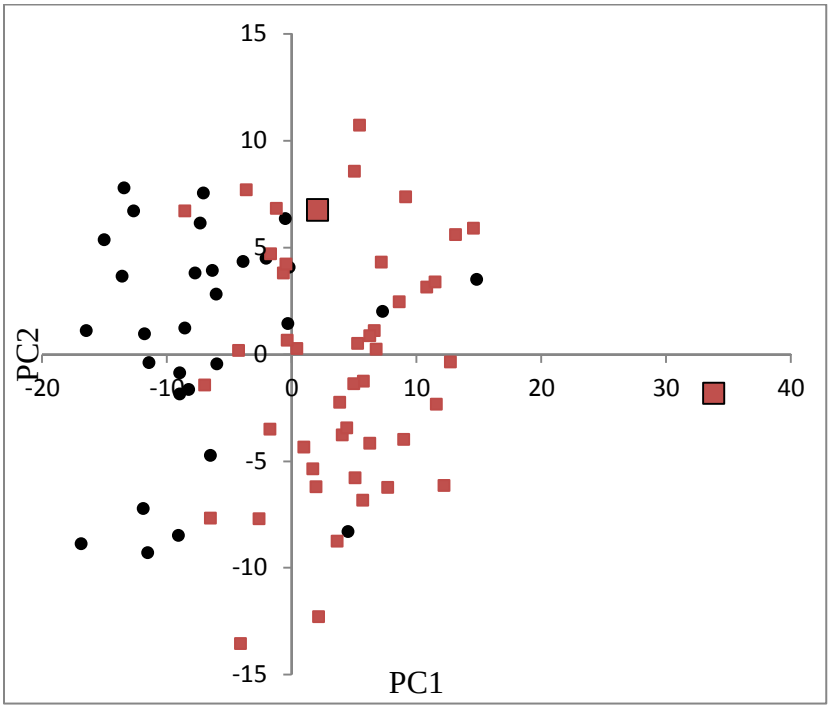


Figure 5. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) for full data set of 210 distances. Irregular fish from Figure 3 are red squares outlined in black.

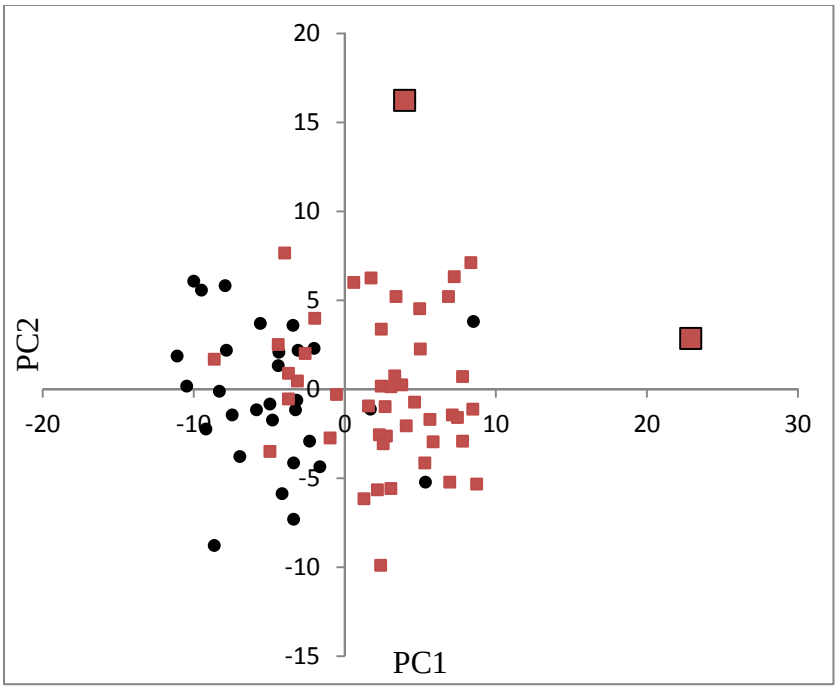


Figure 6. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) for reduced data set of 115 distances. Irregular fish from Figure 3 are red highlighted (■).

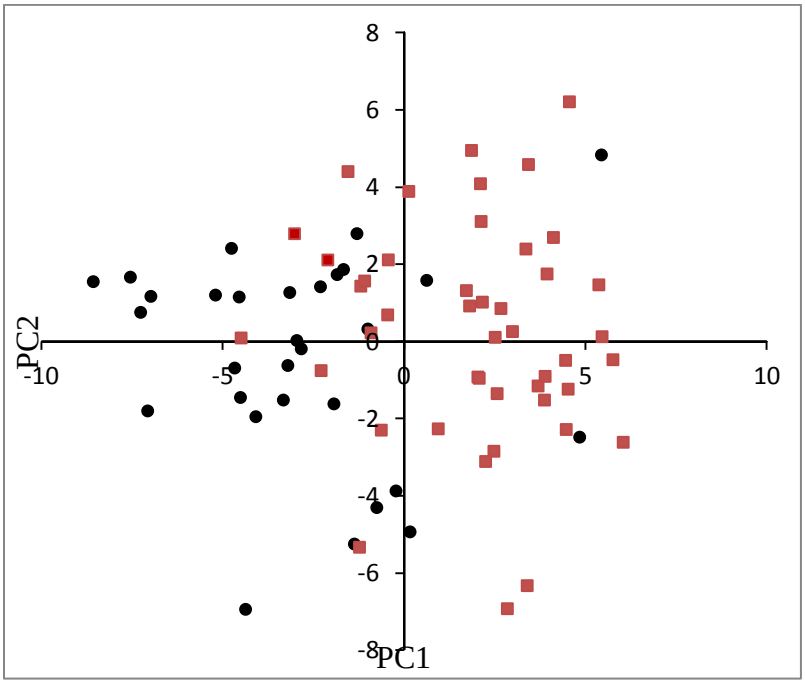


Figure 7. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) with irregular fish removed after data set further reduced to 35 distances.

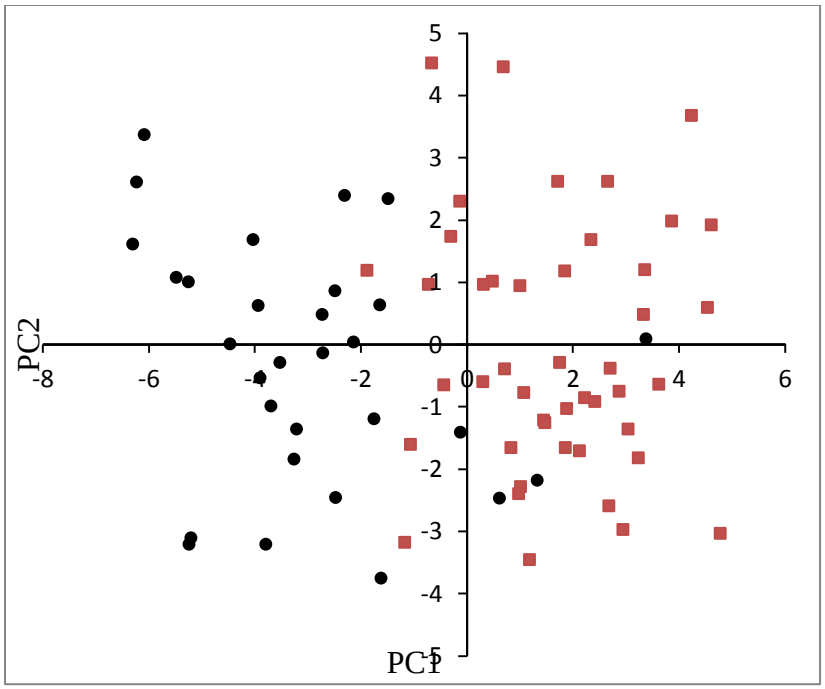


Figure 8. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) of 20 distance measurement data set.

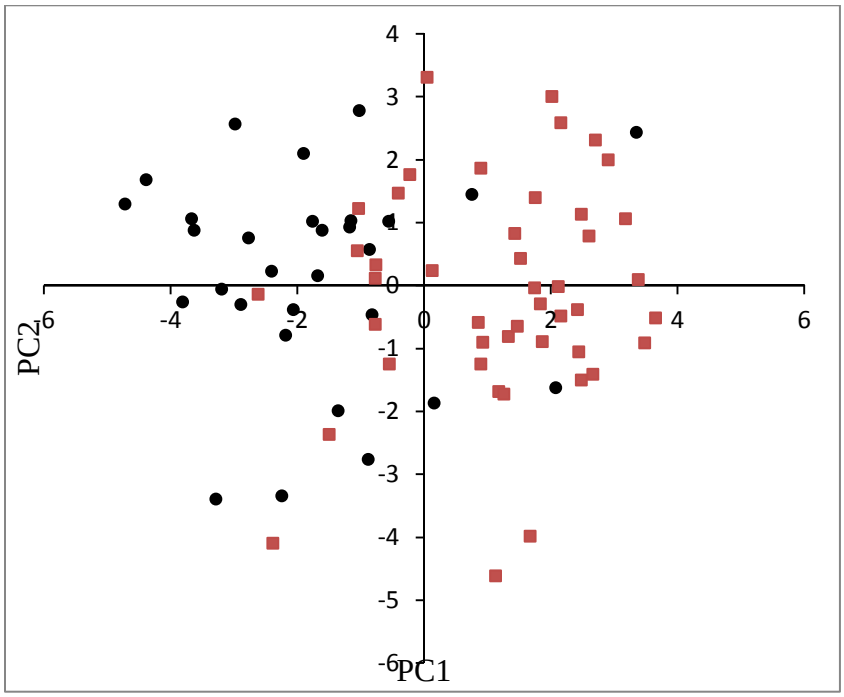


Figure 9. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males) for 11 distance measurements.

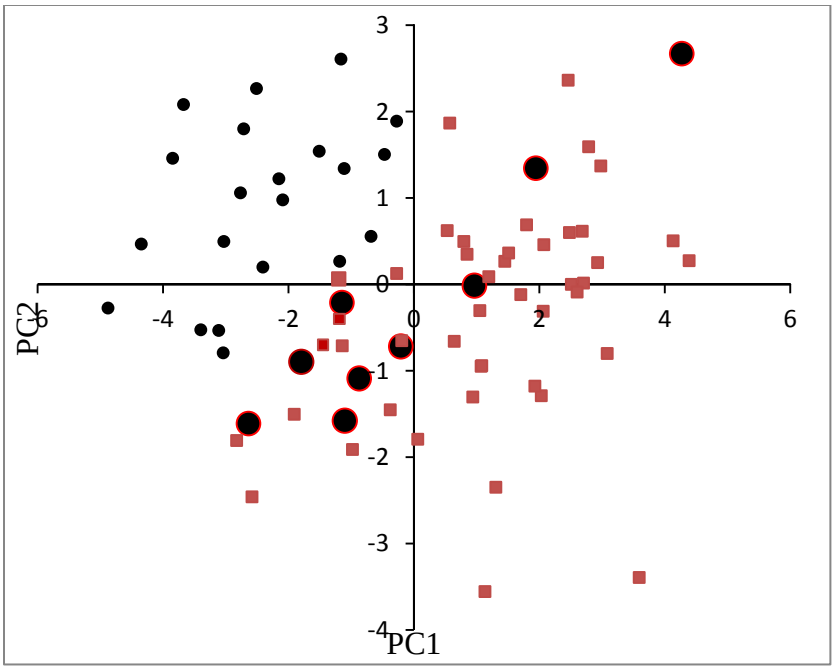


Figure 10. Biplot of the first 2 Principal Components (PC1 and PC2) by sex (■ females; ● males; ● males mixing with females) using the final 11 distances from Table 1.

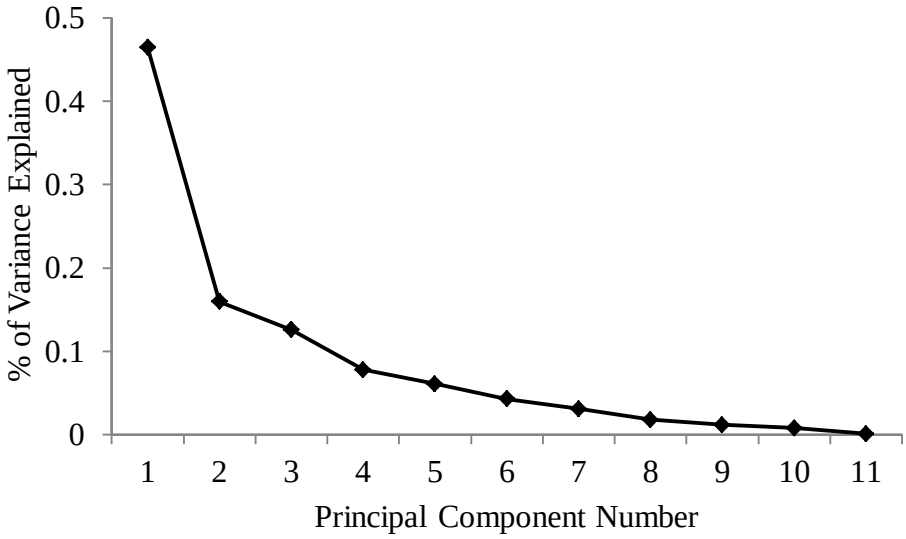


Figure 11. Scree plot of % variance explained per Principal Component from final Principal Component Analysis.

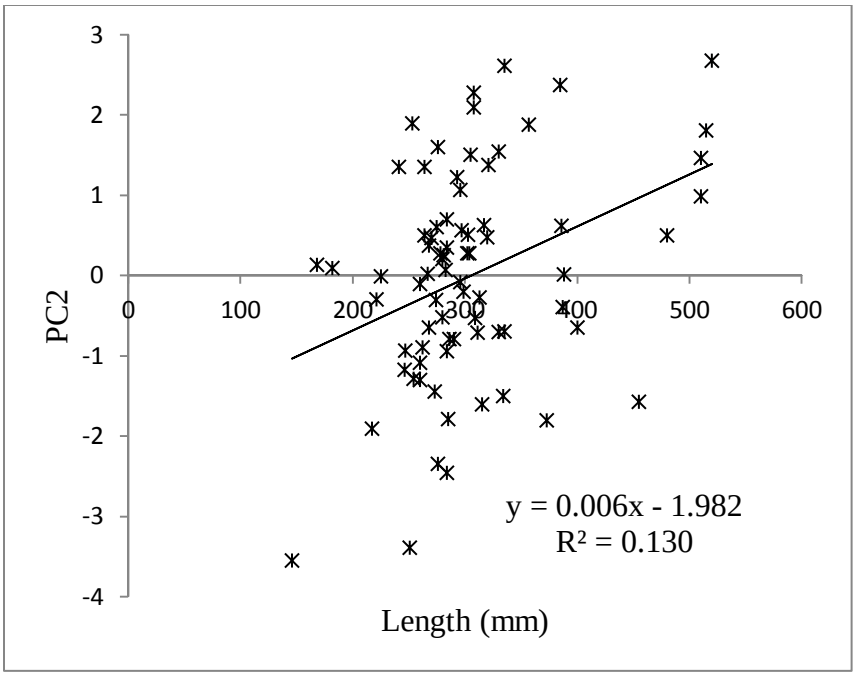
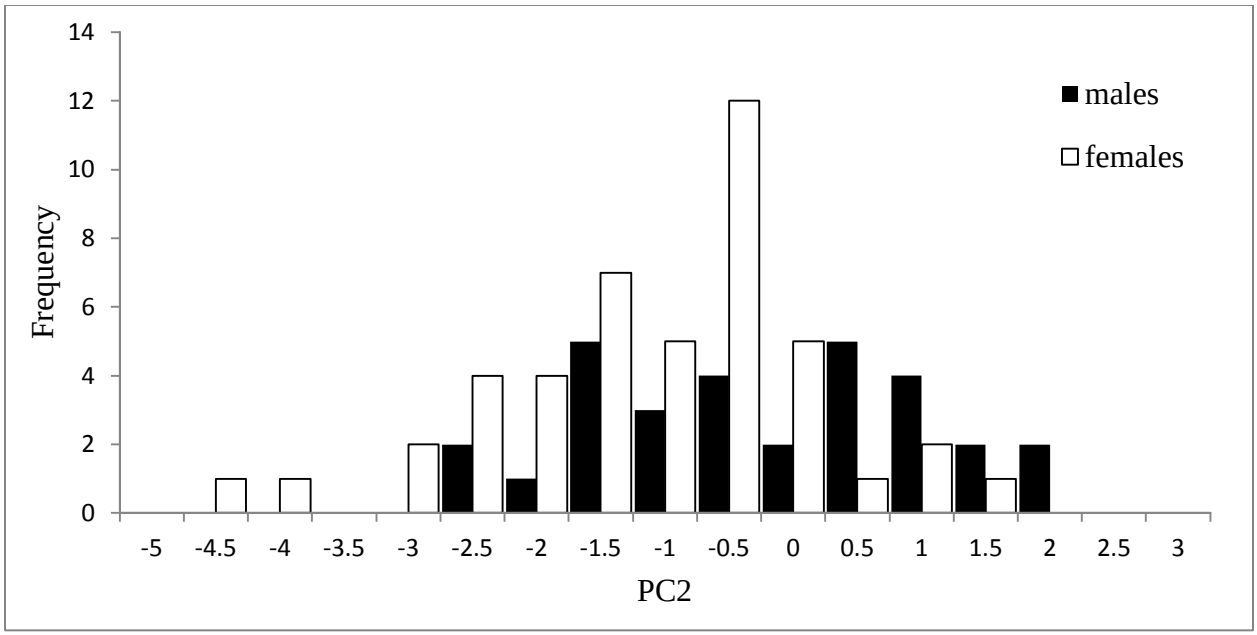


Figure 12. Relationship between total fish length (mm) and Principal Component 2 (PC2) showing little correlation with length.



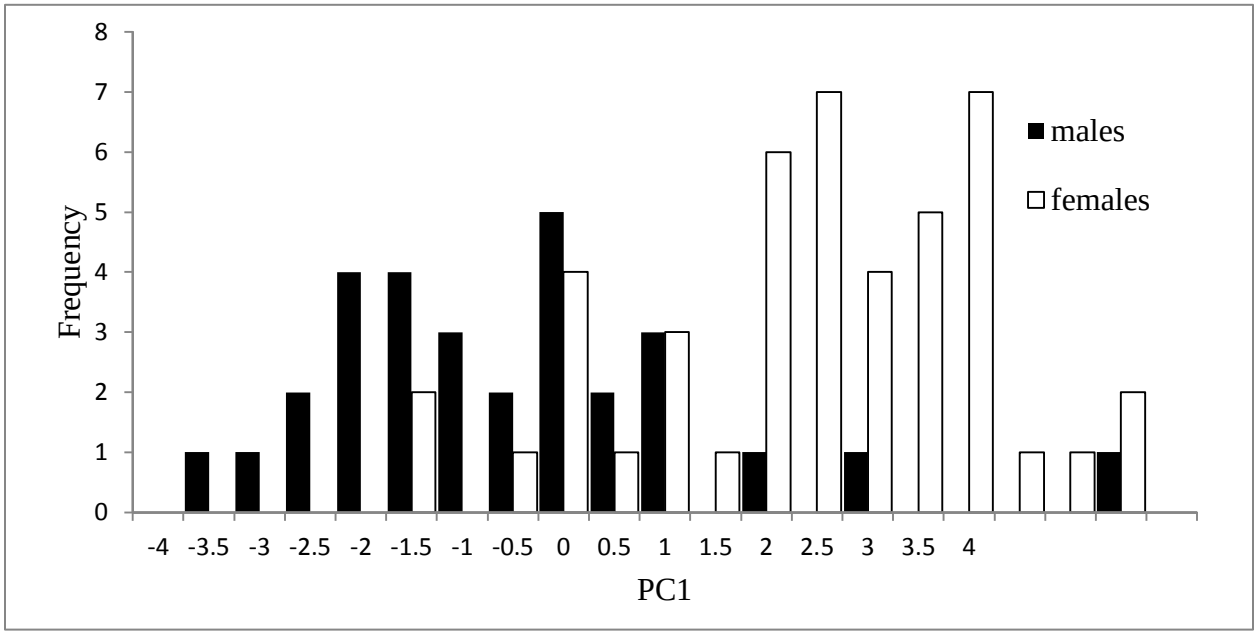


Figure 13. Frequency distribution of Principal Component 1 (PC1) and Principal Component 2 (PC 2) by sex based on final 11 landmark distances.

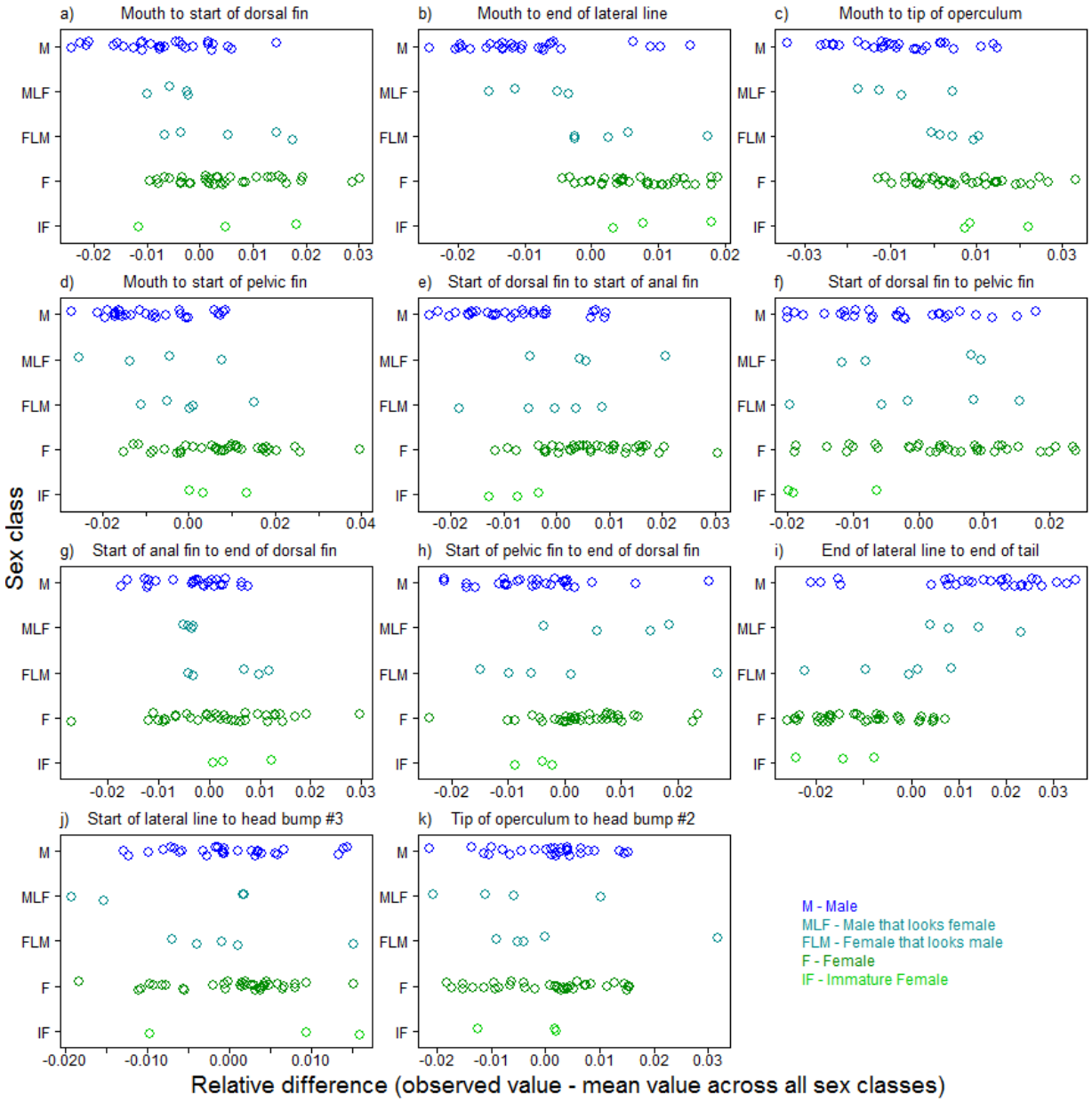


Figure 14. Variation in morphometric distances in relation to sex. For each morphometric length, the relative distance was calculated standardized (to length) differences as the observed – mean across all sex classes.

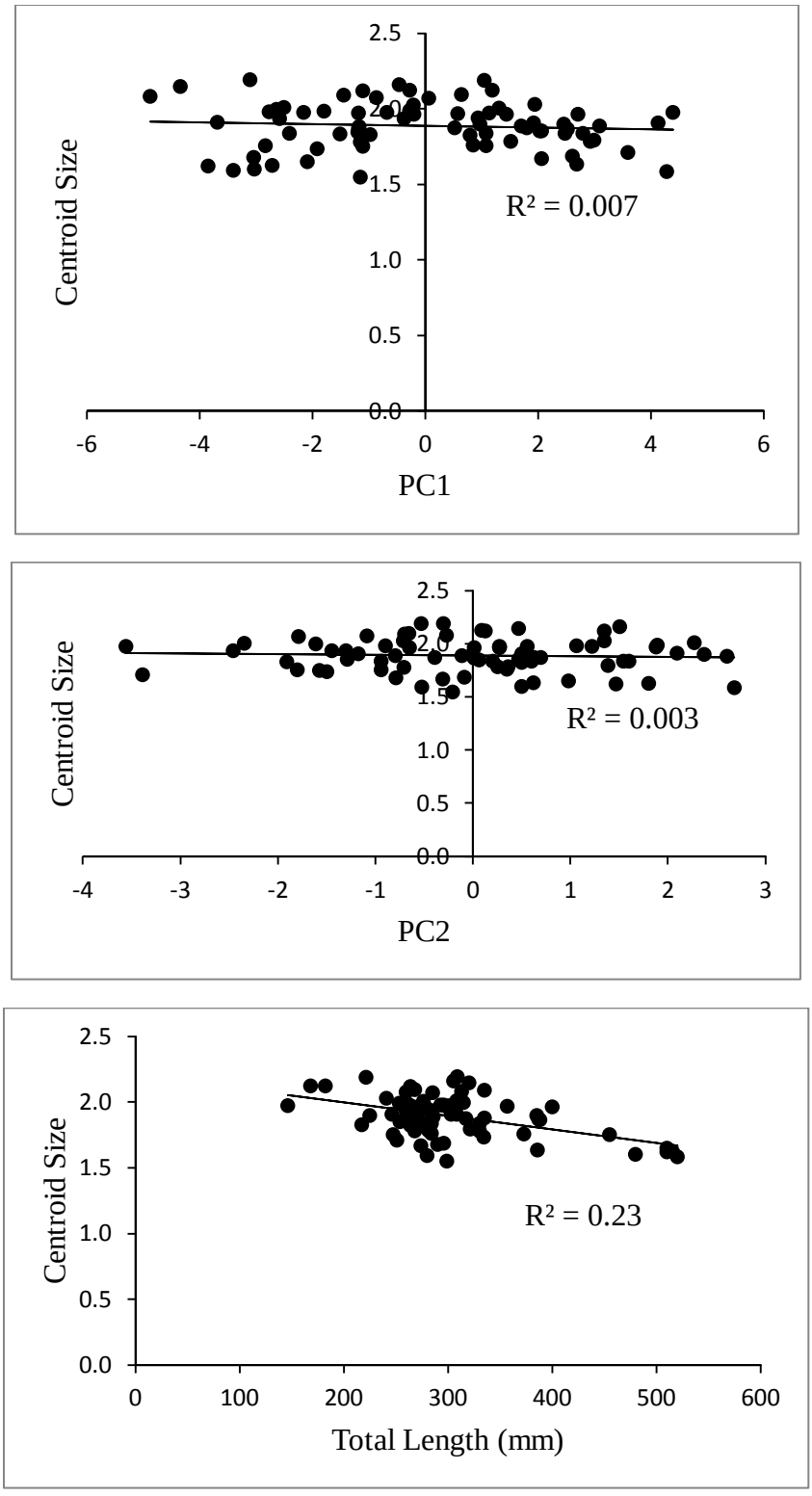


Figure 15. Principal Component 1(PC1), Principal Component 2 (PC2), and total length vs. centroid size. Low R2 values show that centroid size is not a good measurement.

Appendix Table 1. Correlation matrix for the 11 selected mean distances (m_dist)) used in the final Principal Component Analysis. Distances are labeled by number; see Table 1 for description of the distances measured.

	m_dist4	m_dist17	m_dist15	m_dist77	m_dist72	m_dist107	m_dist112	m_dist9	m_dist216	m_dist135	m_dist223
m_dist4	1	0.441	0.718	0.427	0.378	0.293	0.198	0.617	0.079	-0.553	0.149
m_dist17	0.441	1	0.748	0.340	0.405	0.507	0.113	0.576	0.346	-0.575	0.127
m_dist15	0.718	0.748	1	0.257	0.361	0.289	0.130	0.649	0.248	-0.607	0.190
m_dist77	0.427	0.340	0.257	1	0.688	0.440	0.611	0.421	0.340	-0.388	0.464
m_dist72	0.378	0.405	0.361	0.688	1	0.322	0.557	0.631	0.222	-0.631	0.335
m_dist107	0.293	0.507	0.289	0.440	0.322	1	0.243	0.395	0.262	-0.371	0.155
m_dist112	0.198	0.113	0.130	0.611	0.557	0.243	1	0.563	0.117	-0.533	0.258
m_dist9	0.617	0.576	0.649	0.421	0.631	0.395	0.563	1	0.174	-0.985	0.155
m_dist216	0.079	0.346	0.248	0.340	0.222	0.262	0.117	0.174	1	-0.165	0.780
m_dist135	-0.553	-0.575	-0.607	-0.388	-0.631	-0.371	-0.533	-0.985	-0.165	1	-0.137
m_dist223	0.149	0.127	0.190	0.464	0.335	0.155	0.258	0.155	0.780	-0.137	1

Appendix Table 2. Eigenvectors (i.e., Principal Components [Prin] 1-11) of the correlation matrix between the 11 mean distances (m_dist). (Distances are labeled by number; see Table 1 for a description of each distance measurement). Each Prini is an eigenvector.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11
m_dist4	0.3036	-0.2454	0.1362	-0.2342	0.6320	-0.3299	-0.1352	-0.1843	0.2114	0.4066	-0.0600
m_dist17	0.3170	-0.1668	0.3774	0.2512	-0.1786	0.4348	0.3691	-0.1317	-0.2180	0.4929	0.0349
m_dist15	0.3225	-0.2592	0.3772	-0.2187	0.1121	0.0683	0.3013	0.4831	0.1324	-0.5261	-0.0548
m_dist77	0.3115	0.2933	-0.2465	0.1772	0.4765	0.2013	0.2067	-0.4014	-0.3007	-0.4034	0.0121
m_dist72	0.3372	0.1016	-0.2960	-0.0255	0.0452	0.6094	-0.4644	0.2634	0.3437	0.1055	0.0071
m_dist107	0.2488	0.0338	0.1015	0.8205	0.0222	-0.3648	-0.2215	0.2522	0.0622	-0.0522	-0.0233
m_dist112	0.2595	0.1500	-0.5583	-0.0378	-0.1471	-0.2557	0.5866	0.2110	0.2502	0.2350	-0.0602
m_dist9	0.3894	-0.2208	-0.1220	-0.1491	-0.2970	-0.1986	-0.1688	-0.1518	-0.1339	-0.1142	0.7432
m_dist216	0.1862	0.5226	0.3986	-0.0801	-0.2659	-0.0815	0.0124	-0.3929	0.5316	-0.1095	-0.0195
m_dist135	-0.3772	0.2232	0.1287	0.1423	0.3805	0.1313	0.2342	0.2129	0.2475	0.1133	0.6594
m_dist223	0.1865	0.5942	0.1900	-0.2803	0.0333	-0.1540	-0.1405	0.3923	-0.5034	0.2128	0.0161

Procedures for Issuing Manuscripts in the *Northeast Fisheries Science Center Reference Document* (CRD) Series

Clearance

All manuscripts submitted for issuance as CRDs must have cleared the NEFSC's manuscript/abstract/webpage review process. If any author is not a federal employee, he/she will be required to sign an "NEFSC Release-of-Copyright Form." If your manuscript includes material from another work which has been copyrighted, then you will need to work with the NEFSC's Editorial Office to arrange for permission to use that material by securing release signatures on the "NEFSC Use-of-Copyrighted-Work Permission Form."

For more information, NEFSC authors should see the NEFSC's online publication policy manual, "Manuscript/abstract/webpage preparation, review, and dissemination: NEFSC author's guide to policy, process, and procedure," located in the Publications/Manuscript Review section of the NEFSC intranet page.

Organization

Manuscripts must have an abstract and table of contents, and (if applicable) lists of figures and tables. As much as possible, use traditional scientific manuscript organization for sections: "Introduction," "Study Area" and/or "Experimental Apparatus," "Methods," "Results," "Discussion," "Conclusions," "Acknowledgments," and "Literature/References Cited."

Style

The CRD series is obligated to conform with the style contained in the current edition of the United States Government Printing Office Style Manual. That style manual is silent on many aspects of scientific manuscripts. The CRD series relies more on the CSE Style Manual. Manuscripts should be prepared to conform with these style manuals.

The CRD series uses the American Fisheries Society's guides to names of fishes, mollusks, and decapod crustaceans, the Society for Marine Mammalogy's guide to names of marine mammals, the Biosciences Information Service's guide to serial title abbreviations, and the ISO's (International Standardization Organization) guide to statistical terms.

For in-text citation, use the name-date system. A special effort should be made to ensure that all necessary bibliographic information is included in the list of cited works. Personal communications must include date, full name, and full mailing address of the contact.

Preparation

Once your document has cleared the review process, the Editorial Office will contact you with publication needs – for example, revised text (if necessary) and separate digital figures and tables if they are embedded in the document. Materials may be submitted to the Editorial Office as files on zip disks or CDs, email attachments, or intranet downloads. Text files should be in Microsoft Word, tables may be in Word or Excel, and graphics files may be in a variety of formats (JPG, GIF, Excel, PowerPoint, etc.).

Production and Distribution

The Editorial Office will perform a copy-edit of the document and may request further revisions. The Editorial Office will develop the inside and outside front covers, the inside and outside back covers, and the title and bibliographic control pages of the document.

Once both the PDF (print) and Web versions of the CRD are ready, the Editorial Office will contact you to review both versions and submit corrections or changes before the document is posted online.

A number of organizations and individuals in the Northeast Region will be notified by e-mail of the availability of the document online.

Research Communications Branch
Northeast Fisheries Science Center
National Marine Fisheries Service, NOAA
166 Water St.
Woods Hole, MA 02543-1026

MEDIA MAIL

Publications and Reports of the Northeast Fisheries Science Center

The mission of NOAA's National Marine Fisheries Service (NMFS) is "stewardship of living marine resources for the benefit of the nation through their science-based conservation and management and promotion of the health of their environment." As the research arm of the NMFS's Northeast Region, the Northeast Fisheries Science Center (NEFSC) supports the NMFS mission by "conducting ecosystem-based research and assessments of living marine resources, with a focus on the Northeast Shelf, to promote the recovery and long-term sustainability of these resources and to generate social and economic opportunities and benefits from their use." Results of NEFSC research are largely reported in primary scientific media (*e.g.*, anonymously-peer-reviewed scientific journals). However, to assist itself in providing data, information, and advice to its constituents, the NEFSC occasionally releases its results in its own media. Currently, there are three such media:

NOAA Technical Memorandum NMFS-NE -- This series is issued irregularly. The series typically includes: data reports of long-term field or lab studies of important species or habitats; synthesis reports for important species or habitats; annual reports of overall assessment or monitoring programs; manuals describing program-wide surveying or experimental techniques; literature surveys of important species or habitat topics; proceedings and collected papers of scientific meetings; and indexed and/or annotated bibliographies. All issues receive internal scientific review and most issues receive technical and copy editing.

Northeast Fisheries Science Center Reference Document -- This series is issued irregularly. The series typically includes: data reports on field and lab studies; progress reports on experiments, monitoring, and assessments; background papers for, collected abstracts of, and/or summary reports of scientific meetings; and simple bibliographies. Issues receive internal scientific review and most issues receive copy editing.

Resource Survey Report (formerly *Fishermen's Report*) -- This information report is a regularly-issued, quick-turnaround report on the distribution and relative abundance of selected living marine resources as derived from each of the NEFSC's periodic research vessel surveys of the Northeast's continental shelf. This report undergoes internal review, but receives no technical or copy editing.

TO OBTAIN A COPY of a *NOAA Technical Memorandum NMFS-NE* or a *Northeast Fisheries Science Center Reference Document*, either contact the NEFSC Editorial Office (166 Water St., Woods Hole, MA 02543-1026; 508-495-2350) or consult the NEFSC webpage on "Reports and Publications" (http://www.nefsc.noaa.gov/nefsc/publications/). To access *Resource Survey Report*, consult the Ecosystem Surveys Branch webpage (http://www.nefsc.noaa.gov/femad/ecosurvey/mainpage/).

ANY USE OF TRADE OR BRAND NAMES IN ANY NEFSC PUBLICATION OR REPORT DOES NOT IMPLY ENDORSEMENT.