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Assessment of morphological variation between stocks of bluefish, *Pomatomus saltatrix* (Actinopterygii, Perciformes, Pomatomidae), in the Aegean Sea, Black Sea, and Sea of Marmara

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Abstract

The population structure of the bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), in Turkish waters is scarcely described in the literature. To identify any distinct population units of bluefish, and reaffirm the findings of a previous study, four areas were selected: the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara. In this study, truss network morphometrics, meristics, and otolith shape analyses were successfully applied for different population identification of the bluefish. Multivariate analysis of variance (MANOVA) revealed no differences for truss network morphometrics, meristic, and otolith shape characters between males and females. Hence, both sexes were combined for the discriminant function (DFA) and the Principal Component Analysis (PCA). Using univariate ANOVA based on the stepwise method revealed a highly significant difference among different locations for each truss-morphometrics and otolith shape characters. Furthermore, six out of seven meristic characters also showed significant differences between different areas. Based on PCA, 25 out of 27 truss-morphometric characters had a loading value above 0.70, which was considered significant in this study. The results of DFA show clear patterns of truss-morphometric character variations, forming four distinct clusters that were well separated from each other, indicating the existence of four morphologically differentiated populations of the bluefish. The proportion of the correctly classified Aegean Sea, western Black Sea, and eastern Black Sea bluefish samples to their original groups were 100%, demonstrating clear separation of these stocks from each other. Whereas up to 5% of the total samples of the Sea of Marmara were incorrectly classified, assigning to the eastern Black Sea. These findings were supported by meristic and otolith shape characters that also indicated four morphologically differentiated populations of the bluefish. However, their overall proportion of correct classification was relatively lower than the truss-morphometric traits method. The findings suggest the requirement of strategic assessment and management of each bluefish stock separately to use them sustainably in the future.

Keywords

Climate change, factor analysis, Pomatomidae, stock structure, truss network system

Introduction

The bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), is a highly migratory pelagic streamlined predatory species with a wide geographical distribution that occurs in the majority of major ocean basins throughout the world except for the eastern Pacific (Helfman et al. 2009; Carpenter et al. 2015). It comprises an integral part of billfishes, sharks, and tunas' diets, constituting up to 80% of their diets (Feldman 2013). It is also an economically important marine fish species in the temperate and subtropical waters (Shepherd 2010). In the Turkish territorial waters, bluefish begin their spawning migration in spring via the Aegean Sea northwards from the Mediterranean and return south in the early autumn (Ceyhan et al. 2007). Its spawning season is limited to the warmest months in the region at water temperatures of 20–26°C from July to September (Ceyhan et al. 2007; Sabatés et al. 2012).

Bluefish is subjected to over-exploitation threats and has been considered a globally vulnerable species (Carpenter et al. 2015). The overall global landings of bluefish have generally trended from a peak to down over the past 15–24 years, plummeted by 7 percentage points to 46% (Carpenter et al. 2015; MAF 2019). The maximum capture of bluefish was 25 000 tons in 2002 (MAF 2019), and since then, their population has been on a steady decline, hitting its lowest level in 2019 (TÜİK 2020). The total Turkish landings of bluefish from the Aegean Sea, Black Sea, Mediterranean Sea, and Sea of Marmara were 5767 and 1213 tons in 2018 and 2019, respectively (TÜİK 2020).

A previous study by Turan et al. (2006) reported the existence of a total of three morphologically isolated subpopulations of bluefish in the Turkish territorial waters. The first stock was made by the Aegean Sea, the Sea of Marmara, and the western Black Sea, while the two other morphologically isolated subpopulations of bluefish were represented the Mediterranean Sea and the east Black Sea (Turan et al. 2006). However, no other comprehensive research has been undertaken to evaluate the bluefish population structures in the Turkish territorial waters after a study conducted by Turan et al. (2006). According to Rawat et al. (2017) the identification of stock with distinguished phenotypic and genetic differentiation among fish populations within a species may help to effectively: 1) manage the stock separately, 2), achieving biologically sustainable productivity, 3) determine stock-wise population abundance, 4) estimate how each stock respond to fisheries exploitation, and 5) accomplish the objectives of fishery stock assessment by modeling (Rawat et al. 2017). Thus, the presently reported study aimed to investigate the morphological population structure of bluefish for the second time after a decade to determine the possible existence of any new geographically isolated populations of bluefish. In this study, the inter-population morphometric variability of bluefish was investigated in the Aegean Sea, the western Black Sea, eastern Black Sea, and the Sea of Marmara by truss-morphometric traits, meristic characters, and otolith characters.

Materials and methods

Samples of bluefish were collected from four commercial fish landing centers: Aegean Sea (Gulf of İzmir), western Black Sea (Şile İstanbul), eastern Black Sea (Trabzon: Akçaabat), and the Sea of Marmara (Erdek Balıkesir) (Fig. 1). The sampling details of the bluefish are provided in Table 1. Samples were carefully persevered in iceboxes (ca. –20°C) to transfer to the laboratory for further examination.

Figure 1. Map of the study area (Sources: ESRI World Ocean GDAL basemap layer).

Table 1. Descriptive data of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, Western Black Sea, Eastern Black Sea, and Sea of Marmara.

Sea	Location	Coordinates	*n*	Sex ratio (♀:♂)	Date of capture	Sampling gear
Aegean Sea	Gulf of İzmir	38°36′32.8″N, 26°38′53.9″E	31	1.0:1.1	05 Apr. 2014	Fishhook
Western Black Sea	Şile İstanbul	41°13′39.4″N, 29°43′09.1″E	36	1.0:1.1	29 Sep. 2014	Gillnet
Eastern Black Sea	Akçaabat Trabzon	41°02′45.3″N, 39°36′18.5″E	33	1.0:0.9	14 Nov. 2014	Fishhook
Sea of Marmara	Erdek Balıkesir	40°28′59.1″N, 27°33′37.9″E	31	1.0:0.8	29 Feb. 2014	Purse-seine

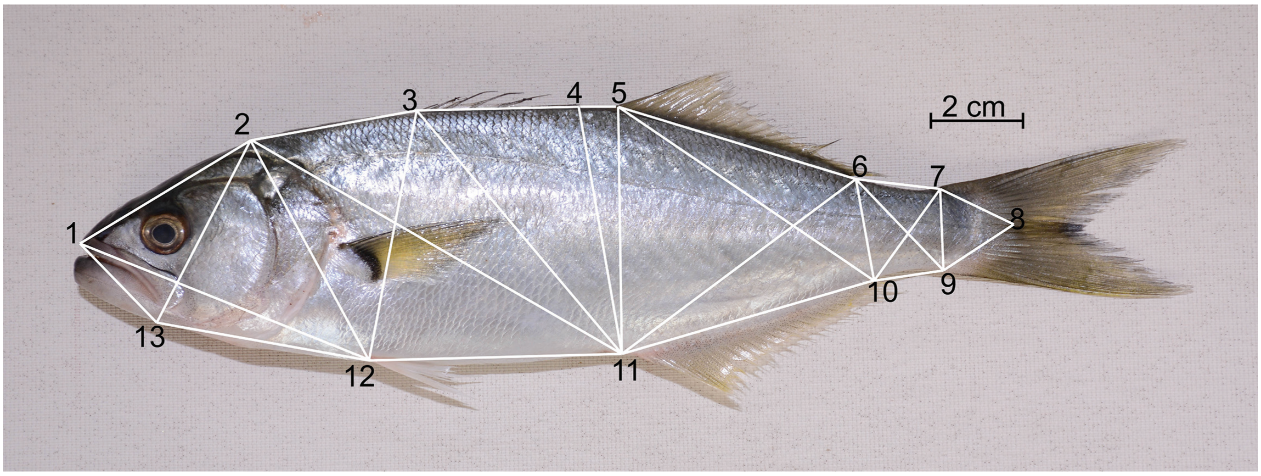

Figure 2. Truss-morphometric characters measured on bluefish, *Pomatomus saltatrix*.

Data acquisition of morphometric traits and meristic characters

Before taking the measurements, the frozen samples of bluefish were thawed for 1 hour under running water, placed on their right side on a water-resistant graph. Body posture and fins were forced into a natural position. Each fish was examined for physical damage, and a sample with any physical damage was removed from the analysis. Furthermore, their sexes were determined by reviewing their gonads under a dissecting microscope.

A total of 13 anatomical landmarks were chosen for the study, and by inter-connecting these landmarks, the box-truss network was produced, representing a truss network of 27 lines (Fig. 2, Table 2). Each landmark line was measured via manual methods by piercing the paper with a needle (Strauss and Bookstein 1982; Hanif et al. 2019).

Using a binocular microscope, the number of branched and un-branched rays in dorsal fin spines, dorsal fin rays, ventral fin rays, pectoral fin rays, and anal fin rays as well as right and left gill rakers were obtained (Turan et al. 2006).

Table 2. Description of morphometric measurements made for each sample of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara between February 2014 and November 2014.

Measurement No.	Distance code	Distance	Landmarks
1	HL1	Head length 1	1–2
2	BL1	Body length 1 (Pre-dorsal length)	2–3
3	DFBL1	First dorsal fin base Length	3–4
4	MDL	Mid dorsal length	4–5
5	DFBL2	Second dorsal fin base length	5–6
6	PDL	Post-dorsal length	6–7
7	CL1	Caudal length 1	7–8
8	CL2	Caudal length 2	8–9
9	BL2	Body length 2	9–10
10	AFBL	Anal fin base length	10–11
11	BL3	Body length 3	11–12
12	BL4	Body length 4	12–13
13	HL2	Head length 2	1–13
14	BD1	Head diagonal 1	2–13
15	BH1	Body height 1	3–12
16	BD2	Body diagonal 2	4–11
17	BH2	Body height 2	5–11
18	BD3	Body diagonal 3	6–11
19	BD4	Body diagonal 4	7–10
20	BD5	Head diagonal2	1–12
21	BD5	Body diagonal 5	2–12
22	BD6	Body diagonal 6	3–11
23	BD7	Body diagonal 7	5–10
24	BH2	Body height 2	6–10
25	BH3	Body height 3	7–9
26	BD8	Body diagonal 8	2–11
27	BD9	Body diagonal 9	6–9

Otolith extraction

The sagittal otoliths were removed from all individuals. Each otolith was carefully wiped, clean, and stored dry in U-plates (Bal et al. 2018a). A digitized image for each otolith was produced using a binocular microscope coupled with a digital camera. The digitized images were then used to measure the otolith dimensions using ImageJ2 software (Rueden et al. 2017). Each otolith was weighed individually to the nearest 0.01 g on a digital balance.

Statistical analysis

Truss-morphometric and otolith variables were standardized separately for each region to eliminate the effect of fish size on these variables. The meristic characters were not standardized as they did not show a significant correlation with the bluefish body size (Turan et al. 2006). The variables were standardized using the following allometric equation (Reist 1986)

$$V_{trans} = \log V - \hat{a}(\log SL - \log SL_{mean})$$

where V_{trans} is the transformed morphometric variable, V is the non-transformed variable, SL is the standard length of each fish, SL_{mean} is the overall mean standard length of all the fish from each group (region), and β is the slope of the relation between log V and log SL.

The modified morphometric variables were tested for normality check, and outliers, if any, were excluded before subsequent analysis. Multivariate analysis of variance (MANOVA) was performed to check significant variation between different sex groups as well as sampling locations based on morphometrics, meristic, and otolith characters. The univariate ANOVA for each variable was then used to test significant differences among different sampling areas. The differences were considered statistically significant at P-values below 0.05. Principal component analysis (PCA) was used to uncover the morphometric variables with a highly influential role in distinguishing between the four populations. Discriminant function analysis (DFA) was used to demonstrate the variations among different bluefish stocks by classifying them to their respective groups based on morphometrics, meristic, or otolith characters. Dendrogram based Euclidean distance method was used to depict similarities between different locations. All statistical analyses were carried out with IBM SPSS Statistics software ver. 25.0.

Results

The size distribution of the bluefish based on total length is presented in Fig. 3. None of the sizes corrected truss measurements showed statistical significance with standard length by using correlation analysis, which indicates the allometric transformation method efficiently removed the effect of body size.

Truss-morphometric traits

There was no statistical difference observed between truss-morphometric characteristics for females and males (one-way MANOVA; $F_{(27, 32)} = 26.4$, Wilk's $\lambda = 0.456$, $P = 0.172$); hence, sexes were combined for further analysis. While there were highly significant differences among the stocks of bluefish from different locations using all data (one-way MANOVA; $F_{(81, 108)} = 26.4$, Wilk's $\lambda = 0.0001$, $P < 0.0001$). Also, the univariate ANOVA based on the stepwise method further revealed a highly significant difference among different locations for each truss-morphometric trait (Table 3). Furthermore, the PCA uncovered the truss-morphometric traits with a highly influential role in distinguishing

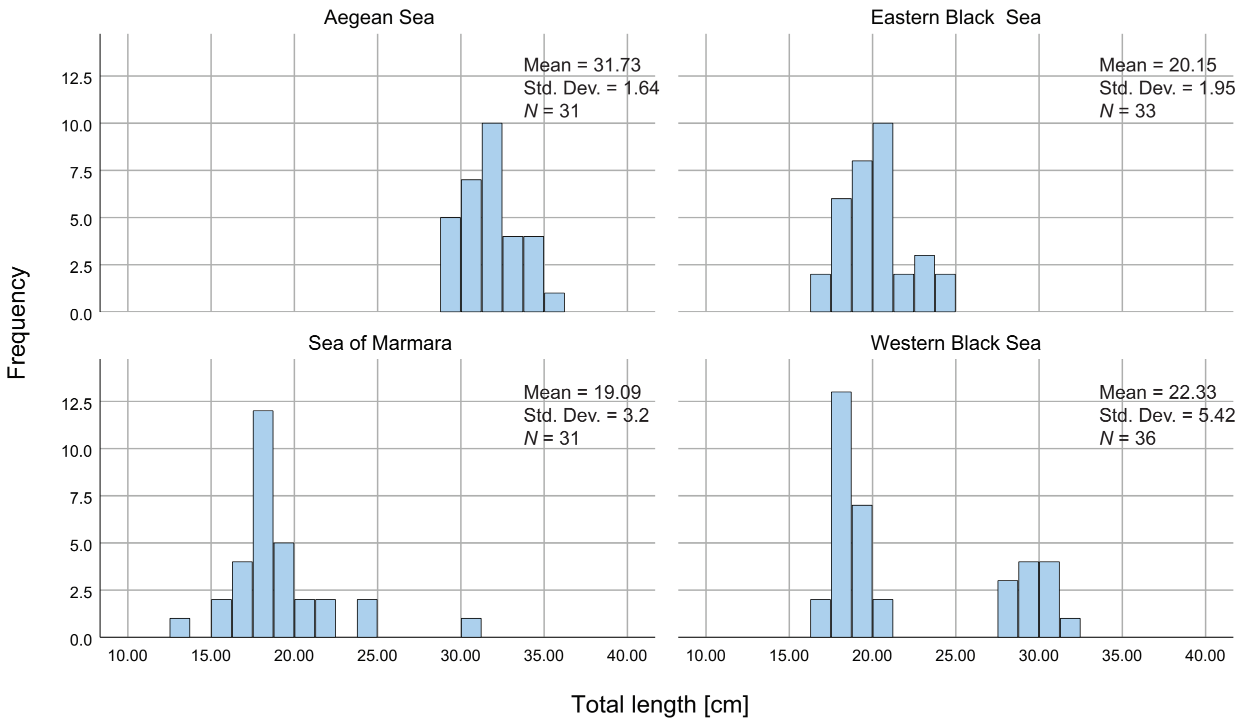

Figure 3. Frequency distribution of bluefish, *Pomatomus saltatrix*, according to their total length.

Table 3. Descriptive statistics of univariate ANOVA based on morphometric characters of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Morphometric characters	Univariate ANOVA						
	Wilks' λ	*F*	Sign.	Morphometric characters	Wilks' λ	*F*	Sign.
1–2	0.276	54.241	<0.0001	3–12	0.068	285.212	<0.0001
2–3	0.085	221.971	<0.0001	4–11	0.056	349.181	<0.0001
3–4	0.163	106.011	<0.0001	5–11	0.070	274.448	<0.0001
4–5	0.315	44.959	<0.0001	6–11	0.035	575.511	<0.0001
5–6	0.036	557.940	<0.0001	7–10	0.093	201.551	<0.0001
6–7	0.176	96.828	<0.0001	1–12	0.067	289.156	<0.0001
7–8	0.215	75.242	<0.0001	2–12	0.067	289.493	<0.0001
8–9	0.174	97.954	<0.0001	3–11	0.038	526.114	<0.0001
9–10	0.228	70.132	<0.0001	5–10	0.025	807.113	<0.0001
10–11	0.033	602.729	<0.0001	6–10	0.072	265.311	<0.0001
11–12	0.055	351.826	<0.0001	7–9	0.132	135.666	<0.0001
12–13	0.113	162.162	<0.0001	2–11	0.022	925.575	<0.0001
1–13	0.308	46.441	<0.0001	6–9	0.157	110.827	<0.0001
2–13	0.201	82.171	<0.0001				

Abbreviations of morphometric characters are given in Fig. 2.

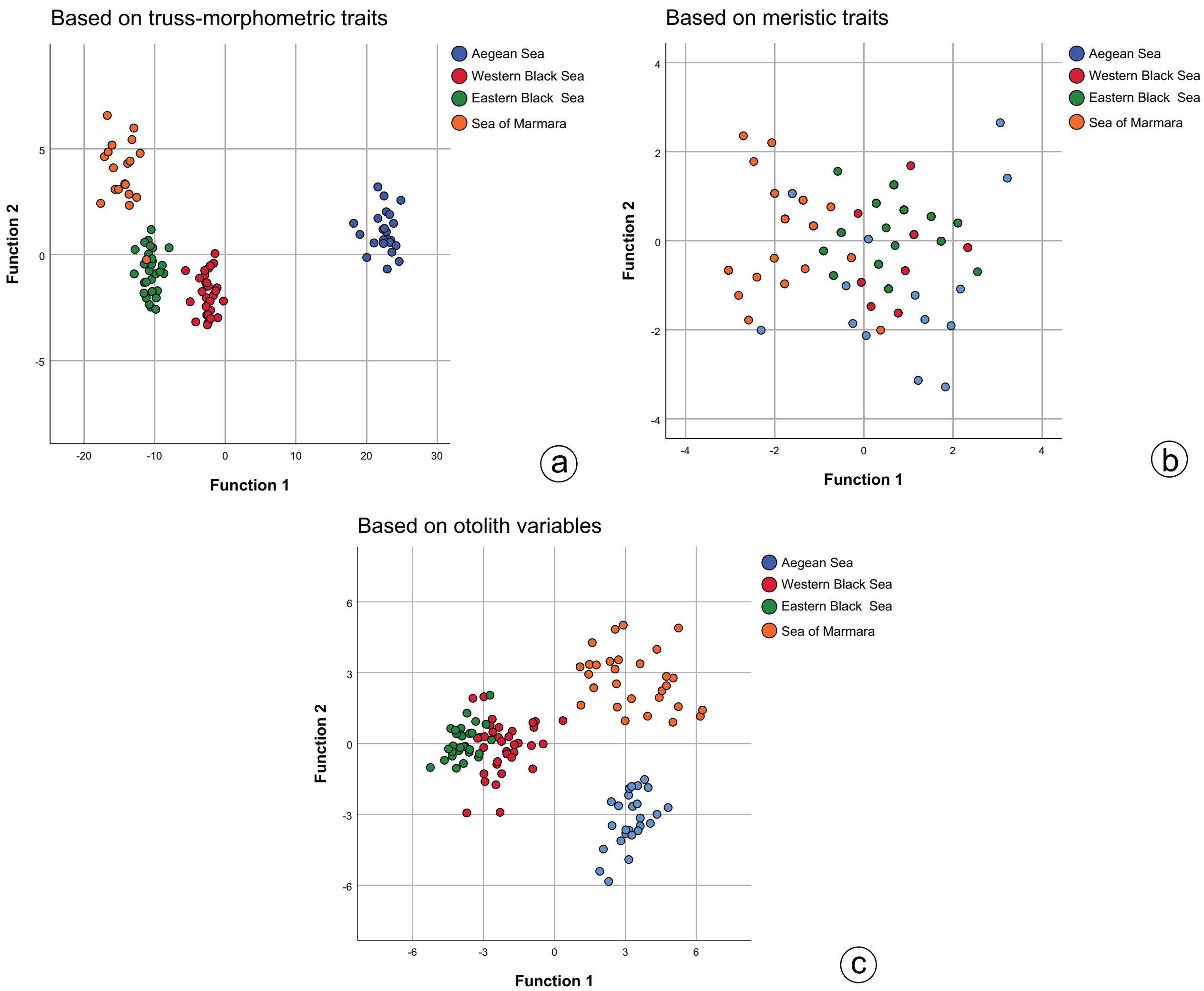

Figure 4. Discriminant function analysis (DFA) of bluefish, *Pomatomus saltatrix*, populations based on the truss-morphometric traits, meristic characters, and otolith variables.

between the four populations. The estimated value of Kaiser–Meyer–Olkin (KMO) was 0.911, suggesting that the data was appropriate for factor analysis. The first two principal components accounted for 84.81% (PC1) and 5.27% (PC2) of the total variance, explaining 90.1% of the total variation. The truss-morphometric trait that had loadings > 0.70 was considered significant in this study. Except for 1–13 and 4–5, all truss-morphometric traits

Table 4. The first two component-loading scores of principal components based on morphometric characters of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Morphometric characters	Principal component				
	PC1 (84.81%)	PC2 (5.27%)	Morphometric characters	PC1 (84.81%)	PC2 (5.27%)
1–2	**0.757**	0.479	3–12	**0.970**	–0.092
2–3	**0.936**	–0.049	4–11	**0.981**	–0.035
3–4	**0.859**	–0.402	5–11	**0.977**	–0.093
4–5	0.615	0.616	6–11	**0.983**	–0.067
5–6	**0.976**	0.025	7–10	**0.964**	–0.104
6–7	**0.917**	0.077	1–12	**0.969**	0.028
7–8	**0.894**	0.007	2–12	**0.967**	–0.113
8–9	**0.894**	0.144	3–11	**0.984**	–0.079
9–10	**0.888**	–0.200	5–10	**0.986**	0.020
10–11	**0.973**	0.016	6–10	**0.974**	–0.052
11–12	**0.968**	–0.010	7–9	**0.954**	–0.021
12–13	**0.863**	–0.373	2–11	**0.984**	–0.041
1–13	0.676	0.570	6–9	**0.943**	–0.058
2–13	**0.887**	0.242			

Abbreviations of morphometric characters are given in Fig. 2. Bold values indicated significance loading at >0.70.

Table 5. Summary output of stepwise canonical discriminant analysis based on morphometric characters bluefish, *Pomatomus saltatrix*, samples from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara collected between February 2014 to November 2014; Overall, 99.0% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	**100%**				100%
Western Black Sea		**100%**			100%
Eastern Black Sea			**100%**		100%
Sea of Marmara			5.26%	**94.74%**	100%

had a loading value above 0.70 on PC1 (Table 4). The second PC2 was strongly associated with 4–5 and 1–13 truss-morphometric traits, and their loading values were 0.616 and 0.570, respectively.

DFA results show clear patterns of truss-morphometric trait variations, forming four distinct clusters that are well separated from each other (Fig. 4). In DFA, the first DF accounted for 97.7%, and the second corresponded to 1.9% of the between-group variability. The proportion of correctly classified Aegean Sea, western Black Sea, and eastern Black Sea samples to their original groups were 100%, demonstrating clear separation of these stocks from each other. Up to 5% of the Sea of Marmara samples were incorrectly classified (Table 5).

Dendrogram, based on the Euclidean distance method, formed three main clusters (Fig. 5). The first cluster formed by the Aegean Sea was separated with maximum Euclidean distance evincing apparent isolation of the Aegean Sea population from others, which supports the result highlighted by DFA (Fig. 4). The minimum Euclidean distance was found between the western Black Sea and the eastern Black Sea, sharing a high similarity.

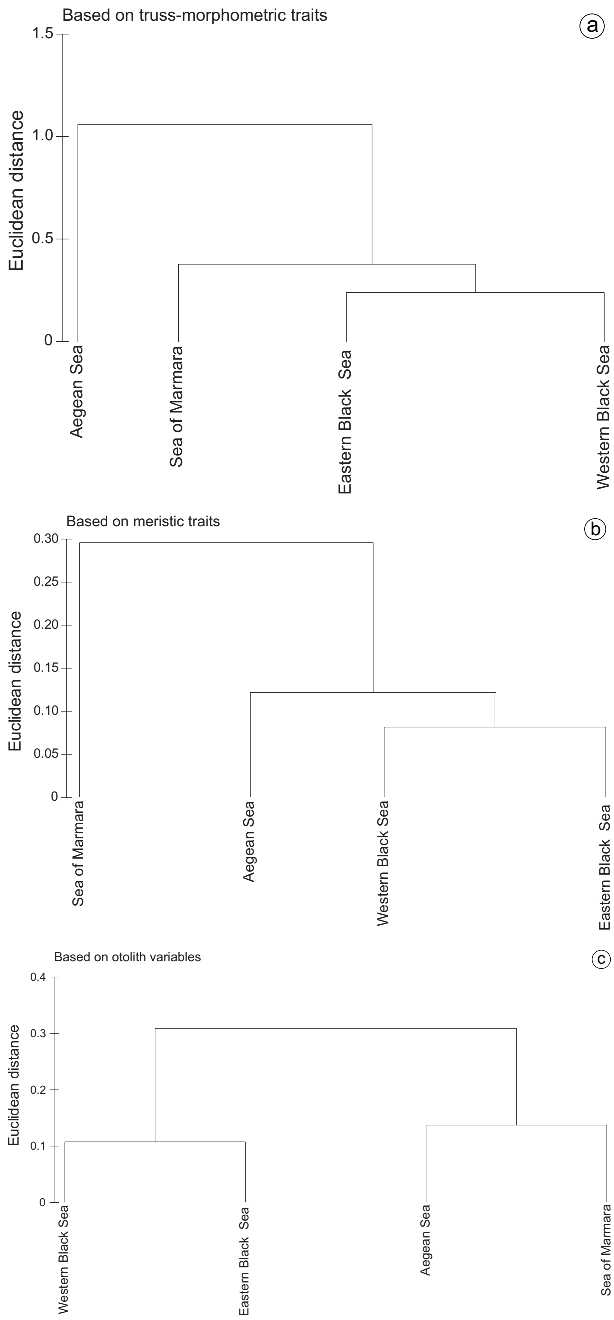

Figure 5. Dendrogram based on the Euclidean distance method depicting the dissimilarity of bluefish, *Pomatomus saltatrix*, populations based on the truss-morphometric traits, meristic characters, and otolith variables.

Meristic characters

The range of the bluefish meristic counts from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara are given in Table 6. The effect of sex on meristic characters were not significant (one-way MANOVA; $F_{(7.0, 123)} = 1.57$, Wilk's $\lambda = 0.918$, $P = 0.150$); therefore, further analysis was done disregarding the sex. The meristic characters showed significant variations for different stocks of the bluefish (one-

Table 6. Descriptive data of the meristic counts of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Meristic characters	Aegean Sea		Western Black Sea		Eastern Black Sea		Sea of Marmara	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Right gill rakers	10.97 ± 0.84	9–12	11.17 ± 0.97	9–13	11.00 ± 0.00	11–11	10.39 ± 1.15	9–12
Left gill rakers	10.87 ± 0.81	9–12	11.25 ± 1.00	9–13	10.91 ± 0.52	10–12	10.39 ± 1.05	9–12
Dorsal fin spines	7.19 ± 0.70	6–10	7.72 ± 0.45	7–8	7.55 ± 0.51	7–8	6.65 ± 0.49	6–7
Dorsal fin rays	24.39 ± 1.17	22–27	24.44 ± 0.88	23–26	24.15 ± 0.94	22–26	22.68 ± 0.79	22–24
Ventral fin rays	11.77 ± 0.72	10–13	12.00 ± 0.00	12–12	12.00 ± 0.00	12–12	12.10 ± 0.30	12–13
Pectoral fin rays	15.13 ± 0.85	12–16	14.97 ± 0.38	14–16	15.18 ± 0.46	14–16	15.03 ± 0.66	14–16
Anal fin rays	25.48 ± 1.09	24–28	25.03 ± 1.03	23–27	24.70 ± 0.88	24–27	24.10 ± 1.14	23–28

Table 7. Descriptive statistics of univariate ANOVA based on meristic characters of the bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara collected between February 2014 and November 2014.

Characters	Wilks' λ	*F*	Significance
Right gill rakers	0.889	5.311	0.002
Left gill rakers	0.884	5.562	0.001
Dorsal fin spines	0.618	26.214	0.000
Dorsal fin rays	0.630	24.873	0.000
Ventral fin rays	0.910	4.195	0.007
Pectoral fin rays	0.982	0.786	0.504
Anal fin rays	0.808	10.083	0.000

Table 8. The component-loading scores of principal components based on meristic characters of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Character	Principal component	
	PC1 (37.45%)	PC2 (20.94%)
Right gill rakers	**0.769**	0.466
Left gill rakers	**0.786**	0.440
Dorsal fin spines	0.626	–0.157
Dorsal fin rays	0.612	–0.574
Ventral fin rays	0.020	0.482
Anal fin rays	0.521	–0.508

Bold values indicated significance loading at >0.70.

Table 9. The summary output of stepwise canonical discriminant analysis based on meristic characters of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014; overall, 64.1% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	**67.74%**	9.68%	9.68%	12.90%	100%
Western Black Sea	16.67%	**50.00%**	27.78%	5.56%	100%
Eastern Black Sea	15.15%	24.24%	**51.52%**	9.09%	100%
Sea of Marmara	6.45%		3.23%	**90.32%**	100%

Table 10. Descriptive data of otolith variables of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Otolith variables	Aegean Sea		Western Black Sea		Eastern Black Sea		*Sea of Marmara	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Otolith length	7.07 ± 0.32	6.28–7.55	5.38 ± 1.09	4.34–7.39	5.02 ± 0.38	4.37–5.95	6.36 ± 0.87	4.65–8.98
Otolith width	2.58 ± 0.14	2.23–2.81	2.16 ± 0.23	1.8–2.58	2.09 ± 0.1	1.91–2.26	2.50 ± 0.26	1.99–3.16
Otolith area	13.03 ± 0.99	10.79–14.94	8.46 ± 2.86	5.53–13.63	7.56 ± 0.97	5.82–9.37	12.63 ± 3.05	7.25–21.63
Otolith perimeter	17.57 ± 1.03	15.66–19.79	14.3 ± 2.87	10.71–19.79	12.47 ± 1.03	10.52–14.48	19.48 ± 3.95	14.51–32.09
Form factor	0.53 ± 0.04	0.46–0.6	0.52 ± 0.06	0.39–0.64	0.61 ± 0.04	0.52–0.72	0.43 ± 0.08	0.18–0.55
Roundness	0.33 ± 0.02	0.3–0.39	0.37 ± 0.03	0.29–0.42	0.38 ± 0.02	0.34–0.41	0.39 ± 0.03	0.33–0.44
Aspect ratio	2.74 ± 0.14	2.36–3.03	2.47 ± 0.26	2.13–3	2.4 ± 0.13	2.16–2.69	2.54 ± 0.17	2.28–3.07

*Bal et al. (2018b).

way MANOVA; $F_{(21, 348)} = 7.259$, Wilk's $\lambda = 0.352$, $P < 0.0001$). Univariate ANOVA, based on the stepwise method, further revealed a highly significant difference among different locations for six out of seven meristic characters (Table 7). The pectoral fin rays were not considered in the PCA analysis as it was constant among different stocks of the bluefish. The estimated value of KMO was 0.596. The PC1 and PC2 accounted for 37.45% and 20.94% of the total variance, explaining 58.4% of the total variation. Only two meristic characters, viz. right and left gill rakers, had a loading value above 0.70 (Table 8).

In DFA, the first DF accounted for 83.0%, and the second corresponded to 16.8% of the between-group variability. Overall, 64.1% of original grouped cases were correctly classified, and the bluefish correct classification into their original population/location ranged from 50.0% to 90.3% by canonical discriminant analysis (Table 9). The remarkably high reclassification rate was recorded by the bluefish individuals from the Sea of Marmara (90.3%) clearly separated from the other stocks (Fig. 4). The dendrogram based on the Euclidean distance method also proved that the stock of the Sea of Marmara was the most clearly distinguished stock isolating it from the other groups with the highest Euclidean distance (Fig. 5). The stocks of the eastern and western Black Sea shared high similarity having the lowest Euclidean distance.

Otolith characters

The mean, standard deviation, minimum and maximum values for each otolith variable of the bluefish (*Pomatomus saltatrix*) are given in Table 10. Similar to truss-morphometric variables, the otoliths variables were free from the influence of body size using the allometric transformation method. The effect of sex on otolith bluefish characters was also not significant (one-way MANOVA; $F_{(4, 110)} = 0.597$, Wilk's $\lambda = 0.979$, $P = 0.666$); therefore, both sexes were combined for further analysis. Similar to truss-morphometric and meristic characters, the otolith variables were showed significant differences among the stocks of bluefish from different locations (one-way MANOVA; $F_{(12, 286)} = 100.275$, Wilk's $\lambda = 0.013$, $P < 0.0001$). Also, univariate ANOVA, based on the stepwise method, further revealed a highly significant difference among different locations for otolith variables (Table 11). This matrix was not positive definite, and hence the KMO was not displayed for otolith variables. The PC1 and PC2 accounted for 66.07% and 21.32% of the total variance, explaining 91.95% of the total variation. Except for CI and RD, all had a loading value above 0.70 (Table 12).

Table 11. Descriptive statistics of univariate ANOVA based on otolith variables of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014 from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Variable	Wilks' λ	*F*	Significance
Otolith length	0.102	324.325	0.000
Otolith width	0.257	107.157	0.000
Otolith area	0.114	288.710	0.000
Otolith circumference	0.121	269.433	0.000
Form factor	0.390	57.986	0.000
Roundness	0.295	88.584	0.000
Aspect ratio	0.425	50.075	0.000

Table 12. The component-loading scores of principal components based on otolith variables of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Variables	Principal component	
	PC1 (66.07%)	PC2 (21.33%)
Otolith length	**0.981**	–0.123
Otolith width	**0.893**	0.181
Otolith area	**0.964**	0.112
Otolith circumference	**0.938**	0.331
Form factor	–0.557	–0.572
Roundness	–0.445	**0.832**
Aspect ratio	**0.741**	–0.551

Bold values indicated significance loading at > 0.70.

In DFA, the first DF accounted for 69.1%, and the second corresponded to 28.9% of the between-group variability. Overall, 96.6% of original grouped cases were correctly classified, and the bluefish correct classification into their original population ranged from 91.4% to 100% by canonical discriminant analysis (Table 13). The bluefish from the Aegean Sea, as well as the Sea of Marmara, each formed a distinct cluster that was well separated from others (Fig. 4). Furthermore, the Aegean Sea and the Sea of Marmara samples' reclassification rate to their original group were 100%, and hence they both were the most clearly isolated groups. Dendrogram, based on the Euclidean distance method, formed two main clusters (Fig. 5). The first cluster formed by the Aegean Sea and Sea of Marmara were separated with maximum Euclidean distance evincing apparent isolation of these populations from others, which supported the result highlighted by DFA (Fig. 4). Similarly, in truss-morphometric traits and meristic characters methods, the minimum Euclidean distance was found between the western Black Sea and the eastern Black Sea, sharing a high similarity.

Discussion

The truss-morphometric characteristics analysis provided evidence of the existence of four morphologically differentiated populations of bluefish, with 95% to 100% correct allocation of bluefish individuals into their original stock. These results are in line with the findings of Turan et al. (2006), who also observed the existence of morphologically differentiated groups of bluefish in Turkish sea waters. Turan et al. (2006) observed three morphologically differentiated groups of bluefish: first included samples from the Aegean Sea, Sea of Marmara, and the western Black Sea and formed a stock, while the other two groups were made by the north-eastern Mediterranean Sea and east Black Sea, and each represented a separate stock. In contrast to Turan et al. (2006), this study evinced the populations of bluefish from the Aegean Sea, the Sea of Marmara, and the western Black Sea did not overlap in DFA analysis, and they are clearly distinct stocks based on truss-morphometric characteristics (Fig. 4a). According to

Table 13. Summary statistics of stepwise canonical discriminant analysis based on otolith variables of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014; overall, 96.6% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	100				100
Western Black Sea		91.43	8.57		100
Eastern Black Sea		3.45	96.55		100
Sea of Marmara				100	100

Turan et al. (2006), the existence of low phenotypic differentiation among the Aegean Sea, Sea of Marmara, and the western Black Sea was attributed to the extensive migration of bluefish in these waters (i.e., Pardiñas et al. 2010), resulted in a higher level of intermingled bluefish stocks.

Several studies suggest that the population structure of highly migratory marine species is strongly regulated by some behavioral traits such as spawning site fidelity, homing behavior (Danancher and Garcia-Vazquez 2011), but can also be promoted by oceanic barriers to gene flow (Machado-Schiaffino et al. 2010), temperature (Crow et al. 2007) or salinity (Nielsen et al. 2004). The Bosphorus is an important migration route for fishes between the Sea of Marmara and the Black Sea (Atilgan et al. 2017; Ceyhan et al. 2007; Kokos 2011). A recent increase in anthropogenic activities, such as an increase in the pollutant loads from industrial and domestic sources, together with high sea traffic and coastal erosion in the Bosphorus, might prevent fish migration (Özsoy and Mikaelyan 1997). They might also restrict the intermingling of bluefish stocks among the western Black Sea, Sea of Marmara, and the Aegean Sea, and consequently showed stock separation.

The use of more than one stock identification approach and comparison between them can enhance the likelihood of extracting differences between classifying for a comprehensive conclusion (Waldman et al. 1988; Begg and Waldman 1999; Cadrin et al. 2014). The truss-morphometric characteristics analysis with meristic characteristics or otolith characters has been used combined to investigate between subpopulations of a fish (Begg and Waldman 1999; Turan et al. 2006; Khan et al. 2012; Bose et al. 2020). The ability of each method to correctly allocate individuals into their original stock change from species to species (Turan et al. 2006; Khan et al. 2012; Hari et al. 2019). In this study, the truss morphometric approach demonstrated a higher success rate (99%) in individuals' allocation to their original locations than the meristic characters method, which had a 64% success rate. The success rate of the otolith characters' approaches demonstrated was higher (97%) than the meristic characters approach. On the contrary, Turan et al. (2006) recorded a higher success rate for meristic characters (64%) than the truss morphometric approach (54%). Consequently, these differences indicate that the ability of a stock identification approach to correctly allocating individuals into their original stock might change over time.

Conclusions

Bluefish stock from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara demonstrated considerable morphometric variations and hence they should be considered as four self-contained stocks that are geographically isolated from each other. Environmental differences between areas probably influence these inter-population morphometric distinctions. This might indicate new environmental consequences hindering the intermingling of bluefish stocks; since the stocks of the Aegean Sea, the Sea of Marmara, and the western Black Sea were observed as a single, morphometrically homogenous stock by Turan et al. (2006). This study suggests the requirement of strategic assessment and management of each bluefish stock separately to use them sustainably in the future.

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References

Atilgan E, Zengin M, Özcan Akpinar I, Kasapoğlu N (2017) Relation between seasonal migration and fishing of bluefish (*Pomatomus saltatrix* L, 1766) population in Turkish coasts. SEAB International Symposium on Euro Asian Biodiversity', 5–8 June 2017, Minsk, Belarus.

Bal H, Yanık T, Türker D (2018a) Growth and reproductive characteristics of the Bluefish *Pomatomus saltatrix* (Linnaeus, 1766) in the Marmara Sea. Ege Journal of Fisheries and Aquatic Sciences 35(1): 95–101. https://doi.org/10.12714/egejfas.2018.35.1.15

Bal H, Yanık T, Türker D (2018b) Relationships between total length and otolith size of bluefish *Pomatomus saltatrix* (Linnaeus, 1766) in the Marmara Sea of Turkey. Natural and Engineering Sciences 3(1): 38–44. https://doi.org/10.28978/nesciences.379319

Begg GA, Waldman JR (1999) An holistic approach to fish stock identification. Fisheries Research 43(1–3): 35–44. https://doi.org/10.1016/S0165-7836(99)00065-X

Bose APH, Zimmermann H, Winkler G, Kaufmann A, Strohmeier T, Koblmuller S, Sefc KM (2020) Congruent geographic variation in saccular otolith shape across multiple species of African cichlids. Scientific Reports 10(1): e12820. https://doi.org/10.1038/s41598-020-69701-9

Cadrin SX, Kerr LA, Mariani S (2014) Interdisciplinary evaluation of spatial population structure for definition of fishery management units. Stock Identification Methods, Elsevier, 537 pp. https://doi.org/10.1016/B978-0-12-397003-9.00022-9

Carpenter KE, Ralph G, Pina Amargos F, Collette BB, Singh-Renton S, Aiken KA, Dooley J, Marechal J (2015) *Pomatomus saltatrix* (errata version published in 2017). The IUCN Red List of Threatened Species 2015: e.T190279A115314064.

Ceyhan T, Akyol O, Ayaz A, Juanes F (2007) Age, growth, and reproductive season of bluefish (*Pomatomus saltatrix*) in the Marmara region, Turkey. ICES Journal of Marine Science 64(3): 531–536. https://doi.org/10.1093/icesjms/fsm026

Crow KD, Munehara H, Kanamoto Z, Balanov A, Antonenko D, Bernardi G (2007) Maintenance of species boundaries despite rampant hybridization between three species of reef fishes (Hexagrammidae): Implications for the role of selection. Biological Journal of the Linnean Society. Linnean Society of London 91(1): 135–147. https://doi.org/10.1111/j.1095-8312.2007.00786.x

Danancher D, Garcia-Vazquez E (2011) Genetic population structure in flatfishes and potential impact of aquaculture and stock enhancement on wild populations in Europe. Reviews in Fish Biology and Fisheries 21(3): 441–462. https://doi.org/10.1007/s11160-011-9198-6

Feldman L (2013) Bluefish United States–Monterey Bay Aquarium's Seafood Watch. Bottom trawl, Bottom gillnet, Handline.

Hanif MA, Chaklader MR, Siddik MAB, Nahar A, Foysal MJ, Kleindienst R (2019) Phenotypic variation of gizzard shad, *Anodontostoma chacunda* (Hamilton, 1822) based on truss network model. Regional Studies in Marine Science 25: e100442. https://doi.org/10.1016/j.rsma.2018.100442

Hari MS, Kathrivelpandian A, Bhavan SG, Sajina AM, Gangan SS, Abidi ZJ (2019) Deciphering the stock structure of *Chanos chanos* (Forsskål, 1775) in Indian waters by truss network and otolith shape analysis. Turkish Journal of Fisheries and Aquatic Sciences 20(2): 103–111. https://doi.org/10.4194/1303-2712-v20_2_03

Helfman G, Collette BB, Facey DE, Bowen BW (2009) The Diversity of fishes: biology, evolution, and ecology. John Wiley & Sons.

Khan MA, Miyan K, Khan S, Patel DK, Ansari NG (2012) Studies on the elemental profile of otoliths and truss network analysis for stock discrimination of the threatened stinging catfish *Heteropneustes fossilis* (Bloch1794) from the Ganga River and its tributaries. Zoological Studies (Taipei, Taiwan) 51: 1195–1206.

Kokos M (2011) Mass fish migration delayed in Bosphorus. FiskerForum, Otto Pedersvej 1, 6960 Hvide Sande, Denmark.

Machado-Schiaffino G, Juanes F, Garcia-Vazquez E (2010) Introgressive hybridization in North American hakes after secondary contact. Molecular Phylogenetics and Evolution 55(2): 552–558. https://doi.org/10.1016/j.ympev.2010.01.034

MAF (2019) Ministry of Agriculture and Forestry: Su Ürünlerİ İstatİstikleri (Seafood Statistics), 9 pp.

Nielsen EE, Nielsen PH, Meldrup D, Hansen MM (2004) Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. Molecular Ecology 13(3): 585–595. https://doi.org/10.1046/j.1365-294X.2004.02097.x

Özsoy E, Mikaelyan A (1997) Sensitivity to Change: Black Sea, Baltic Sea and North Sea. Sensitivity to Change: Black Sea. Baltic Sea and North Sea 27. https://doi.org/10.1007/978-94-011-5758-2

Pardiñas A, Campo D, Pola I, Miralles L, Juanes F, Garcia-Vazquez E (2010) Climate change and oceanic barriers: genetic differentiation in *Pomatomus saltatrix* (Pisces: Pomatomidae) in the North Atlantic Ocean and the Mediterranean Sea. Journal of Fish Biology 77(8): 1993–1998. https://doi.org/10.1111/j.1095-8649.2010.02774.x

Rawat S, Benakappa S, Kumar J, Naik K, Pandey G, Pema C (2017) Identification of fish stocks based on truss morphometric: A review. Journal of Fisheries and Life Sciences 2(1): 9–14.

Reist JD (1986) An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Canadian Journal of Zoology 64(6): 1363–1368. https://doi.org/10.1139/z86-203

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18(1): e529. https://doi.org/10.1186/s12859-017-1934-z

Sabatés A, Martín P, Raya V (2012) Changes in life-history traits in relation to climate change: Bluefish (*Pomatomus saltatrix*) in the northwestern Mediterranean. ICES Journal of Marine Science 69(6): 1000–1009. https://doi.org/10.1093/icesjms/fss053

Shepherd G (2010) Status of fishery resources off the northeastern US. Bluefish. NEFSC, Resource Evaluation and Assessment Division.

Strauss RE, Bookstein FL (1982) The truss: Body form reconstructions in morphometrics. Systematic Zoology 31(2): 113–135. https://doi.org/10.2307/2413032

TÜİK (2020) Fishery Products, 2019.

Turan C, Oral M, Öztürk B, Düzgüneş E (2006) Morphometric and meristic variation between stocks of bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean seas. Fisheries Research 79(1–2): 139–147. https://doi.org/10.1016/j.fishres.2006.01.015

Waldman JR, Grossfield J, Wirgin I (1988) Review of stock discrimination techniques for striped bass. North American Journal of Fisheries Management 8(4): 410–425. https://doi.org/10.1577/1548-8675(1988)008<0410:ROSDTF>2.3.CO;2