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Morphometric Structuring of the Anchovy (*Engraulis encrasicolus* L.) in the Black, Aegean and Northeastern Mediterranean Seas

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Abstract: The status of populations of anchovies in Turkish terrestrial waters was preliminarily investigated using morphometric characters with the truss network system. Samples were taken from the main fishing areas of each sea, comprising the central (Sinop) and eastern (Trabzon) Black Sea, the Aegean Sea (İzmir) and the eastern Mediterranean (İskenderun). Plotting discriminant functions 1 and 2, explaining 93% of between-group variability, revealed a high degree of dissimilarity among the anchovy samples, indicating that the anchovies in each sea represent different aggregations. The overall random assignment of individuals into their original group was high (80%). Pairwise comparisons using multivariate analysis of variance (MANOVA) showed highly significant differences between all the samples ($P < 0.001$). Univariate analysis of variance (ANOVA) revealed significant differences with varying degrees between the means of the 4 samples for 16 out of 25 standardized morphometric measurements. Principal components analysis (PCA) indicated that the observed differences were mainly from the measurements taken from the head.

Key Words: Anchovy, *Engraulis encrasicolus*, stock identification, morphometric characters, the truss network system

Karadeniz, Ege ve Kuzeydoğu Akdeniz Hamsi (*Engraulis encrasicolus* L.) Populasyonlarının Morfometrik Yapılanması

Özet: Truss Network Sistemi ile morfometrik karakterler kullanarak Türkiye karasularında bulunan hamsi populasyonlarının durumu ilk olarak incelenmiştir. Örnekler Orta (Sinop) ve Doğu (Trabzon) Karadeniz, Ege Denizi (İzmir), Kuzeydoğu Akdeniz (İskenderun) gibi her bir denizin ana balıkçılık alanı limanlarımızdan toplanmıştır. Kümelerarası Korelasyon Analizi'nde, birinci ve ikinci varyasyonların kümeleştirilmesi sonucu gruplar arasındaki varyasyonun % 93'ü kullanıldığında örnekler arasında yüksek derecede farklılığın olduğu gözlenmiştir. Buna göre her bir denizdeki hamsi populasyonları arasında morfometrik yapılaşmanın varlığı tespit edilmiştir ($P < 0,001$). Morfometrik karakterler bakımından, balıkların kendi orijinal grubuna doğru olarak sınıflandırılması, % 80 olarak yüksek bulunmuştur. Çok Değişkenli Varyans Analizi ile dört örneğin toplam 25 morfometrik karakterinin 16'sı arasında istatistiksel olarak önemli derecede farklılıkların olduğu bulunmuştur. Ana Bileşenler Analizi ise, populasyonlar arasında gözlenen farklılıkların genelde baş bölgesinden olduğunu göstermiştir.

Anahtar Sözcükler: Hamsi, *Engraulis encrasicolus*, stok belirleme, morfometrik karakterler, Truss Network Sistemi

Introduction

The anchovy, *Engraulis encrasicolus* L., has a widespread distribution and inhabits the eastern Atlantic coast from Scandinavia to West Africa and the Mediterranean Sea (1). In Turkey, the anchovy is found in the Mediterranean, Aegean, Marmara and Black seas and is the most exploited fish species in the Marmara and Black seas. The dynamics of the anchovy stocks are extremely irregular over time, with numerous poorly understood features that prevent its efficient

management on the basis of conventional methods. Stock identification is an integral component of modern fisheries stock assessment studies, and, in turn, of effective fisheries management (2). A number of studies have reported population differences within and between Black Sea and Sea of Azov anchovies (3-5). Spanakis et al. (6) found significant genetic and morphometric differences between the Aegean and Ionian Sea populations. Garcia et al. (7) examined *E. encrasicolus* from the northwestern Mediterranean, and were unable

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to differentiate between samples from sites covering an area from Barcelona on the Spanish coast to close to the island of Elba on the western Italian coast. Bembo et al. (8) found significant genetic differentiation between *E. encrasicolus* collected from sites in the Aegean and Tyrrhenian seas.

Morphometric characters have been successfully used for stock identification. There are many well-documented morphometric studies that provide evidence for stock discrimination (8-14). When morphology was investigated, morphometric measurements were typically limited to select body structures such as the fins with poor or no ability to quantify body shape (15). These measurements tended to concentrate along the body axis with only sampling from depth and breadth, and most measurements were from the head. A new system of morphometric measurements called the truss network system (16) has been increasingly used for stock identification (8,9,13,15,17-21). This system covers the entire fish in a uniform network, and theoretically should increase the likelihood of extracting morphometric differences between stocks. A regionally unbiased network of morphometric measurements over the 2 dimensional outline of a fish should give more information about local body differences than a conventional set of measurements (15,16).

In the present study, the analysis of the stock structure of the anchovy is carried out from a phenotypic point of view to determine the morphological differences associated with the origins of individuals from different areas with distinct environmental conditions.

Materials and Methods

Samples were taken from commercial purse seine fishing in 4 different localities in the northeastern Mediterranean (NMS), Aegean (AS) and Black seas (CBS & EBS) (Figure 1). The sampling locations and certain biological aspects of the samples are shown in Table 1.

The fish were thawed, placed on their right side on acetate sheets, and the body posture and fins were teased into a natural position. Each landmark was obtained by piercing the acetate sheet with a dissecting needle, defining 12 landmarks (Figure 2). Additional data, such as eye diameter and head width, were also recorded. Only undamaged fish were included in the analyses.

Prior to the analysis, it was necessary to eliminate any size effect in the data set. Variation should be attributable to body shape differences, and not related to the relative size of the fish. In the present study, there were significant linear correlations between all measured characters and the standard length of the fish. Therefore it was nec-

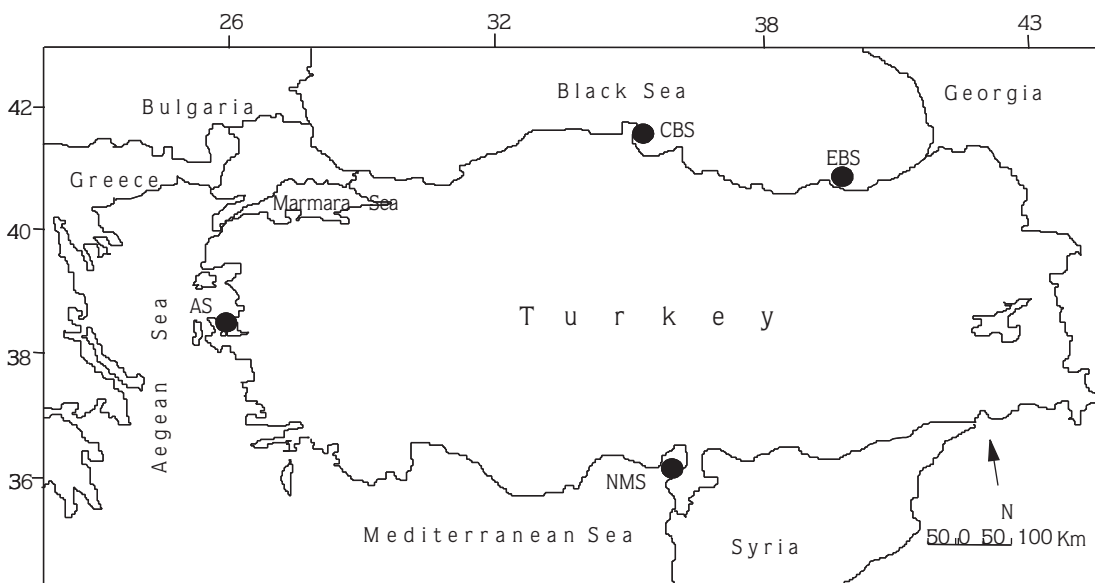


Figure 1. The map of the sampling of anchovy (•: indicates sampling location). NMS: northeastern Mediterranean; AS: Aegean Sea; CBS: central Black Sea; EBS: eastern Black Sea.

Table 1. Location, sampling gear, and biological features of anchovy samples. (Standard deviations of means are given in brackets. STL: standard length (mm) of samples).

Sample Sites	Locations	Collection Time	Gear	Sample size	Mean STL
Eastern Black Sea (EBS)	39°04' N 41°08' E	05.11.1999	Purse seiners	40	10.10 (±0.41)
Central Black Sea (CBS)	35°15' N 41°75' E	05.11.1999	Purse seiners	40	9.20 (±0.36)
Aegean Sea (AS)	26°85' N 38°35' E	22.10.1999	Purse seiners	39	9.19 (±0.47)
NE Mediterranean Sea (NMS)	36°05' N 36°65' E	23.12.1999	Purse seiners	40	10.63 (±0.47)

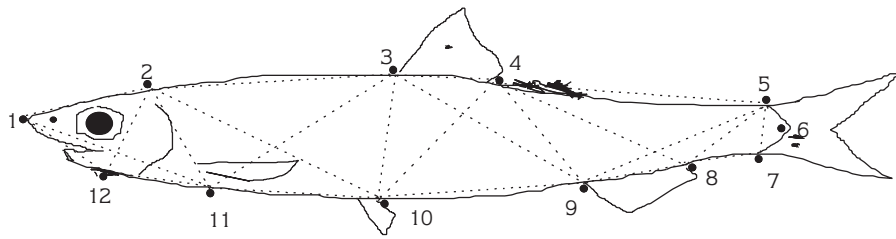


Figure 2. Locations of the 12 landmarks for constructing the truss network on anchovy illustrated as black dots and morphometric distance measures between the dots as lines. Landmarks refer to (1) anterior tip of snout at upper jaw, (2) most posterior aspect of neurocranium (beginning of scaled nape), (3) origin of dorsal fin, (4) insertion of dorsal fin, (5) anterior attachment of dorsal membrane from caudal fin, (6) posterior end of vertebrae column, (7) anterior attachment of ventral membrane from caudal fin, (8) insertion of anal fin, (9) origin of anal fin, (10) insertion of pelvic fin, (11) insertion of pectoral fin, (12) posteriormost point of maxillary.

essary to remove size-dependent variation for the morphometric characters. An allometric formula given by Elliott et al. (22) was used to correct for length effects in samples.

$$M_{adj} = M (L_s / L_o)^b$$

where M: original measurement, M_{adj} : size adjusted measurement, L_o : standard length of fish, L_s : overall mean of standard length for all fish from all samples in each analysis. Parameter b was estimated for each character from the observed data as the slope of the regression of $\log M$ on $\log L_o$, using all fish in all groups. The efficiency of size adjustment transformations was assessed by testing the significance of the correlation between transformed variables and standard length.

Standard length (landmark distance between 1 and 6, Figure 2) was excluded from the final analyses. Both univariate and multivariate analysis of variance (ANOVA and MANOVA) were carried out to test the significance of morphological differences. In addition, size adjusted data were standardized and submitted to discriminant function analysis (DFA) using SPSS v9, and graphs were generat-

ed using SYSTAT v5.0. Population centroids with 95% confidence ellipses derived from the DFA were used to visualize the relationships among the individuals of groups. Individuals were assigned to the samples using the discriminant functions, and the percentage of correctly assigned fish was an additional measure of differentiation among samples.

Results

None of the 25 transformed morphometric characters gave a significant correlation with standard length, and thus the allometric formula was successful in removing the size effect from the data. Univariate statistics (ANOVA) showed that 16 of 25 truss measurements were significantly different among samples in varying degrees (Table 2). Pairwise comparisons using multivariate ANOVA (MANOVA) revealed a highly significant inter-sample variation ($P > 0.001$) between all samples.

Plotting DF1 and DF2 showed a clear between-sample differentiation (Figure 3). The first DF accounted for 71% and the second accounted for 22% of the between-

Table 2. Univariate statistics (ANOVA) testing differences between samples from all truss measurements. Significance levels, * P < 0.05, ** P < 0.01, *** P < 0.001.

Characters	Wilks' Lambda	F	Significance
1-2	0.840	9.818	0.000***
1-11	0.931	3.854	0.011*
1-12	0.968	1.717	0.166
2-12	0.981	1.008	0.391
2-11	0.956	2.396	0.070
11-12	0.880	7.053	0.000***
2-3	0.925	4.219	0.007*
2-10	0.996	0.222	0.881
3-10	0.885	6.743	0.000***
3-11	0.921	4.422	0.005*
10-11	0.925	4.212	0.007*
3-4	0.933	3.697	0.013*
3-9	0.965	1.871	0.137
4-9	0.887	6.574	0.000***
4-10	0.934	3.638	0.014*
10-9	0.813	11.877	0.000***
4-5	0.978	1.188	0.316
4-8	0.951	2.676	0.049
5-8	0.989	0.583	0.627
5-9	0.834	10.265	0.000***
8-9	0.772	15.272	0.000***
5-7	0.864	8.146	0.000***
8-7	0.964	1.919	0.129
EY	0.704	21.698	0.000***
HD	0.804	12.600	0.000***

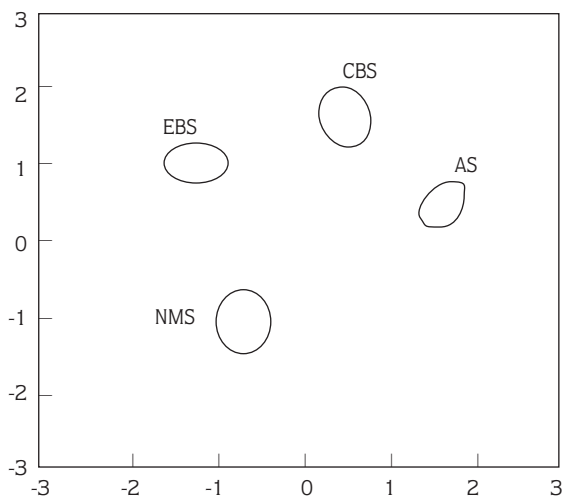


Figure 3. Sample centroids and 95% confidence ellipses of discriminant function scores. Samples referred to in the text were from the eastern Black Sea (Trabzon) (EBS), central Black Sea (Sinop) (CBS), Aegean Sea (AS), eastern Mediterranean (EMS).

group variability, explaining 93% of the total between-groups variability (Figure 3). All the samples were clearly separated from each other in the discriminant space, suggesting that there is limited intermingling among populations. The Mediterranean sample was most isolated from the Aegean and Black Sea samples. The Black Sea samples were also clearly separated from the others, but were closer to each other in comparison.

Pooled within-group correlations between discriminating variables and DFs revealed that the posterior body measurements (9-8, 9-10, 5-9, 5-7) dominantly contributed to first DF (Table 3), and the anterior body measurements (3-11, 11-10, 1-2, 2-11, 2-12) contributed to the second DF, implying that these characters are the most important in the description of population characteristics.

Table 3. Pooled within-groups correlations between discriminating variables and discriminant functions (variables ordered by size of correlation within function. * denotes the largest correlation between each variable and discriminant functions).

Characters	Discriminant Functions		
	1	2	3
8-9	0.349*	0.111	-0.141
10-9	-0.300*	-0.158	-0.130
5-9	0.279*	0.158	-0.023
5-7	0.259*	0.042	-0.065
11-12	0.239*	0.014	-0.121
2-3	-0.166*	-0.155	-0.064
3-4	0.162*	0.099	0.129
3-11	-0.047	0.308*	-0.247
10-11	0.097	0.289*	0.065
1-2	0.45	0.263*	0.096
2-11	-0.038	-0.243*	-0.085
1-11	0.138	-0.184*	0.169
4-5	-0.046	-0.160*	-0.024
3-10	0.011	0.104	-0.745*
4-9	0.001	-0.227	-0.642*
HD	0.118	-0.449	0.562*
4-10	-0.009	0.167	-0.479*
EY	-0.404	0.032	0.423*
4-8	0.057	-0.151	-0.358*
1-12	-0.041	-0.105	0.312*
3-9	0.027	-0.147	-0.296*
8-7	-0.090	0.009	0.288*
2-12	0.078	0.048	0.132*
5-8	-0.005	0.106	0.122*
2-10	-0.028	-0.049	-0.061*

A correct classification of individuals into their original population varied between 70% and 82% by discriminant analysis and 78% of individuals could be classified in their correct a priori grouping (Table 4). The proportion of correctly classified eastern Black Sea samples (EBS) into their original group was the highest (82%).

Discussion

The present morphometric study revealed evidence of highly significant morphometric heterogeneity among anchovy populations. Findings generated by both DFA and MANOVA suggest 4 phenotypically distinct local samples varying in the degree of differentiation. Interestingly there were clear differences between the samples taken from the eastern (Trabzon) and central (Sinop) Turkish Black Sea coast. These morphometric differences may be attributed to the fact that anchovies in the Trabzon and Sinop regions originated from different spawning grounds, located on the north and northwestern coast of the Black Sea. Several researchers have reported population differences within and between Black Sea and Sea of Azov anchovies (3-5). Kalnina and Kalnin (5) found significant genetic differences between Black Sea and Sea of Azov anchovies and suggested that there are 2 distinct populations of anchovy in the Black Sea. The morphometric dissimilarity of the Aegean Sea sample may suggest that this anchovy is self-recruiting and comes from the Marmara Sea, because there is a discrete spawning ground of anchovy in the Marmara Sea, and anchovy eggs in this spawning aggregation are different from those in the Black Sea spawning aggregations in dimension (23). Spanakis et al. (6) and Bembo et al. (8) found significant genetic and morphometric differences between the

Aegean and Ionian seas and Aegean and Tyrrhenian seas anchovy populations, respectively. The morphometric discreteness of the Mediterranean anchovy samples in the present study also may suggest that this population comes from the Mediterranean part of Africa and therefore was different from Aegean and Black Sea samples. Otherwise, there may be a self-recruiting population of anchovies in this region, because Ak et al. (24) collected anchovy eggs in the Mersin region that were morphologically different (in dimension) from those in the Aegean and Black seas.

The pattern of morphometric distinctness detected among the anchovy samples suggests a direct relationship between the extent of morphometric divergence and geographic separation. The detected pattern of high inter-sample variation may indicate reproductive isolation among local anchovy populations, which would confirm the genetic bases of observed morphometric differentiation among samples. However, in general, fishes demonstrate greater variance in morphological traits both within and between populations than other vertebrates, and are more susceptible to environmentally-induced morphological variation (25,26). Therefore, the effects of some environmental factors on spawning groups such as temperature, salinity, food availability or migration distance may determine the potential phenotypic discreteness of the anchovy.

The present findings reveal the potential power of the truss method for the identification of anchovy stocks. An unbiased network of morphometric measurements over the 2 dimensional outline of a fish removes the need to find the types of characters and optimal number of characters for stock separation, and provides information over the entire fish form.

Table 4. Correct classification of individuals into their original population.

	Sample	NMS	CBS	EBS	AS	Total
Original Count	NMS	28	5	0	7	40
	CBS	2	32	3	3	40
	EBS	1	6	32	0	39
	AS	5	2	1	32	40
%	NMS	70	12	0	18	100
	CBS	5	80	8	7	100
	EBS	3	15	82	0	100
	AS	13	5	2	80	100

The management implications of the detected morphological discreteness of the anchovy depend on the extent to which structuring persists over time. If the phenotypic differences found are genuine and not affected environmentally, then they should be repeatable in further analyses. Consistent differences in at least 2 repeated analyses between 2 groups of fish may indicate their temporal and spatial integrity. The genetic bases of the morphometric discreteness were not examined here. The application of genetic markers (27-29) would be an effective

method of examining the environmental component of phenotypic discreteness among geographic regions and facilitating the development of management recommendations.

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