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-RESEARCH ARTICLE-

Morphological Comparison of Thinlip mullet *Liza ramada* (Risso, 1827) Populations using the BioMorph Software

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Abstract

The development of image analysis systems has facilitated progress and diversification of morphological methods and expands the potential for using morphometry as a tool for fisheries management. This study aims to elucidate morphological structure of Thinlip mullet *Liza ramada* from Samandag and Arsuz Coasts in Northeastern Mediterranean using BioMorph software. The morphometric data set were generated from each sampled individual images, and the obtained morphometric landmarks on fish image were subjected to multivariate analyses to elucidate population differentiation. In discriminant function analysis, overall random assignment of individuals into their original population was 95% that 86.4% and 100% of cross-validated grouped individuals of Arsuz and Samandag populations were classified into their original population, respectively. In Principal component analysis, 19 principal components were produced of which first and second PCs explained 77 and 5 % of the variation. Examination of the contribution of each morphometric character to the first and second PCs indicated that the observed differences were mainly from the dorsal and height measurements, demonstrating these characters to be important in the description of the population specifications.

Keywords:
Mullet, *Liza ramada*, morphological comparison, image analysis.

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Introduction

Mullets have worldwide distribution and inhabit tropical and temperate seas; a few spend their lives in freshwater (Nelson, 2006). The Mugilidae family includes 17 genera and 72 species in the world (Harrison and Senou, 1997; Nelson, 2006). Eight species of Mugilidae inhabit the Mediterranean Sea, and originally classified as part of the single genus Mugil, under different

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names *(M. cephalus, M. ramada, M. labrosus, M. labeo, M. aurata, M. abu, M. saliens, M. carinata*). Lately, *M. ramada* was revised to *Liza ramada* (Turan, 2015). The thinlip mullet (*Liza ramada*) is one of the most appreciated fish in Mediterranean Sea (Nelson, 1994). *L. ramada* (Risso, 1827), which is a member of Mugilidea, is an euryhaline, eurythermic and pelagic coastal marine species which usually lives in inshore waters, entering lagoons and estuaries. It rarely enters freshwater and prefers a muddy bottom and feed on periphyton, detritus and small invertebrates (Fazli et al., 2008; Turan, 2015).

Morphometric characters have been most frequently employed to delineate stocks of fish (Begg & Waldman, 1999; Turan et al., 2004). Morphometric characters are continuous characters describing aspects of body shape (Ihssen et al., 1981).

Although a large number of studies on the taxonomic status of *Liza ramada* conducted on a large scale comprising several countries, there has been relatively very limited study on population structure of *Liza ramada* in Turkish marine waters.

Therefore, this study aims to elucidate morphological structuring of Thinlip mullet *Liza ramada* from Arsuz and Samandag coasts in Northeastern Mediterranean using BioMorph software.

Material and Methods

In total, 25 specimens of *Liza ramada* were collected from Arsuz and Samandag coasts in Northeastern Mediterranean between August and September 2019 (Figure 1).

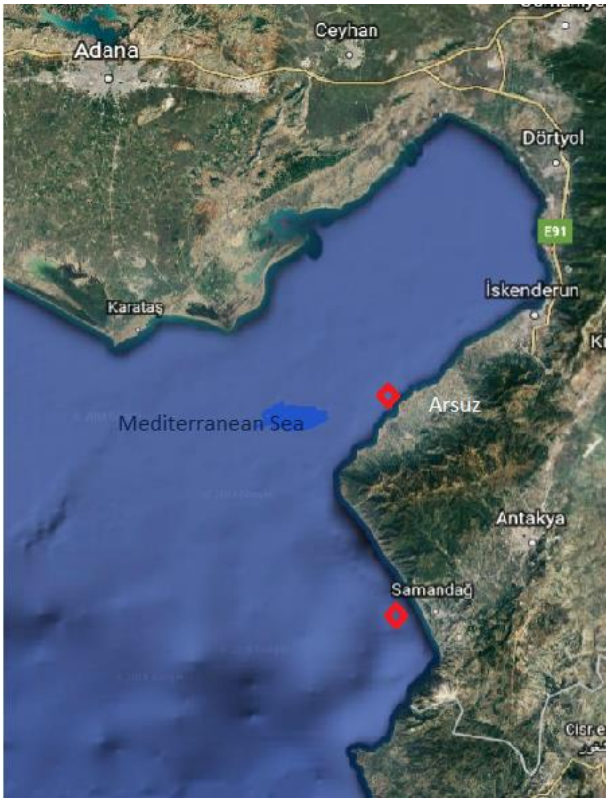

Figure 1. Map of the sampling locations of Thinlip mullet *Liza ramada*.

Morphological data were generated from each sampled individual photos. Thirteen morphometric landmarks were obtained as descriptive on fish image with the BioMorph (Figure 2).

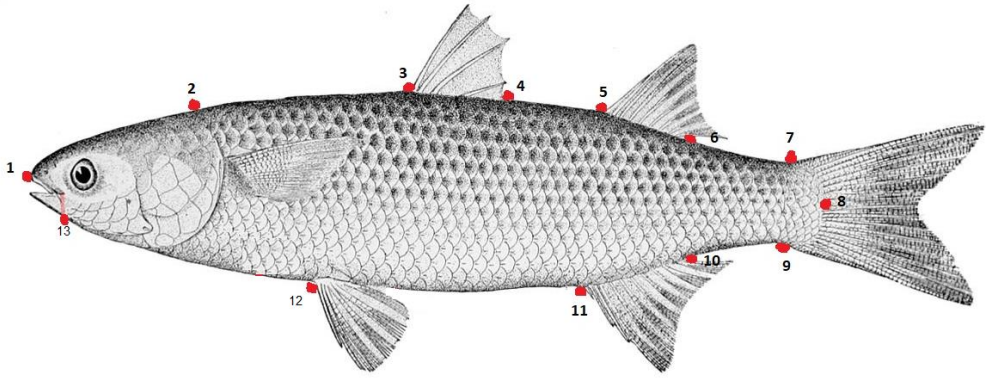

Figure 2. Thirteen landmarks obtained as descriptive on Thinlip mullet *Liza ramada* image.

In order to determine the biometric measurements, digital photographs from the bio-objects were taken first, then these images were transferred to the computer from which measurements were made using computer aided software. The landmarks were determined on the bio-object surface on digital photo. In order to eliminate the process of marking manual land marking for each fish, automated predictions of the 13 landmarks were determined by BioMorph (Kutlu & Turan, 2018) for each sample after the first determination of the landmarks on the fish image.

Prior to analyses, morphometric data were checked for normal distribution. Mean of ratios of the characters of the samples were calculated according to Mayr (1969) to compare degree of differentiation of characters among samples. In order to eliminate any size effect in the data set, allometric size effect on the data set were removed using the Allometry function in the BioMorph since the variation should be attributable to body shape differences, and not related to the relative size of fish. In the present study, there were no significant correlation between standard length and all morphometric measurements after the Allometry function.

Univariate analysis of variance (ANOVA) was used to test the significance of morphological differences. Size adjusted data were submitted to Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) using SPSS. Individuals were assigned to the samples using the discriminate functions, and the percentage of correctly assigned fish was an additional measure of differentiation among samples.

Results

BioMorph Landmark Distance Measurements Analysis produced 77 morphometric measurements from the combination of 13 landmarks. Morphometric data revealed normal distribution for two populations. In the Variance Analysis Technique using BioMorph, morphometric characters (2-3, 3-4, 5-8, 6-8, 7-8) revealed significant differences ($P<0.05$) between the locations.

In PCA, the first principal components (PC) explained 77% of morphometric variation and the second principal component explained 5% of morphometric variation.

Table 1. Distribution of variance explained for each component by principal components analysis.

	Total Variance Explained		
	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	60.349	77.371	77.371
2	4.187	5.368	82.739
3	3.171	4.066	86.804
4	2.995	3.839	90.643
5	2.198	2.818	93.461
6	1.559	1.999	95.460
7	1.054	1.351	96.811
8	0.955	1.224	98.035
9	0.535	0.685	98.720
10	0.302	0.387	99.108
11	0.221	0.283	99.391
12	0.165	0.211	99.602
13	0.124	0.159	99.762
14	0.080	0.103	99.865
15	0.056	0.072	99.936
16	0.028	0.035	99.972
17	0.013	0.016	99.988
18	0.006	0.008	99.995
19	0.004	0.005	100.000

Plotting PC1 (77%) and PC2 (5%) explained 82% of total variance showed vectorial importance of the morphometric characters for population differentiation. The characters such as 2_5, 3_7, 4_9, 2_8 were important for the detected population differentiation.

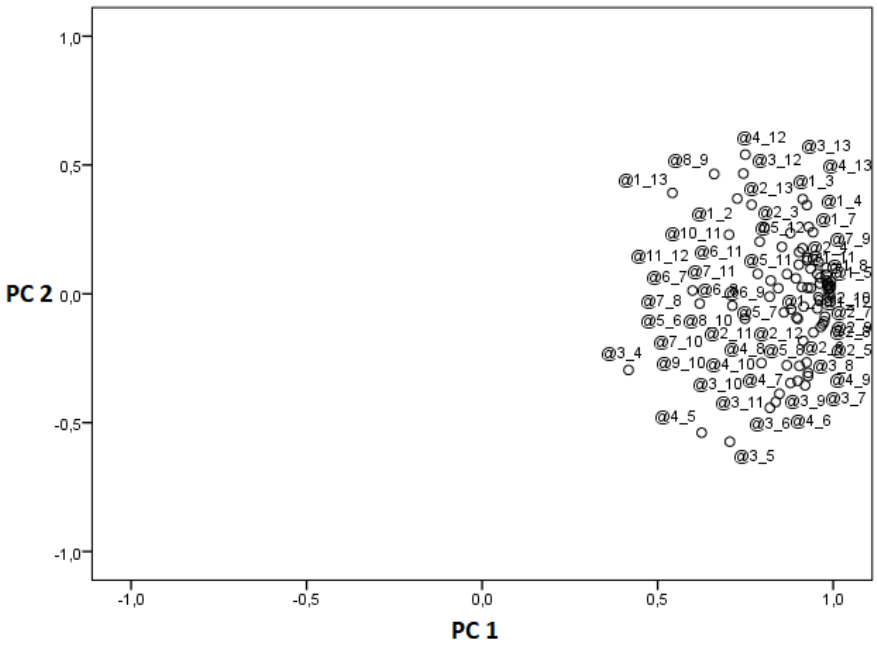

Figure 3. Contribution of morphometric characters to the first two principal components for population differentiation.

In Discriminant Function Analysis (DFA), only one discriminant function was produced and 100% of the between group variation was explained in the first DF. The overall random assignment of individuals into their original populations was high that the proportion of 95 % of cross-validated grouped cases correctly classified into their original group, showing a clear separation. 86.4% of cross-validated grouped individuals of Arsuz population was classified into its original population and 13.6% were classified into Samandag population (Table 2). 100% of cross-validated grouped individuals of Samandag population was classified into its original population.

Table 2. Cross-validated grouped individuals of populations classified into its original population.

		Predicted Group Membership	
Samples		Arsuz	Samandağ
Arsuz	Numbers	22	3
Samandağ		0	25
Arsuz	%	86.4	13.6
Samandağ		0	100

Discussion

In present study, highly significant morphometric variation was detected between populations of *Liza ramada* from Arsuz and Samandag coasts. The detected pattern of phenotypic discreteness also suggests a direct relationship between the extent of phenotypic divergence and geographic separation, indicating that geographic separation is a limiting factor to migration among stocks.

Environmentally induced phenotypic variation, however, may have advantages in the stock structure analysis of exploited species, especially when the time is insufficient for significant genetic

differentiation to accumulate among populations. However phenotypic markers may detect morphological differentiation due to environmental differences in the habitats of partially-isolated stocks, which may be a practical level of partitioning among self-recruiting stocks. Such self-recruiting stocks may react independently to exploitation (Begg &Waldman, 1999). Morphometric analysis could thus be a first step to investigating the stock structure of species. Therefore, in the future studies using genetic markers may reveal genetic heterogeneity among the populations which support the detected phenotypic differentiation.

Comparison of the morphometric characters among samples (Figure 3) revealed that body height measurements play an important role for population differentiation. The differences between these characters may be resulted from environmental factors such as over fishing and/or pollution.

In this present study, the findings reveal the potential power of morphometric network system generated by BioMorph for identification of phenotypic stocks of *L. ramada*. An unbiased network of morphometric measurements over two-dimensional outline of *L. ramada* 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.

Consequently, morphometric characters suggest high phenotypic differentiation between *L. ramada* stocks from Arsuz and Samandag coasts. However, it should be emphasized that application of a genetic techniques would be very beneficial to support the detected phenotypic variation.

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