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Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters

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Morphologic differentiation among stocks of Mediterranean horse mackerel, *Trachurus mediterraneus*, throughout the Black, Marmara, Aegean and Eastern Mediterranean Seas, was investigated using morphometric and meristic characters. Discriminant function analysis of both morphometric and meristic characters suggested that there is restricted migration of mackerel among the adjacent seas. Overlapping of four Black sea samples on the discriminant space in morphometric and meristic characters suggested that there is one self-recruiting population in the area. The Marmara sea samples were the most isolated samples from all others for both morphometric and meristic characters, which may indicate existence of a distinguishable mackerel stock in the area. The sample from the Aegean Sea was grouped with one geographically close Mediterranean sample based on morphometrics, and separated from all other Mediterranean samples based on meristic characters, suggesting some degree of intermingling between these areas. Examination of the contribution of each morphometric variable to canonical functions indicated that differences among samples seemed to be associated with the anterior part of the body. In meristic analyses, highest contributions to canonical functions were associated with the number of gill rakers and pectoral fin rays.

Keywords: Mediterranean horse mackerel, meristics, morphometrics, stock identification, *Trachurus mediterraneus*.

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Introduction

Mediterranean horse mackerel, *Trachurus mediterraneus* (Steindachner 1868), is one of the most important fishing resources in the Mediterranean Sea. This species is a semi-pelagic carnivore fish distributed throughout the Mediterranean, Marmara and Black Seas, and along the eastern Atlantic coast from Morocco to the English Channel (Smith-Vaniz, 1986; Fischer *et al.*, 1987). The contribution of *Trachurus mediterraneus* to local fisheries differs in each sea; 54% of the total catch in the Black Sea (2919 t), 39% in the Marmara Sea (562 t), 4% in the Aegean Sea (247 t) and 3% in the Northeastern Mediterranean Sea (272 t) (DIE, 2001). The total catches of *T. mediterraneus* have drastically declined to nearly zero in the Black and Marmara Seas in 1988 due to overfishing in previous years (DIE, 2001).

For effective fishery management and implementation of worthwhile stock rebuilding programs, knowledge of stock structure, distribution of fishing effort and mortality amongst the various components are essential, since each stock must be managed separately to optimize their yield (Grimes *et al.*, 1987; Carvalho and Hauser, 1994; Begg *et al.*, 1999). Poor understanding of the fish and fishery management can lead to dramatic changes in the biological attributes and productivity of a species (Altukhov, 1981; Ricker, 1981; Smith *et al.*, 1991).

The potential capacity of populations to adapt and evolve as independent biological entities in different environmental conditions is restricted by the exchange of individuals between populations. A sufficient degree of isolation may result in notable phenotypic and genetic differentiation among fish populations within a species, which may be recognizable as a basis for separation and management of distinct populations. Morphological characters, such as body shape and meristic counts, have long been used to delineate stocks (Heincke, 1898), and continue to be used successfully (Villaluz and Maccrimmon, 1988; Haddon and Willis, 1995; Silva, 2003). Variation in such characters was assumed to be entirely genetic in early studies (Heincke, 1898; McQuinn, 1997), but is now known to have both environmental and genetic components (Foote *et al.*, 1989; Robinson and Wilson, 1996; Cabral *et al.*, 2003). Studies of morphologic variation among populations continue to have an important role to play in stock identification, despite the advent of biochemical and molecular genetic techniques which accumulate neutral genetic differences between groups (Swain and Foote, 1999). Morphometric and meristic are the two types of morphologic characters that have been most frequently employed to delineate stocks of fish. Morphometric characters are continuous characters describing aspects of body shape. Meristic characters are the number of discrete, serially repeated, countable structures that are fixed in embryos or larvae.

The fishing effort is different among the adjacent seas of occurrence of *T. mediterraneus,* being considerably higher in the Black Sea (5160, total number of fishing vessel; DIE, 2001) and lower in the Northeastern Mediterranean Sea (1574). Therefore, the evaluation of the exchange of individuals between these North and South coastal areas would be particularly important for fisheries management purposes.

There is currently no knowledge of horse mackerel stock structure among fishing areas of the Eastern Mediterranean Sea. This study aims to investigate the morphologic population structure of *T. mediterraneus* based on morphometric characters using truss network system and meristic characters from Turkish territorial waters.

Material and methods

Trachurus mediterraneus were collected by commercial fishing vessels from ten fishing ports in the Black, Marmara, Aegean, and Northeastern Mediterranean Seas during the feeding season (Table 1; Figure 1). Following capture, samples were placed individually into plastic bags and were kept deep-frozen (−20°C) for transport to the laboratory. Thirty animals were collected at each site, based on Reist's (1985) recommendation that at least 25 animals be used for morphological analyses. The age of fish was determined by identifying and counting annuli of otoliths (Waldron and Kerstan, 2001).

Multivariate analyses

A stepwise multivariate discriminant analysis was used separately for morphometric and meristic data to identify the combination of variables that best separate *T. mediterraneus* samples, since predictive ability of morphometric and meristic characters are statistically different (Ihssen *et al.*, 1981; Hair *et al.*, 1996).

In morphometric analyses, allometric growth, i.e. heterogeneity in body size among samples, can result in heterogeneity of shape without providing information on differences in body proportions among populations (Reist, 1985). In the present study, there were significant correlations with body length for morphometric and meristic characters. Therefore, transformation of absolute measurements to size-independent shape variables was the first step of the analyses. Size-dependent variation for morphometric and meristic characters was removed using the formula by Elliott *et al.* (1995):

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

where M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_o the standard length of fish, and L_s the overall mean of standard length for all fish from all samples for each variable. The 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 specimens. Correlation coefficients between transformed variables and standard length were calculated to check if the data transformation was effective in removing the effect of size in the data.

Table 1. Sampling details of *T. mediterraneus* used in this study. MSL, mean standard length (cm). Standard deviations of MSL are given in brackets.

Sample	Abbreviation	Date of capture	Age	Sampling gear	MSL
Black Sea (Trabzon)	BS1	21 March 2002	1	Purse seiners	12.3 (1.07)
Black Sea (Ordu)	BS2	24 December 2002	1+	Purse seiners	10.8 (0.7)
Black Sea (Sinop)	BS3	12 February 2002	1	Purse seiners	13.21 (0.7)
Black Sea (Zonguldak)	BS4	25 February 2002	1	Gill nets	10.0 (0.9)
Marmara (Istanbul)	MS1	11 October 2002	1+	Purse seiners	12.3 (1.0)
Marmara (Çanakkale)	MS2	20 April 2002	1	Purse seiners	14.2 (0.5)
Aegean Sea (Izmir)	AS	03 December 2002	1+	Purse seiners	11.5 (0.4)
N. Mediterranean (Antalya)	NMS1	16 December 2002	1+	Gill nets	14.9 (0.9)
N. Mediterranean (Mersin)	NMS2	10 December 2002	1+	Purse seiners	15.1 (1.0)
N. Mediterranean (Iskenderun)	NMS3	15 December 2002	1+	Gill nets	16.1 (0.8)

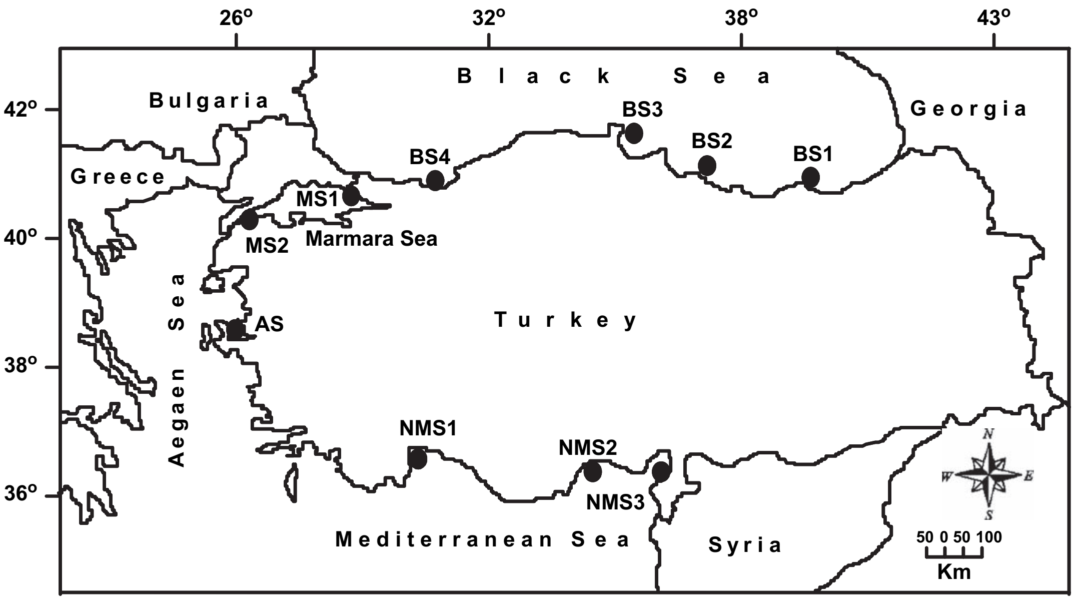

Figure 1. The map of the sampling of *T. mediterraneus*. ● indicates sampling location.

The degree of similarity among samples in the overall analysis and relative importance of each measurement for group separation were assessed by stepwise discriminant function analysis (DFA) with cross-validation or jackknifed classification. Population centroids with 95% confidence ellipses derived from the DFA were used to visualize relationships among the individuals of groups. Mahalanobis distance between centroids and its associated probability were also evaluated. Morphometric and meristic characters were analyzed together in multivariate analysis of variance (MANOVA) to test the significance of differences among groups. *Post hoc* multiple comparison tests using least significance difference (LSD; $\alpha = 0.05$) were also performed to identify the number of significant morphometric and meristic characters between pairs of locations. The relative importance of morphometric and meristic characters in discriminating populations was assessed using the *F*-to-remove statistic. Collinearity among variables used in the discriminant function model was evaluated using the tolerance statistic.

Morphometric variables

Truss measurements were made on specimens by collecting X−Y co-ordinate data for morphological landmarks. Fish were thawed, placed on their right side on acetate sheets on which body posture and fins were teased into a natural position. Each landmark was obtained by piercing the acetate sheet with a dissecting needle, defining 14 landmarks (Figure 2). Additional data, such as eye diameter (EYE), head width (HW) and pectoral fin length (PFL), were also recorded. Only undamaged fish were included in the analyses.

Meristics

Numbers of unbranched and branched rays in the first and second dorsal (DFR1, DFR2), first and second anal (AFR1, AFR2), pectoral (PFR), ventral (VFR) fins and gill rakers on the upper limb and lower limb of the first gill arch (GRN) were obtained under a binocular microscope.

Results

Morphometrics

None of the standardized truss measurements showed significant correlation with standard length. These indicate that the effect of body length had been successfully removed with the allometric transformation. The effect of sex on the truss measurements was not tested since horse mackerel do not exhibit sexual dimorphism (Shaboneyev and Kotlyar, 1979; Borges, 1996) and samples were collected during the feeding season. Univariate statistics (ANOVA) revealed highly significant ($P < 0.001$) differences among locations from 32 out of 33 truss measurements. The length of the first dorsal fin measurement was not significantly different among locations.

In DFA, the first DF accounted for 45% and the second accounted for 30% of the between-group variability, showing clear between-sample differentiation (Figure 3). Black Sea samples (BS1, BS2, BS3 and BS4) together with

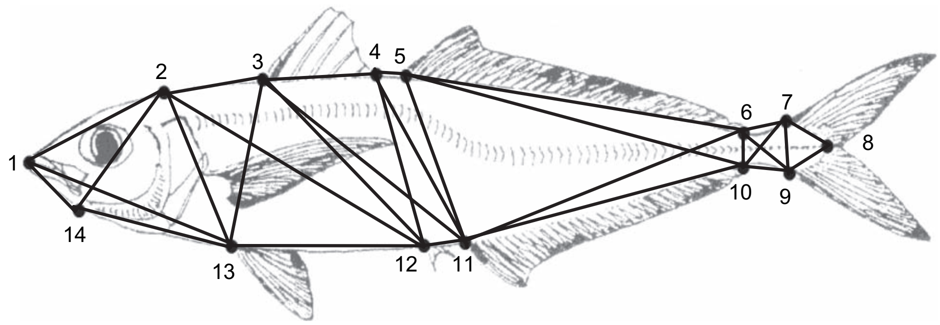

Figure 2. Locations of the 14 landmarks defining the truss network on *T. mediterraneus*.

one of Marmara Sea samples (MS2) formed one group. Another Marmara sea sample (MS1) was highly isolated from all other samples in discriminant space, which may indicate existence of a distinguishable mackerel stock in the Marmara Sea. The Aegean Sea sample (AS) was grouped together with one geographically close Mediterranean sample (NMS1), suggesting a sufficient degree of intermingling of mackerel between these areas. The other Mediterranean samples (NMS2 and NMS3) overlapped each other and were differentiated from all other samples.

The overall assignment of individuals into their original sample by DFA was 57% (Table 2). Most misassignments within groups were observed between the Black sea samples. The proportion of correctly classified Marmara sample (MS1) to their original group was highest (88%), showing a clear separation from all others. A multivariate ANOVA (MANOVA) using morphometric and meristic characters together revealed highly significant inter-sample variation (Pillai's trace = 4.01, $P < 0.001$).

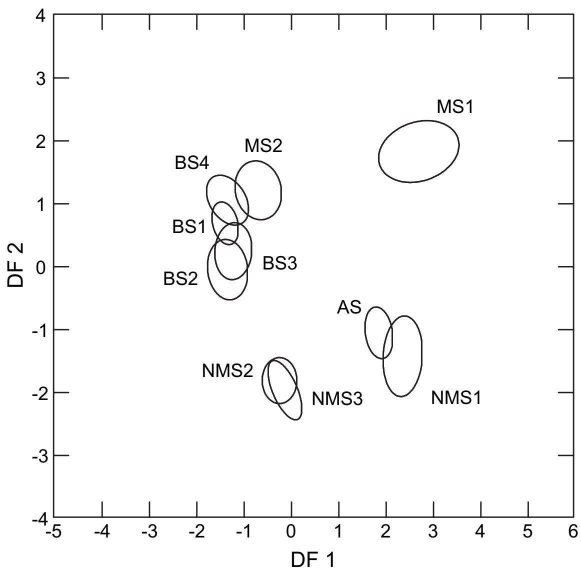

Figure 3. Ninety-five percent confidence ellipses of DFA scores for morphometric analysis.

Examination of the contribution of each variable to the first canonical function showed high contributions from measurements 1_2, HW, 1_14, EYE and 2_14 (head of fish) (Figure 4). The contribution of variables to the second canonical function was also mostly from the measurements taken from anterior part of body 3_11, 6_10, 3_4, 2_12.

Meristics

The range of the meristic counts of the *T. mediterraneus* samples are given in Table 3. After the allometric transformation, the correlation results revealed that all of the meristic variables studied were free from the influence of size. Univariate comparisons between populations were highly significant ($P < 0.001$) for 5 out of 7 meristic characters. AFR1 and VFR were not considered in the analysis because these variables were constant among groups. High tolerance statistics were observed for the five meristic characters (0.913−0.997) and *F*-to-remove values from the discriminant model were also high for these characters.

The first DF accounted for 44% and the second accounted for 42% of the between-group variability (Figure 5). As observed in morphometric data, the Black Sea samples (BS1, BS2, BS3 and BS4) formed a group with the Marmara sea sample (MS2). However, the other Marmara

Table 2. Percentage of individuals classified correctly into their original group for morphometric characters. Samples are identical to those defined in Table 1.

Sample	BS1	BS2	BS3	BS4	MS1	MS2	AS	NMS1	NMS2	NMS3
BS1	**34**	13	20	20	3	10	0	0	0	0
BS2	17	**50**	20	10	0	3	0	0	0	0
BS3	17	20	**31**	13	0	10	3	0	3	3
BS4	13	10	17	**40**	0	20	0	0	0	0
MS1	3	0	0	3	**88**	3	3	0	0	0
MS2	7	7	7	23	7	**49**	0	0	0	0
AS	0	3	0	0	0	0	**63**	20	7	7
NMS1	0	0	0	0	0	0	17	**66**	7	10
NMS2	0	3	0	0	0	0	3	3	**71**	17
NMS3	0	0	0	0	0	0	3	7	13	**77**

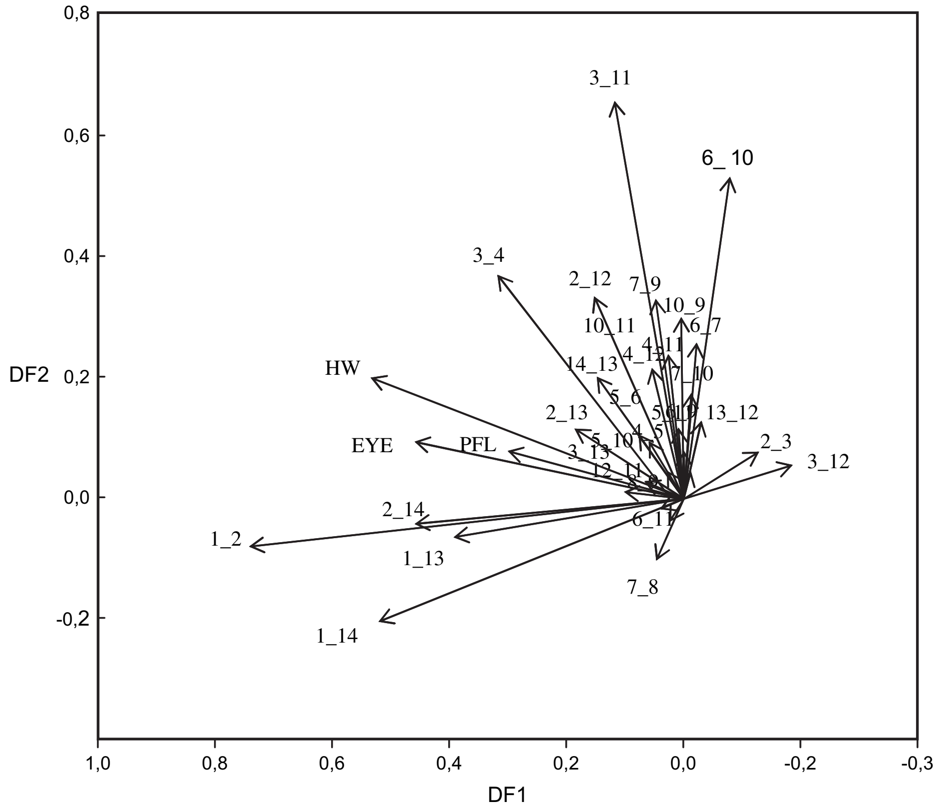

Figure 4. Contribution of morphometric variables to the canonical functions. Vectors indicate the loadings of the scores for each variable on the first two discriminant functions.

sea sample (MS1) was clearly different from all other samples. The Aegean sea sample was isolated from all other samples, but close to the Mediterranean samples (NMS1, NMS2, NMS3), which were positioned together as one group in the discriminant space.

A correct classification of individuals into their original population varied between 31% and 91% by canonical analysis and 58% of individuals could be classified into their correct *a priori* grouping (Table 4). The highest reclassification rate was shown by individuals from the MS1 (91%) and AS (87%) samples, the most clearly isolated groups.

Examination of the contribution of each meristic character to the first and second canonical functions revealed that the gill raker numbers and pectoral fin rays contributed heavily to the meristic differentiation (Figure 6).

Table 3. Range of the meristic counts of *T. mediterraneus* samples.

Sample	DFR1	DFR2	AFR1	AFR2	PFR	VFR	GRN
BS1	7–8	28–33	2	27–31	20–23	6	51–62
BS2	7–8	28–33	2	25–30	19–20	6	52–60
BS3	7–8	27–32	2	23–29	18–21	6	51–55
BS4	7–8	28–32	2	25–28	19–22	6	50–58
MS1	7–9	26–31	2	24–28	17–18	6	51–54
MS2	7–8	27–32	2	24–30	20–23	6	50–56
AS	7–8	27–32	2	26–29	20–21	6	57–61
NMS1	7–8	27–35	2	21–30	19–23	6	52–59
NMS2	7–8	28–35	2	22–32	20–23	6	51–58
NMS3	7–9	28–35	2	21–32	20–21	6	54–60

Discussion

Morphometric and meristic characters showed significant phenotypic heterogeneity among Mediterranean horse mackerel samples. The results of DFA suggest at least three phenotypically distinct local populations. The differentiation may suggest a relationship between the extent of phenotypic divergence and geographic distance, indicating that migration among populations of the Black, Marmara, Aegean and Northeast Mediterranean Seas may be limited. There are notable differences among the regions in environmental factors such as temperature, salinity, food availability, and fishing intensity (DIE, 2001).

The samples from the Black Sea (BS1, BS2, BS3, BS4) were similar to each other for both morphometric and meristic characters. There may be enough mixing among these locations to prevent differentiation.

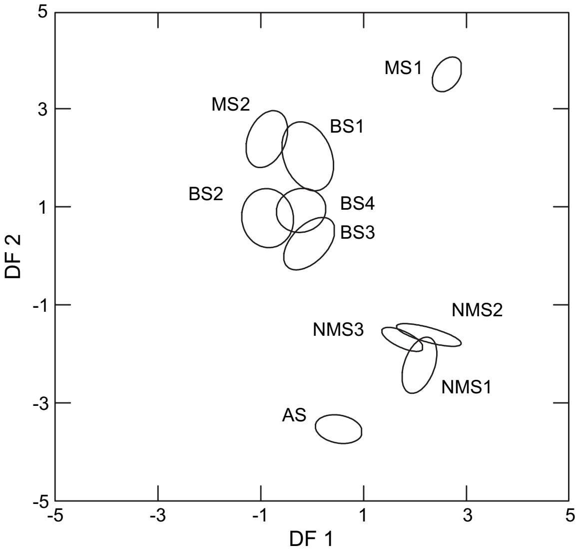

Figure 5. Ninety-five percent confidence ellipses of DFA scores for meristic analysis.

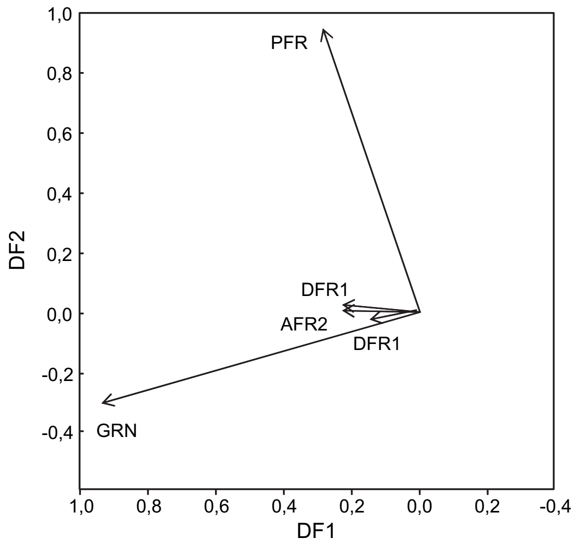

Figure 6. Contribution of meristic variables to the canonical functions. Vectors indicate the loadings of the scores for each variable on the first two discriminant functions.

The strong morphometric and meristic differentiation of the Marmara sample (MS1) suggests that there may be a self-recruiting population or sub-species of horse mackerel in the Marmara Sea. The closed geographic structure and environmental discreteness of the Marmara Sea, such as warm temperature and low salinity may lead to isolation of the population. On the other hand, one of the Marmara Sea sample (MS2) was similar to the Black Sea samples for both morphometric and meristic analyses, which may be attributable to possible inadvertent sampling of migratory Black Sea mackerel in the Marmara.

The Aegean sea sample was morphometrically similar with samples from one of the nearby Mediterranean locations. However, meristic data did not support the homogeneity between Aegean and Mediterranean mackerel samples. The disparity between morphometric and meristic data suggests that meristic characters may be more influenced by local environmental conditions, which increases their differentiation at small geographic scales. Alternatively, there may be some migration between these areas and the meristic data are more sensitive to detect low number of migrants between the areas (Fahy, 1983; Lindsey, 1988; Hulme, 1995).

Table 4. Percentage of individuals classified correctly into their original group for meristic characters.

Sample	BS1	BS2	BS3	BS4	MS1	MS2	AS	NMS1	NMS2	NMS3
BS1	**63**	3	3	13	0	17	0	0	0	0
BS2	7	**63**	10	17	0	3	0	0	0	0
BS3	3	10	**70**	17	0	0	0	0	0	0
BS4	13	23	17	**31**	0	7	0	3	3	3
MS1	3	0	0	3	**91**	3	0	0	0	0
MS2	17	6	0	10	0	**67**	0	0	0	0
AS	0	0	0	0	0	0	**87**	7	3	3
NMS1	0	0	0	0	0	0	3	**71**	13	13
NMS2	0	0	0	0	0	0	0	13	**70**	17
NMS3	0	0	0	0	0	0	0	15	17	**68**

The Mediterranean samples (NMS2, NMS3) from Iskenderun Bay were clustered together for both morphometric and meristic analyses. The consistent differentiation of these samples in morphometric and meristic analyses may support the existence of Iskenderun Bay mackerel population and lack of intermingling between the Iskenderun Bay and the other populations.

The pattern of high inter-sample variation may indicate reproductive isolation between local populations that would confirm the genetic basis of observed morphometric differentiation among samples. However, fish are known to exhibit a large component of environmentally induced morphological variation (Allendorf, 1988; Wimberger, 1992), which might reflect different feeding or developmental environment. Therefore, environmental factors such as temperature, salinity, food availability or prolonged swimming may determine the phenotypic differentiation in horse mackerel, especially in the Black, Marmara and Mediterranean populations which experience specific environmental conditions (Artuz, 1992; Komakhidze *et al.*, 1998). Thus, significant morphological differences do not necessarily demonstrate restrictions of gene flow among populations, though they do suggest that fish in each group may not mix extensively. As morphology is especially dependent on environmental condition during early life-history stages (Ryman *et al.*, 1984; Cheverud, 1988), morphological differentiation may indicate that the majority of fish spend their entire lives in separate regions.

Discriminant function analyses indicated that morphometric differentiation between the samples was largely due to differences in the head characters of fish, which may reflect differential habitat use. Relative head length may be related to prey size (Gatz, 1979). Eye diameter, which also contributed heavily to regional differentiation, may reflect differences in turbidity (Matthews, 1988). Moore (1950) described increases in cutaneous sense organs to compensate for reduced eye diameter in cyprinids adapted to turbid streams in North America.

At present, *T. mediterraneus* has been treated as a single stock in Turkish territorial waters. From a management viewpoint, the present morphometric and meristic analyses suggest that there may be some population structuring of horse mackerel populations among three areas, the Black Sea, Marmara Sea and Northeast Mediterranean Sea (Iskenderun Bay), including Aegean Sea. The management implications depend on the extent to which such structuring persists over time. If the phenotypic differences found are not accidental, then they should be repeatable in further analyses. Consistent differences from repeated analyses between two groups of fish may indicate their temporal and spatial integrity. Persistence would warrant separate management, because any depletion in one of these stocks is unlikely to be compensated by immigration from other units, at least at a sufficiently rapid rate.

For a reliable identification of a stock a variety of methods should be used, since different methods may produce different results (Fournier *et al.*, 1984; Shaw *et al.*, 1999). The results from this work should be compared with data obtained from other phenotypic and genetic studies to confirm stock identity. For example, otolith chemistry is increasingly used as a natural tag or chemical signature that reflect differences in the chemical compositions of the individual's habitat, and assess relative contribution of the different nursery areas to mixed adult stocks (Campana and Thorrold, 2001; Rooker *et al.*, 2003).

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