

Use of a Discriminant Function for the Morphometric and Meristic Separation of Whiting Stocks, *Merlangius merlangus euxinus*, along the Turkish Black Sea Coast

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Abstract: Stock differentiation studies of whiting, *Merlangius merlangus euxinus*, from the Turkish Black Sea coast were carried out using morphometric and meristic characters and applying the generalised distance of Mahalanobis. Fifteen morphometric measurements, together with eleven meristic characters, were used in the analyses. Insufficient differences ($P>0.01$) in general phenotypic and genotypic characteristics implied the existence of a single unit stock. Samples taken in different periods showed lower similarity than those taken in the same periods.

Key Words: whiting, *Merlangius merlangus euxinus*, morphometrics, meristics, stock differentiation

Karadeniz'in Türkiye Kıyılarında Bir Ayırışım Fonksiyonu Kullanarak Mezgit *Merlangius merlangus euxinus* Stoklarının Morfometrik ve Meristik Ayırımı

Özet: Karadeniz'in Türkiye kıyılarında mezgitlerde (*Merlangius merlangus euxinus*), morfometrik ve meristik verilere Mahalanobis'in genelleştirilmiş aralığını uygulayarak stok ayırımı çalışması yapılmıştır. Analizlerde, 11 meristik ve 15 morfometrik ölçüm kullanılmıştır. Genel fenotipik ve genotipik özelliklerdeki farklılıkların azlığı ($P>0.01$) tek bir stoğun bulunduğunu göstermiştir. Farklı peryotlarda alınan örnekler, aynı peryotta alınanlara göre daha az benzerlik göstermiştir.

Anahtar Sözcükler: mezgit, *Merlangius merlangus euxinus*, morfometrik karakterler, meristik karakterler, stok ayırımı

Introduction

The differences in the morphometric and meristic characters of a species between regions may result from differences in genotype, or environmental factors operating on one genotype, or both of these acting together (1). While both morphometric and meristic characters respond to changes in environmental factors, their responses are different in some situations. The meristic characters, e.g., numbers of vertebrae and keeled scales, are fixed in the early embryonic stage of the individual, which is a short period of the life span, and afterwards remain unchanged. On the other hand, morphometric characters are not sensitive to short-term, local fluctuations, and reflect average differences over long periods between environmental factors in different areas (1).

Morphometric and meristic characters have often been used in discrimination and classification studies by statistical techniques, e.g., Sharp et al. (2), Pope & Hall (3), Jahnson & Barnett (4), Burd (5), Gamble (6,7), Boonstra (8), Agnew (9) and Avsar (10).

The whiting, *Merlangius merlangus euxinus*, is a common gadoid fish in the North-eastern Atlantic, the Mediterranean (the Aegean Sea and the Adriatic), the Black Sea and adjacent parts of the Azov Sea (11,12,13). Earlier studies on biology, distribution and population parameters of whiting in the Black Sea were carried out by Burdak (14), Prodanov (15,16) and Ivanov & Beverton (17). Studies conducted around the Turkish Black Sea coast are on the distribution, abundance and stock assessment of whiting, e.g., Akşiray (18); Kutaygil & Bilecik (19); DBT-DBE (20); SUAE (21); Uysal (22) and

Bingel et al. (23, 24). There are no data on differentiation of whiting stocks along the Turkish Black Sea coast.

Similarities or dissimilarities and geographical distribution of the stocks are important for the achievement of a rational exploitation. However, the fisheries along the Turkish Black Sea coast are currently managed as one unit, i.e., controls on mesh size, fishing effort, seasonal fishing time and minimum saleable sizes are applied in a blanket policy, without regional distinction. Rational exploitation depends on determining the number of unit stocks present. In this study, morphometric and meristic discrimination of whiting was carried out along the Turkish Black Sea coast.

Materials and Methods

Samples were collected from 66 stations located along the continental shelf of the Turkish Black Sea coast (Figure 1). Stations were sampled using bottom trawl nets by R/V BILIM in April and September 1990. In September 1991 and October 1992, sampling was performed by R/V SURAT-1. A total of 6067 individuals was examined (Table 1).

In poor hauls, the total catch was considered as a single sample for further analysis. For larger catches, subsampling was carried out according to the procedure described by Holden & Raitt (25). The samples were preserved in solutions of 4% formaldehyde buffered with borax.

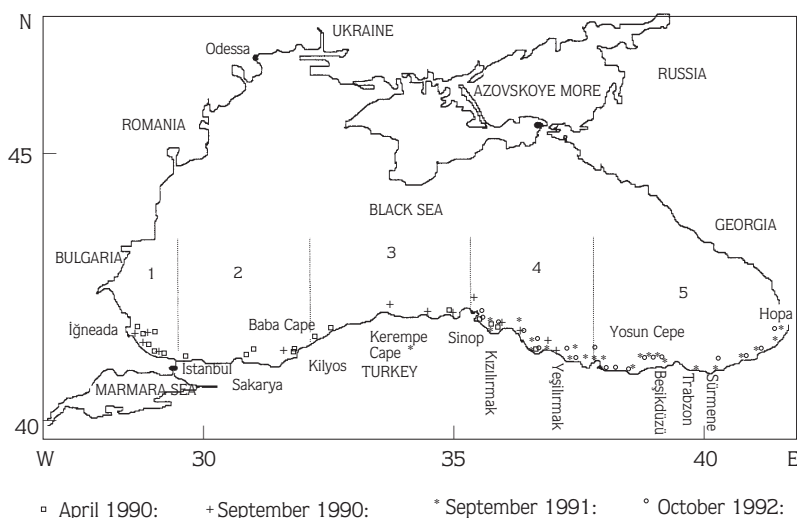


Figure 1. Locations of the sampling stations along the Turkish Black Sea coast.

Sampling Period	Male		Female		Total
	min-max	n	min-max	n	
April 1990	53-193	675	62-230	771	1446
September 1990	74-203	656	71-228	723	1379
September 1991	78-221	767	83-293	905	1672
October 1992	90-258	634	85-288	936	1570
Total	53-258	2732	62-293	3335	6067

Table 1. The minimum and maximum total length measurements (mm) of whiting and number of individuals analysed in each sex and in all sampling periods.

Morphometric measurements were made using a measuring board and calipers. Overall length measurements were made with the fish lying on its right side, snout to the left, on a measuring board consisting essentially of a wooden piece with a centre scale and a headpiece (nose block) against which the snout is gently pressed. The mouth was closed, the fish body and tail were straightened along the mid-line and the reading was taken from the scale. Longitudinal, vertical and lateral measurements other than overall length were made using a caliper as a straight line between perpendiculars along the median vertical and lateral body axes. Fifteen length measurements were taken from each fish (Figure 2). All morphometric measurements were determined to the nearest millimetre.

Eleven meristic characters consisting of gill rakers, gill filaments, vertebrae and all fin rays (dorsal-D1, D2, D3; anal-A1, A2; caudal, pectoral and pelvic fin) were counted after all morphometric measurements were taken. The counts were made using a stereozoom microscope (magnification 10x). To count gill rakers and gill filaments, the first gill arch under the left operculum was removed using a scalpel and forceps and put in a petri

dish containing water. The vertebrae were removed by cutting with a scalpel from the posterior side to the anterior margin. The vertebral column was then cleaned of the remains of flesh and was counted immediately. The fin rays were counted again. The rays on the paired fins were counted on one side only.

For the discrimination of morphometric and meristic characteristics, the generalised distance function analysis developed by Mahalanobis et al. (26) was used. This form of multivariate analysis gives a measure of distance between pairs of groups in units of standard deviation (27,28) and a mean for determining whether two groups represent discrete stocks or the same stock. The quantitative separation of the whiting stocks was made by using the equation given by Weber (26):

$$D^2 = (b_1d_1 + b_2d_2 + b_3d_3 + b_4d_4 + \dots + b_md_m)/m$$

where m is the number of measured and counted characters, D^2 the generalised Mahalanobis distance, b_i the discriminant functions of the i^{th} measurement and d_i the mean difference of the i^{th} measurement. A significance test described by Sneath & Sokal (29) was used to qualify the statistical difference between two groups.

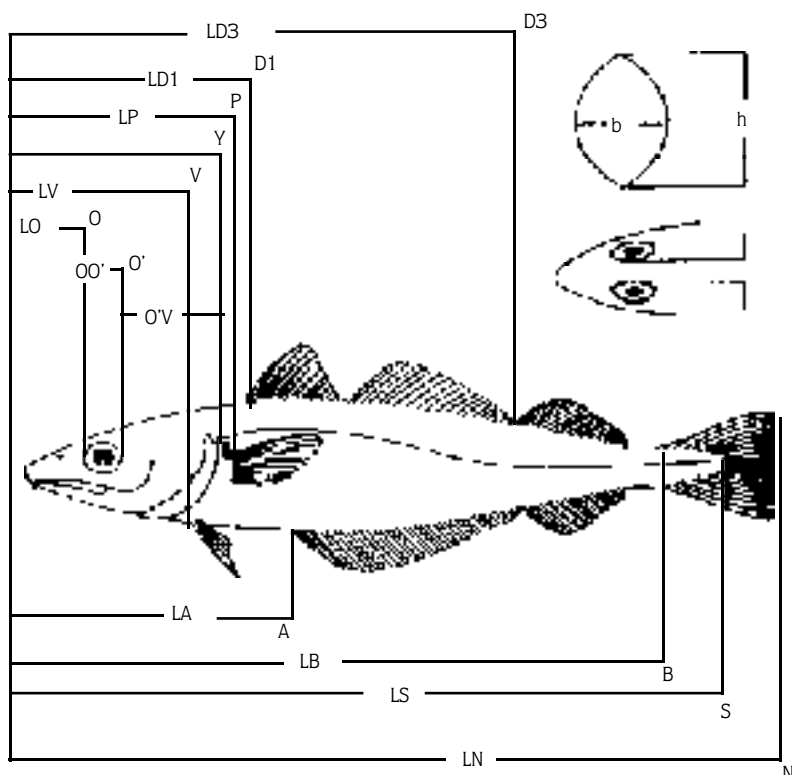


Figure 2. Measured morphometric characteristics of *Merlangius merlangus euxinus*. LN, Total length; LS, Standard length; LB, Body length; LO, Preorbital length; LY, Head length; OO', Orbital diameter, O'Y, Preorbital distance; LP, Prepectoral distance; LV, Preventral distance; LD1, Preanterior dorsal distance; LD3, Last dorsal distance; LA, Preanal distance; h, Greatest depth; b, Greatest breadth; OO, Interorbital distance.

Arrangement of the data for discriminant analysis

Before running the discriminant analysis, morphometric measurements should be standardised either by directly multiplying or dividing by size or by any measurable character related to length (30). In the present study, there were considerable differences between the minimum and maximum total lengths of both sexes in each sampling period, therefore, morphometric measurements were standardised by dividing with the eviscerated weight.

Similarly, there were obvious differences between the sex and age compositions of the samples and, especially, between the sex compositions of age group III and higher age groups in all regions. The difference between the sex ratios of age groups I and II was smaller than in age group III and higher (Table 2). Therefore, comparison of the samples for the discrimination was meaningless prior to standardisation of the data set. In general, males and females of a fish species show different growth characteristics (25) and individuals of a species exhibit different annual growth characteristics from one age to another, and successive cohorts may grow differently depending on environmental conditions (31), and generalised distance (D^2) increased with increasing differences in total lengths (28). Therefore, it was

assumed that such differences either between age groups or sexes will much affect the results of discriminant analysis. To eliminate age-influenced size differences for each morphometric measurement and prevent any error which might result from the differences in percentage ratios of males and females, the number of individuals of the same age group in each sex were equalised.

Selection of the groups compared

The whiting, which is a cold-water demersal species distributed throughout the continental shelf in the Black Sea, has pelagic eggs. Reproduction takes place the whole year round with a maximum from September to March. They do not need to migrate to wintering grounds. In winter, mature fish move into the deeper part of their range (i.e., 10 to 130 m) for spawning. The youngest and smallest fishes (post larvae, age 0+) are found in summer in the coastal waters and in the surface layers down to 65 m over depths of 1000-2000 m of the open sea (17). The distribution and orientation of the eggs and larvae are much affected by the existing current system in the area. The fish eggs and larvae may be transported and dispersed long distances and over large areas by currents (32). All the features mentioned above were considered, and sampling stations were grouped into 5 regions to obtain reasonable results from discriminant analysis. The

Table 2. The numbers (n) and differences in percentage ratios (%) of males and females of whiting in different sampling periods and age groups in five sampling areas.

Sampling periods	REGIONS														
	1			2			3			4			5		
	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%
APR 1990	155	138	6	277	339	10	85	109	12	158	185	8	-	-	-
SEP 1990	180	202	6	48	45	4	129	125	2	299	351	8	-	-	-
SEP 1991	-	-	-	-	-	-	-	-	-	282	358	12	485	547	6
OCT 1992	-	-	-	-	-	-	-	-	-	302	418	16	332	518	22
Agegroup															
I	242	215	6	135	178	6	128	119	4	771	741	2	482	445	4
II	86	113	6	173	167	2	82	96	8	262	445	26	305	372	10
III	5	22	62	16	32	34	4	12	50	3	120	96	33	186	70
IV	0	1	100	0	8	100	0	7	100	0	9	100	2	34	88
V	-	-	-	-	-	-	-	-	-	0	2	100	1	15	88
VI	-	-	-	-	-	-	-	-	-	-	-	-	0	7	100
VII	-	-	-	-	-	-	-	-	-	-	-	-	0	5	100
IX	-	-	-	-	-	-	-	-	-	-	-	-	0	2	100

April 1990 and September 1990 samples were collected from between the first and fourth regions while the September 1991 and October 1992 samples were collected from the fourth and fifth regions (Figure 1).

The first region was chosen as the area from the Bulgarian-Turkish border to the Bosphorus entrance of the Black Sea since the more saline deep waters, originating from the Mediterranean, flow along the Bosphorus and the rim current bifurcates to form an anticyclonic eddy along the western side of the Bosphorus entrance (33). This more saline water and anticyclonic eddy might be considered the oceanographic barrier along the eastern border of this group. The second region is located between the Black Sea entrance of the Bosphorus and Zonguldak. This area is influenced by anticyclonic eddies along the shelf in the Sakarya region (33) and by the Sakarya and Kilyos rivers. The region from Cape Baba to Cape İnce may be classified as the third group, which contains the widest continental shelf towards the west side of Sinop. Analysis of hydrographic data and satellite images from 1992 showed persistent upwelling with surface temperatures as low as 12°C between Cape Baba and Cape İnce (34). In this region, the flow continues further eastwards along the coast without significant changes in its structure until offshore of Cape İnce. The region located between Cape İnce and Cape Yasun (Ordu) may be considered the fourth group. The main oceanographic conditions of the area are influenced by high freshwater flow of the Kızılırmak and

Yeşilirmak rivers. The flow going eastward forms an anticyclonic eddy to the east of the Sinop Cape and another eddy located off the Yeşilirmak-Kızılırmak rivers region (33). In the region, Sinop and Samsun bays constitute the two wide continental shelves of the eastern Black Sea coast of Anatolia. The fifth group covers the region between Cape Yosun and the Georgian border. The rest of the flow continuing eastward gives the large scale anticyclonic eddy located between 37° E and 41° E longitudes along the shelf (33). The continental shelf in the region becomes narrower and deepens suddenly.

Results

Significance tests ($P > 0.01$) and generalised distance of Mahalanobis analysis, compared for the same and different sampling periods, indicate a single unit stock along the Turkish Black Sea coast (Table 3).

The generalised Mahalanobis distances between the regions for different periods were higher than those found for the same period. Fish from the same period showed discriminant values ranging from 0.0265 to 0.1506, while fish from different periods showed values ranging from 0.0632 to 0.7701 (Table 3). This may be due to the differences in the growth rates, which are influenced by seasonal and annual differences in the environmental conditions between the regions.

Tablo 3. Generalised Distance of Mahalanobis values compared for the same and different sampling period and their significance test results. The Generalised Mahalanobis Distance are given above the diagonal and the results of the significance test below the diagonal. (-) No significant difference at $P > 0.01$.

GROUP		APR90	APR90	APR90	APR90	SEP90	SEP90	SEP90	SEP90	SEP91	SEP91	OCT92	OCT92
		I	II	III	IV	I	II	III	IV	IV	V	IV	V
APR 1990	I	*****	0.0623	0.1506	0.0913	0.1463	0.2622	0.1783	0.0883	0.2454	0.1429	0.1501	0.1403
	II	(-)	*****	0.0670	0.0641	0.2409	0.3442	0.2742	0.1637	0.1396	0.1777	0.2239	0.1667
	III	(-)	(-)	*****	0.0745	0.4379	0.7701	0.4374	0.2399	0.2523	0.2822	0.4273	0.3385
	IV	(-)	(-)	(-)	*****	0.2155	0.4196	0.3111	0.2039	0.1566	0.2054	0.2612	0.1944
SEP 1990	I	(-)	(-)	(-)	(-)	*****	0.0699	0.0488	0.0391	0.1061	0.0796	0.0972	0.0918
	II	(-)	(-)	(-)	(-)	(-)	*****	0.0602	0.0703	0.1927	0.1782	0.1868	0.1914
	III	(-)	(-)	(-)	(-)	(-)	(-)	*****	0.0510	0.1333	0.0911	0.1295	0.1112
	IV	(-)	(-)	(-)	(-)	(-)	(-)	(-)	*****	0.0822	0.0632	0.1064	0.0844
SEP 1991	IV	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	*****	0.0876	0.1129	0.0842
	V	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	*****	0.1438	0.0968
OCT 1992	IV	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	*****	0.0265
	V	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	*****

Discussion

The degree of discrimination, D^2 , between two groups or more is directly related to the intermingling of specimens of the considered groups (30). Mais (35) conducted a subpopulation study on Pacific sardines, *Sardinops caeruleus*, inhabiting the west coast of North America and Mexico and found that California and central Baja California stocks were closely related and undoubtedly intermingled to a considerable degree. Fish from these areas overlapped so greatly that no inference of separate stocks can be made. The D^2 values of these stocks were calculated to be 0.309-1.353. Ehrich & Rempe (28) studied morphometric discrimination between hake populations (*Merluccius productus*) from the North-east Pacific using size-independent discriminant analysis and stated that the groups which include generalised distance values D^2 ranging from 0.3 to 2.9 belong to one stock. Avsar (10) studied the stock differentiation of the sprat off the southern Black Sea coast and obtained D^2 values varying from 0.04 to 0.64. The D^2 values in the present study (Table 3) are smaller than those of the minimum D^2 values given by Mais (35), Ehrich & Rempe (28) and Avşar (10). The calculated D^2 range in the present study implied that all the Turkish Black Sea whiting collected in the 1990-92 period can be considered a single unit stock. It is worthy of note that the significance test result obtained from all subregions compared also supports this conclusion.

Relatively larger D^2 values occurred between regions for different sampling periods. This may be explained by the fluctuations in temperature and other environmental factors during the spawning and post-spawning periods and be related to differences in the growth characteristics influenced by seasonal as well as annual changes in environmental conditions. Parrish & Sharman (1) stated that the meristic characters are fixed in early embryonic life of the individual and remain unchanged thereafter. Thus they respond to environmental factors only for a short period of time, after which their values are unaffected by environmental fluctuations and the short-term local fluctuations in environmental factors may give rise to wide variations in their values amongst members of the same and different year-classes of a single unit stock. They also stated that the physiological characteristics, of which the rate and pattern of growth are the most important, are not susceptible to short-term local fluctuations, and reflect more the average

differences over longer periods between environmental factors in different areas. Villaluz & Macrimmon (36) stated that meristic characters are often subject to early phenotypic modifications by environmental variations (e.g., temperature, salinity, food).

Transportation and mixing processes (e.g., rim current) can provide an exchange of genes between the fishes inhabiting the basin of the Black Sea despite the border of the eastern and western cyclonic gyres, which could be considered an oceanographic barrier for eggs and larvae of the eastern and western Black Sea whiting. Ivanov & Beverton (16) stated that whiting is distributed over the entire continental shelf in the Black Sea and reproduction takes place the whole year round, reaching a maximum from September to March. The eggs are pelagic and spawned in batches, and the post larvae are found in summer in the coastal waters, the open sea and in the surface layers down to 65 m over depths of 1000-2000 m. Laevastu (32) mentioned that the eggs and larvae may be transported long distances and dispersed over large areas by currents. Oğuz et al. (37) described upper ocean circulation of the Black Sea by a well defined cyclonic boundary current approximately following the narrow continental slope region and a series of semipermanent anticyclonic eddies between the boundary current and the undulations of the coast. They stated that geostrophic surface currents exceeded 30 cm/s in the south-eastern corner of the Black Sea Basin. Oğuz et al. (38) found that the rim current had a width of about 50 km with a maximum current speed of about 30 cm/s in the multi-national coordinated surveys of September 1991 and the rim current along the southern coast indicated small amplitude meanders with a length scale of about 125 km. Bingel et al. (24) reported that the ADCP measurements (Acoustic Doppler Current Profiler) in April 1993 indicated a current speed of about 50 cm/s at the core of the Rim current and meandering currents were found along the south-western coast, with speeds reaching 100 cm/s and larger values. The rim current flowing along the continental slope region can transport eggs and larvae over a short time from spawning grounds to new inshore or offshore areas all along the Turkish Black Sea coast. That is, eggs and larvae of whiting may be transported in as little as about 54 days along approximately 1400 km of Anatolia shoreline at a mean current speed of 30cm/s. Hislop (39) stated that an individual female spawns in batches and its spawning

season lasts at least ten weeks (75 days). Russell (40) stated that the incubation period of the eggs based on their temperature is generally 12-15 days and that 5.5 mm length is reached within 5 days after hatching, when the postlarval stage begins. During the time periods mentioned by Hislop (39) and Russell (40), eggs and larvae may be transported and mixed either completely or partly. These processes may allow the exchange of genetic characteristics between fish inhabiting the western and eastern basins of the Black Sea and, as a result, may sufficiently dilute any differences in general phenotypic and genotypic characteristics, so as to imply the existence of a single unit stock.

Garrod & Gambell (41) conducted a study on the distinction of whiting stocks by tagging experiments in the Irish Sea and showed that whiting may move considerable distances within the Irish Sea and that various populations fished in the Irish Sea intermingled, and therefore were not completely distinguishable from

adjacent stocks. However, the migrations and distribution of whiting have not as yet been sufficiently understood to enable the stock to be evaluated (17). Besides the egg and larvae transport, the migration of adult whiting over long distances contributes to the mixing of the entire population in the Black Sea.

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