A stock differentiation study of the sprat (Sprattus sprattus phalericus Risso) off the southern coast of the Black Sea

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Abstract

Stock differentiation studies of sprat (Sprattus sprattus phalericus Risso) from the Black Sea coast of Turkey have been carried out morphometrically and meristically applying the generalised distance of Mahalanobis (GDM) and also physiologically using three-level nested ANOVA (TLNA). Fifteen morphometric measurements, together with nine meristic counts and fat concentration of wet tissues of sprat have been used in the analyses of GDM and TLNA, respectively. Insufficient differences in general phenotypic, genotypic (P > 0.01) and physiological (P > 0.05) characteristics implied the existence of a single unit stock. Samples collected in the same period showed greater similarity than those taken from the same areas in different periods.

Introduction

Variability in morphological characteristics is considered to be a result of the degree of isolation and localisation of a fish population (Blackith and Albrecht, 1959). Morphometric characteristics in direct response to environmental factors may create some differences that reflect average differences over longer periods between environmental factors in different areas. In such cases, morphometric measurements have been used widely in fisheries and particularly in stock differentiation studies (Parrish and Sharman, 1958; Amos et al., 1963; Pope and Hall, 1970, 1972; Mais, 1972; Yeh and Liu, 1973; Panhorst and Becker, 1976; Sharp et al., 1978; Ehrich and Rempe, 1980; Avsar, 1987; Avsar et al., 1988a,b, 1990).

Unlike morphometric characteristics or coloration, meristic characters are usually fixed before metamorphosis and remain constant throughout the life span of the individuals (Parrish and Sharman, 1958). Variations in meristic characteristics result from both genetic variations between populations and

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species, and from environmental variations, which can directly affect the number of parts formed in developing embryos and larvae within genetically controlled limits (Parrish and Sharman, 1958). Therefore, meristic characteristics have also been widely used in stock differentiation studies (Aasen and Akyuz, 1956; Parrish and Sharman, 1958; Demir, 1963; Mais, 1972; Van den Boonstra, 1977; Sharp et al., 1978; Ehrich and Rempe, 1980).

Fish store large quantities of fat during the feeding season as an energy resource when the food is less available (Iles and Wood, 1965), and wide variations in fat content are therefore recorded during a single year of life. If sprat has more than one unit stock, then such stocks might show spatial differences in their fat content. The fat content of a fish species has been used as an indicator of physiological heterogeneity of populations (Shul'man et al., 1989).

The sprat (Sprattus sprattus phalericus Risso) is an abundant and widely distributed fish along the European coastlines of the Mediterranean, especially the Adriatic (Fischer, 1973; Fischer et al., 1987) and in the Black Sea (Slastenenko, 1956; Ivanov and Beverton, 1985). Its distribution in the Black Sea has been demonstrated in detail by Ivanov and Beverton (1985) and studies on population parameters and nutritional condition have been carried out by Bulgarian (Stoyanov, 1965; Ivanov, 1983), Romanian (Cautis, 1971) and Russian (Berg et al., 1949; Aslanova, 1954; Slastenenko, 1956; Domashenko and Yurev, 1978; Sirotenko and Sorokalit, 1979; Shul'man et al., 1985; Shchepkin and Minyuk, 1987) authors along their respective coasts. To the author's knowledge, only one study (Aksiray, 1954) relates to the identification and geographical distribution of sprat on the Black Sea coast of Turkey, but studies on the identification and differentiation of the other fish stocks have been carried out (Demir, 1963; Mengi, 1971; Payza, 1983; Avsar, 1987; Avsar et al., 1988a,b; Avsar et al., 1990).

Knowledge of the similarities and geographical distribution of local stocks may be important for their rational exploitation and management. However, the commercial pelagic fishery along the Turkish Black Sea coast has been regarded as a single unit, controlled by seasonal fishing bans and minimum landing sizes without regional distinction. Rational exploitation depends on determining the number of unit stocks present. To achieve this, morphometric, meristic and physiological discrimination of sprat has been carried out for specimens from the Turkish Black Sea coast.

Materials and methods

Material for this study was collected from 57 sampling stations located along the continental shelf area of the Black Sea coast of Turkey (Fig. 1). Stations were sampled using both mid-water and bottom trawls. Sampling by bottom trawl was performed in April and September 1990 and in September 1991. Mid-water trawl surveys were carried out in December 1990 and January 1992.

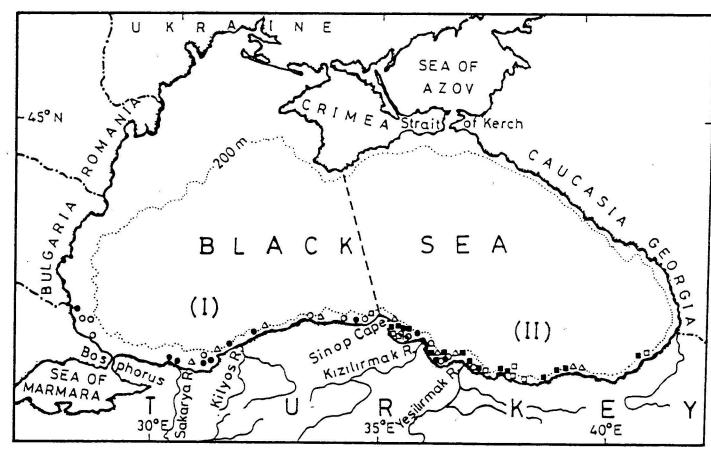


Fig. 1. Locations of the sampling stations along the Turkish Black Sea coast: (I) southwestern area; (II) southeastern area. The dashed line represents the border between the two areas; solid circles, April 1990; open circles, September 1990; open squares, December 1990; solid rectangles, September 1991; triangles, January 1992.

After each haul, the catch was sorted for sprat specimens. In poor hauls, total catch was considered as the sample size for further analyses. For larger catches, sub-sampling was carried out according to the procedure described by Holden and Raitt (1974). The number of specimens analysed and the length ranges for each sex and each sampling period and method of analysis are given in Table 1. Fishes taken for morphometric and meristic studies were preserved in solutions of 4% formaldehyde buffered with borax (Ferrerio and Labarta, 1988). Fishes taken for lipid studies were placed in a sterile plastic bag and frozen (-20°C) on-board until they could be transferred to the laboratory where they were kept at -20°C until analysis.

Morphometric measurements and meristic counts were made on the same individuals. Fifteen morphometric measurements were taken from each fish (Fig. 2), which was placed on its right side with the mouth closed, except for measurements of the interorbital distance, greatest depth and greatest breadth, which were made by holding the dorsal side of the fish facing up in the hand. All morphometric measurements were made using a calliper as a straight line between perpendiculars along the median vertical and lateral body axes, and recorded to the nearest millimetre.

With the exception of vertebral counts and counts of gill rakers and flaments, meristic counts were made directly on each fish under a binocular microscope (×20 magnification) with transmitted light. Vertebral counts were made directly with the naked eye, and gill flaments and gill rakers were counted after removing the left operculum and placing the first left gill arch in a petri dish with distilled water. Nine meristic characteristics were examined together with fifteen morphometric measurements (Fig. 2) in separating stocks of sprat. These were as follows.

Table 1
The minimum and maximum total length measurements (mm) and number of specimens analysed in each sex and in each sampling period

Sampling period	Juvenile min-max	n	Male min-max	n	Female min-max	n	Examined fish
Discriminant anal	ysis						
April 1990	H	_	58-130	190	58-120	344	534
September 1990	34-46	17	51-119	159	52-125	427	603
December 1990	28-45	37	34-105	118	35-112	238	393
September 1991	-	_	67-130	47	65-141	295	342
January 1992		_	49-109	570	49-137	928	1498
Total	28-46	54	34-130	1084	35-141	3232	3370
Lipid analysis							
April 1990	-	_	61-115	96	52-119	128	224
September 1990	_	-	80-114	36	70-115	143	179
January 1992	-	_	60-108	177	60-109	245	422
Total			60-126	309	52-129	516	825

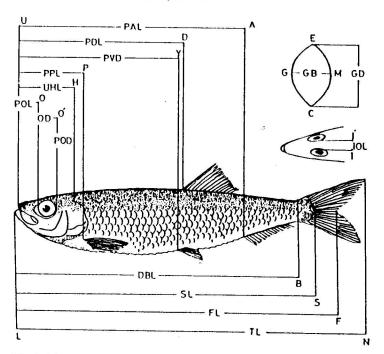


Fig. 2. Measured morphometric characteristics of Sprattus sprattus phalericus (Risso). TL, total length; FL, fork length; SL, standard length; DBL, dorsal body length; PDL, pre-anterior dorsal length; PAL, pre-anal length; PVD, pre-ventral distance; PPL, pre-pectoral length; UHL, upper head length; POD, post-orbital distance; OD, orbital diameter; POL, pre-orbital length; IOL, inter-orbital distance; GD, greatest depth; GB, greatest breadth. Picture redrawn from Tortonese (1970).

- (1) Dorsal fin rays were counted by considering the terminally divided dorsal fin ray as a single ray.
- (2) Pectoral fin rays were counted on the left fin using the same criteria as those for the dorsal fin.
- (3) Anal fin rays were counted by considering the terminally divided anal fin ray as a single ray and the last two shortest fins on the back side of this ray.
 - (4) Vertebrae were counted from skull to urostyle.
- (5) Gill-rakers were counted on the ventral arm of the first left gill arch from base to bend. Branched rakers having a common base were counted as one.
- (6) Gill-flaments were counted on the ventral arm of the first left gill arch using the same criteria as those for the gill-raker.
- (7) Pelvic fin rays were counted on the left fin by considering the terminally divided ray as a single ray.
 - (8) Caudal fin ray counts included rays on both the upper and lower lobes.

(9) Keel scales were counted between the ventral edge of the operculum and cloaca.

For the quantification of lipid concentration in wet tissue, a fillet was cut off the left side from the vertebral column, starting from near the gills. If one fillet did not yield enough material for fat analysis, a second was removed from the right side of the same fish. The weight of each tissue sample was between 8 and 10 g (Harwitz, 1980). Each fillet made an equal contribution to the total weight of the composite sample. The total weight of the tissue was recorded at an accuracy of 0.0001 g. Twenty grams of dry and anhydrous sodium sulphate were added to the composite sample to absorb the water from the muscle tissue. This was homogenised in a blender. The homogenate was then transferred to a thimble and extracted with hexane for 6 h in a Soxhlet apparatus as described by Harwitz (1980). After extraction, the solvent was evaporated using rotovapour and the remaining constituent was reweighed.

The following formula was used to obtain the percentage of fat on a wet weight basis

Percent fat in wet tissue =
$$\frac{\text{Fat weight (g)}}{\text{Weight of composite sample}} \times 100$$

The generalised distance function developed by Mahalanobis et al. (1949, cf. Weber, 1972) was used for the morphometric and meristic discrimination. This form of multivariate analysis gives a measure of the distance between pairs of groups in units of standard deviation (Lerman, 1965). The quantitative separation of the stocks of the sprat was made using the formula given by Weber (1972)

$$D^{2} = (b_{1}d_{1} + b_{2}d_{2} + b_{3}d_{3} + b_{4}d_{4}... + b_{m}d_{m})/m$$

where m is the number of characters measured and counted, D^2 is the generalised Mahalanobis distance, b_i is the discriminant function of the *i*th measurement and d_i is the mean difference of the *i*th measurement.

A significance test given in Sneath and Sokal (1973) was used to qualify the statistical difference between two groups.

Arrangement of data for discriminant analyses

According to Blackith and Rayment (1971), morphometric measurements should be standardised either by directly multiplying or dividing by size or by any measurable character related to length before running the discriminant analysis. As can be seen in Table 1, there were considerable differences between the minimum and maximum total length measurements of both sexes in each sampling period. Standardisation was done by dividing any morphometric measurement of each individual with its eviscerated weight as described by Avsar et al. (1988a).

The sex and age compositions of the samples were widely scattered so the comparison of the samples for the discrimination was meaningless prior to standardisation of the data set. As can be seen from Table 2, the differences in percentage ratios of males and females show significant variations (e.g. 72% in September 1991). In general, each sex of a fish species shows different growth characteristics (Holden and Raitt, 1974). Therefore, it is assumed that the results of discriminant analysis will be much affected by the differences in percentage ratios of males and females. To eliminate this error, the numbers of individuals of each sex were equalised.

Similarly, the differences in percentage ratios of age groups of both sexes were appreciably high between the samples of the southwestern and south-eastern Black Sea coast (Table 2). Fish exhibit different annual growth characteristics with age and also successive cohorts may grow differently depending on environmental conditions (Sparre et al., 1989). Therefore, the data should be age independent, so the equal number of fishes of the same age group in each sex was taken into account and age-influenced size differences of each morphometric measurement were eliminated using the equal number of fishes of the same age group.

Table 2 The numbers (n) and the differences in percentage ratios of males and females in different sampling periods and age groups in two sampling areas

	Group I	Group I ¹			Grroup II ²			
	Male (n)	Female (n)	Percentage difference	Male (n)	Female (n)	Percentage difference		
Sampling perio	od							
Apr. 1990	138	245	28	52	00			
Sept. 1990	79	181	40		99	32		
Dec. 1990		_	40	80	246	52		
Sept. 1991			-	118	238	34		
Jan. 1992	100	-	_	47	295	72		
Jan. 1772	189	289	20	381	639	26		
Age group								
0	15	15	0	170	271			
I	238	334	16	14 (0.102)	271	22		
П	133	280	man hard	413	689	26		
Ш			36	77	328	62		
IV	14	66	64	11	152	86		
	3	17	70	5	62	86		
v	3	3	0	2	15	76		

Sampling stations in Group I were located between the Bulgaria-Turkey border and Sinop Cape (southwestern area of the Black Sea).

²Sampling stations in Group II were located between Sinop Cape and the Turkey-Georgia border (southeastern area of the Black Sea).

Results

Selection of the groups compared

The members of a unit stock should have the same spawning ground (Cushing, 1968). According to this idea, the sampling area must be divided into possible spawning grounds. A brief account of the spawning grounds of the Black Sea sprat (Sprattus sprattus phalericus Risso), is given by Ivanov and Beverton (1985). They state that sprat spawn all around the inshore waters of the Black Sea but that intensive spawning occurs in the northwestern shelf basin. Since this species prefers waters with relatively low salinity, river deltas are also possible spawning grounds, and the big river deltas such as the Sakarya and Kilyos deltas in the western Black Sea and Kizilirmak-Yesilirmak Rivers in the eastern Black Sea coast of Turkey form suitable small spawning grounds for Black Sea sprat (Fig. 1). Therefore, the sampling stations among these local spawning grounds were divided into two groups. Stations in the area between the Bulgaria-Turkey border to Sinop Cape (southwestern area) were designated Group I, and those in the area between Sinop Cape and the Turkey-Georgia border (southeastern area) were designated Group II. Both groups were in direct contact with the persisting cyclonic gyres of the Black Sea (Aasen and Akyuz, 1956). The April 1990, September 1990 and January 1992 samples were collected from both areas while the December 1990 and September 1991 samples were collected from the southeastern area (Fig. 1).

Generalised distance analyses

Significance and generalised distance of Mahalanobis analyses compared for the same sampling period and for the different sampling periods are given in Table 3, where the generalised Mahalanobis distances (D^2) were given above the diagonal and the results of the significance test below the diagonal.

As can be seen from Table 3, the calculated D^2 values varied from 0.04 to 0.64. The minimum (D^2 =0.04) and maximum (D^2 =0.64) values were calculated between Groups I and II for the sampling period April 1990 and the samples of April 1990 (Group I) to September 1991 (Group II). The D^2 values obtained between the southwestern and southeastern regions for different periods are higher than those for the same period. This contradiction may be related to the differences in the growth characteristics which are, in turn, influenced by the seasonal as well as annual differences in the environmental conditions of both regions.

Significance test results showed no significant differences at the P < 0.01 level between groups of the same period and between the different periods.

Table 3
Generalised distance of Mahalanobis between and within sampling periods and their significance levels

Apr. 1990 I - Sept. 1990 I -				Sept. 1990		Sept.	Jan. 1992	
Apr. 1990 II -	 Group I	Group II	Group I	Group II	1990 Group II	1991 Group II	Group I Group II	
Sept. 1990 II = Dec. 1990 I = Sept. 1991 II = Jan. 1992 I = Jan 1992 II =	0.0353	0.2971 0.4926	0.4369 0.4802 0.0526	0.4610 0.5433 0.3055 0.3532	0.6349 0.5597 0.1229 0.1141 0.3367	0.2744 0.4856 0.1232 0.2624 0.1658 0.1584	0.3883 0.6413 0.2089 0.3125 0.1857 0.1875 0.0732	

¹Group I, sampling stations located between the Bulgaria-Turkey border and Cape Sinop (southwestern area of Black Sea); Group II, sampling stations located between Cape Sinop and Turkey-Georgia border (southeastern area of Black Sea).

-. No significant difference (P > 0.01).

Table 4
Three-level nested ANOVA (with unequal sample sizes) for the percentage of fat in the tissues obtained in three different sampling periods

Source of variation	d.f.	SS	MS	Fs	Significance
April 1990	· · · · · · · · · · · · · · · · · · ·				
Among regions Among sexes within regions Among length groups within sexes Within length groups	1 2 17 4	16.32 3.74 116.55 1857.54	16.32 1.87 6.86 5.33	8.74 0.27 1.28	NS NS NS
Total	61	1877.59	3.33		
September 1990 Among regions Among sexes within regions Among length groups within sexes Within length groups Total	1 2 14 16 33	0.62 172.94 107.38 4897.41 5070.96	0.62 86.47 7.67 15.07	0.007 11.27 0.51	NS ** NS
January 1992 Among regions Among sexes within regions Among length groups within sexes Within length groups Fotal	1 2 15 86 104	33.01 51.91 91.39 1709.30 1794.23	33.01 25.96 6.09 4.66	1.27 4.26 1.31	NS + NS

^{*}Significant (P < 0.05); **highly significant ($P \ll 0.05$); NS, not significant (P > 0.05).

Nested ANOVA analyses

The results of TLNA obtained on the percentage of fat of the wet tissues for April 1990, September 1991 and January 1992 are given in Table 4. There is no significant variance (P>0.05) in the percentage fat levels of Sprattus sprattus phalericus between the southeastern and southwestern Black Sea coast

within three sampling periods. This is in a good agreement with data from discriminant analysis. However, while there are also no significant differences (P > 0.05) among length groups within sexes in April 1990, September 1991 and January 1992, highly significant (P < 0.05) and significant (P < 0.05) differences are calculated among sexes within regions in September 1991 and January 1992 respectively.

Discussion

In discriminant analysis, the quantity of the discrimination (D^2) obtained between two or more groups is directly related to the degree of their resemblance to each other (Blackith and Rayment, 1971). Apart from this, Ehrich and Rempe (1980) have studied the morphometric and meristic discrimination of Pacific hake (Merluccius productus) and concluded that if the D^2 values are between 0.3 and 2.9, all groups belong to one population. In addition to this, Mais (1972) studied the Pacific sardine (Sardinops caeruleus) inhabiting North Central Baja California and southern California. He obtained a D^2 value of 0.9 and so decided that there is great similarity among the samples. The calculated D^2 values in the present study (Table 3) are smaller than those of the minimum D^2 values given by Ehrich and Rempe (1980) and Mais (1972). The calculated D^2 range in the present study implies that all the Turkish Black Sea sprat collected in 1990, 1991 and 1992 can be considered as one unit stock. It is worthy of note that the significance test result obtained from all groups compared (Table 3) also supports this conclusion.

According to Lerman (1965), the changes in the dimensions of morphometric characteristics (or in the number of meristics) in evolving stocks are transferred from generation to generation in an increasing manner. Individuals of a given species or race may create new local races within a few generations (Kosswig, 1974). Thus, the λ zov Sea form of the Black Sea sprat may create polymorphism in the morphometric characteristics or meristic counts under the different conditions of this sea.

The fact that larger D^2 values were obtained between Groups I and II for different sampling periods than for the same period may be explained by the intermingling of individuals during spawning and feeding migrations of Azov Sea and Black Sea sprats. The Azov Sea form of the Black Sea sprat may have some specific characteristics in their phenotypic and genotypic peculiarities. Of course, there might be genetic exchange between sprats inhabiting both Azov Sea and eastern Black Sea during joint spawning and feeding migrations. Additionally, there is neither geographical barrier nor a considerable distance between the groups compared in the Black Sea.

It is clear that in areas influenced by the river inflows, specific temperature and salinity values are the most important factors influencing species distribution and growth characteristics. Such areas can be considered as a distinct faunal sub-province with different physical and chemical conditions. However, according to Ivanov and Beverton (1985), the sprat is an euryhaline species thriving even in 4% salinity. However, sprat migrate at a rate of about 8 miles in 24 h (610 m h⁻¹) (Bleil and Kastner, 1987). Therefore, distance is not a barrier in the exchange of fauna between the estuaries located along the Black Sea coast of Turkey. This is due not only to their wide salinity tolerance, acting as a homogeniser in adaptation but also to their great mobility.

According to Ivanov and Beverton (1985), sprat spawn all around the offshore waters of the Black Sea but heavily in less saline delta regions and also in the northwestern shelf area. The existing main cyclonic current system may cause the eggs and larvae to drift from spawning areas to new inshore or offshore areas of the Black Sea. These transportation and mixing processes must allow the exchange of genetic characteristics between the fish inhabiting southwestern and southeastern basins of the Black Sea. However, the borders of eastern and western cyclonic gyres seem to form an oceanographic barrier for the eggs and larvae of the eastern and western Black Sea sprats.

The sprat is a pelagic fish and feeds on both phytoplankton and zooplankton, but mainly on copepods. According to Sorokin (1983), the number and biomass of zooplankton can exceed the average by several times in the coastal waters, where the circular current is distributed by ridges, or in the shallow northwest part of the sea including the coastal areas near the Crimea, the coastal regions of Turkey, of Caucasia, the pelagic regions at the boundary of the east and west cyclonic eddies between the Crimea and Sinop Cape, and in the anticyclonic area in the southeast part of the Black Sea. In the Black Sea, the borders between the above-mentioned cyclonic eddies together with these eddies and the anticyclonic gyres encourage the intermingling of planktonic organisms, so there are no marked differences between these regions on the basis of planktonic productivity (Sirotenko and Sorokalit, 1979). Therefore, the sprat is expected to disperse all around the Black Sea showing a similar distribution to their planktonic food organisms. It can be suggested that this fish is able to evolve stocks with large habitats and all of the sprats inhabiting southwestern and southeastern basins can be regarded as a single stock.

The position of the Bosphorus may have an effect on the genetic shape of the Black Sea sprat. The eggs and larvae originated from the main spawning ground may drift by the surface current from the northwestern shelf of the Black Sea to the south and be distributed to the east. During this transportation, some eggs and larvae may be carried through the Bosphorus upper layer current to the Sea of Marmara. According to Ivanov and Beverton (1985), juveniles prefer warmer water than adults. Therefore, the relatively warmer waters of Marmara provide the most suitable wintering area for juvenile sprat. However, adults (more than I year old) prefer cold water (Ivanov and Beverton, 1985), so there are two possibilities: (i) adults may stay in the layer

below the thermocline in the Sea of Marmara or (ii) they may migrate through the Bosphorus from the Sea of Marmara to the relatively cold waters of the Black Sea. The second idea is supported by Sorokin (1983).

In temperate aquatic environments, the availability of food generally varies seasonally and annual cycles of fattening are normally observed in teleost fishes (Nikolsky, 1963). Apart from this, a comparison of the lipid content of different sampling periods (seasons) is not very meaningful. For this reason, a comparison of the total lipid content of different regions in each sampling period was taken into consideration (Table 4). A comparison of the same sampling period for different years (e.g. southeastern area in September 1990 and September 1991) was not carried out because of differences in the rates of fattening from year to year (Shul'man et al., 1985). According to these authors, the level of fat in the fish tissue is related to their feeding condition and the feeding itself is directly related to the availability of food that varies with the annual productivity. However, it was considered that the possible differences between the samples of two localities may result from the sexes or length groups in each region. Therefore, the above-mentioned factors which are assessed by means of TLNA analysis of Sokal and Rohlf (1969) were taken into consideration to estimate the possible differences among sprat samples.

Although the Black Sea sprat spawns throughout the year in the Black Sea, the most intensive spawning takes place from November to March (Ivanov and Beverton, 1985), and September, January and April are the pre-intensive, intensive and post-intensive periods, respectively. The differences between sexes within regions increased considerably ($P \ll 0.05$) during the preintensive season while it decreased in the intensive period (P < 0.05) and there was no significant difference in post-intensive period (Table 4). Lipid reserves of fish are used to meet energy demands for reproduction (Nikolsky, 1963). Therefore, the changes in seasonal fattening of these fish are directly related to their sexual cycles. Additionally, the fattening normally occurs in the early phases of gonadal development and the body-fat stores are progressively depleted from the pre-intensive period to the intensive spawning season (De Vlaming, 1975). The highly significant and significant differences obtained between sexes within regions in September 1990 and January 1992 may indicate either the dissimilarity on the level of fattening of both sexes or possible differences between the timing of sexual maturity of males and females. De Silva (1973) states that the males of Atlantic sprat tend to mature sexually earlier than the females. However, in order to reach a reliable positive conclusion for the Black Sea sprat, it seems that repeated sampling must be carried out on a monthly basis throughout the year.

However, as stated by Parrish and Sharman (1958), a unit stock has its own specific spawning time. Their argument and the results obtained in this study clearly demonstrate that sprat should form a unit stock along the Turk-

ish Black Sea coasts as there were no significant differences between regions in three sampling periods.

Conclusion

There is no comprehensive study on stock differentiation of the clupeid species inhabiting the Black Sea coast of Turkey at present. As a pioneering work, the Black Sea sprat was studied. The generalised distance of Mahalan-obis and three-level nested ANOVA were employed to test the significance of differences between sprat stocks. The effects of several factors on variations in the stocks of Black Sea sprat are discussed. The similarities between southwestern and southeastern sprat samples, considerably influenced by oceanographic, physiological and adaptational mechanisms, nutritional conditions and some physical factors (such as the prevailing current system), are discussed in detail.

The analysis of samples collected from the southwestern and southeastern Black Sea coast indicates that the differences between phenotypic and genotypic peculiarities of the species are generally small (Table 3) in comparison with the generalised distances either in the same or in different sampling periods. This conclusion is supported by results obtained from the application of three-level nested ANOVA with unequal sample size on the total lipid contents of samples collected from the same localities in each sampling period (Table 4). Thus, neither of the two methods applied provided a sufficient discrimination between the individuals of Black Sea sprat inhabiting the Black Sea coast of Turkey and therefore it is concluded that sprat form only one unit stock in the study region.

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