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What does external morphometry tell us about stock discrimination of *Sardina pilchardus* stocks from Türkiye?

by

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Abstract

The analysis of shape is a fundamental part of much biological research. Morphometrics, which incorporates concepts from biology, geometry, and statistics, is the study of the geometrical form of organisms. In addition, morphometric characteristics can be used to differentiate 'phenotypic stocks' as groups with similar growth, mortality, and reproductive rates. In this study, 25 morphometric characters were used to discriminate European pilchard (*Sardina pilchardus*) stocks from Türkiye. Intraspecific variation of the European pilchard was investigated based on morphometric characters. Samples were collected from the Aegean Sea (AS; N = 54), the Sea of Marmara (MS; N = 50) and the Mediterranean Sea (MEDS; N = 50) during the 2019 fishing season. Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) were used for stock discrimination of the European pilchard. Univariate statistics (ANOVA) showed that 24 of the 25 measurements differed significantly between samples to a varying degree ($p < 0.001$). According to CDA, an overall classification success rate of 87.7% was achieved using 12 morphometric parameters. All samples were clearly separated from each other in the discriminant space, suggesting that there was no strong intermingling between populations. In addition, we would like to conclude that the morphological difference is not at the genetic level, and would like to emphasize the need for additional stock discrimination methods.

Key words: *Sardina pilchardus*, stock discrimination, morphometrics, multivariate analysis, Türkiye

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1. Introduction

The European sardine or pilchard, *Sardina pilchardus* (Walbaum, 1792), is one of the most popular sea fish worldwide. *S. pilchardus* is a fast-growing and short-lived small pelagic fish species. This clupeiform species has a wide distribution range in the NE Atlantic Ocean, from the Celtic Sea and the North Sea to Mauritania and Senegal (including the Azores, Madeira, and the Canary Archipelagos). It is also found in the Mediterranean, Marmara, and Black seas (Parrish et al. 1989). Its populations represent some of the most valuable fish resources throughout its range in the NE Atlantic, from the North Sea to the Senegalese coast, including the Mediterranean Sea and the Black Sea (Castalago & Palomera 2014). According to the IUCN (IUCN 2023), the conservation status of the European sardine is LC.

Commercially valuable marine pelagic fish populations are frequently driven to extinction due to excessive overfishing (Hutchings 2000; Myers & Worm 2003; Pauly et al. 2003; Atarhouch et al. 2006). European pilchard catches have decreased in several areas over the past ten years, and almost all of its geographic distribution has been identified as having completely or extensively fished stocks (FAO 2018, 2019; ICES 2018). The amount of sea fish caught in Türkiye is approximately 328 165 tonnes. Anchovies account for a large part of this amount (151 598 tonnes), but sardines also occupy an important place in Türkiye's fisheries with an annual catch of 15 800 tonnes (ranked 4th; Anonymous 2022). Sardines, which are mostly caught by Türkiye from the Aegean Sea, vary in size depending on the sea from which they are caught. In addition to contributing to the economies of many countries around the world through its production, the species has a significant impact on the biodiversity and ecological balance of our seas (Williams 2003).

Understanding the population structure or stock identification of fish species is fundamental to developing appropriate fisheries management strategies. Stock identification is a multidisciplinary subfield of fisheries science that uses genetic, biometric and life history analyses (Ihssen et al. 1981; Pawson & Jennings 1996; Begg & Waldman 1999). For this purpose, several approaches (meristics, morphometrics, traditional tags, parasites as natural tags, otolith chemistry and several molecular markers, e.g. protein allozymes, mitochondrial DNA, nuclear DNA, microsatellite DNA) are presently used to describe fish stocks or populations (Begg & Waldman 1999; Cadrin & Friedland 1999; Cadrin 2000; Sajina et al. 2011; Reis-Santos et al. 2015; Mounir et al. 2019; Moura et al. 2020; Muniz et al. 2021; Neves et al. 2021; Caballero-Huertas et al. 2022; Labonne et al. 2022). Among these methods, morphometry is the baseline methodology and a cost-effective method. Although it is now widely acknowledged that morphological variation involves both environmental and genetic components, persistent variations in form between fish groups may reflect various growth, mortality, or reproduction rates, which are important for the classification of stocks (Swain & Foote 1999; Cadrin 2000). Morphometrics can be used to differentiate 'phenotypic stocks' as groups with similar rates of growth, mortality and reproduction (Mounir et al. 2019). Morphometric characters are defined by several (no fewer than two) anatomical landmarks. The choice of landmarks, characters, and variables depends on the concept of homology to justify comparability of attributes across species (Smith 1990). Phenotypic differences do not always result from genetic divergence, and groups defined by morphometric differences may not indicate reproductively isolated populations. For more than a century, geographic variation in morphometry has been used to distinguish between regional varieties of fish. The historical evolution of stock identification methods and the development of morphometric approaches have been closely linked. Morphometric variations can be an indication that groups had different environments or habitats at an important stage of development (Cadrin 2000).

There are several published studies on *S. pilchardus*, and these studies address stock discrimination (Mounir et al. 2019), population structure (Tinti et al. 2002; Neves et al. 2021), age (Silva et al. 2015), growth (Dahel et al. 2016), phylogeny and genetic (Atarhouch et al. 2006; Sarmaşık et al. 2008; Machado et al. 2018), morphometric and meristic parameters (Mustać and Sinovčić 2010; Geladakis et al. 2018), migration (Giannoulaki et al. 1999) otolith shape (Jemaa et al. 2015; Neves et al. 2023) and feeding ecology (Costalago & Palomera 2014) of this popular commercial fish species.

The present study aims (i) to examine the morphological differentiation and (ii) to determine the intraspecific variation in stocks of the European pilchard sampled from the Aegean, Marmara and Mediterranean seas of Türkiye.

2. Materials and methods

2.1. Sampling

Samples of sardines were collected from commercial fishermen from catches made in the

Aegean Sea (AS-Edremit; N = 54, 12.88 ± 0.12 cm), the Sea of Marmara (MS-Bandırma; N = 50, 13.01 ± 0.1 cm), and the Mediterranean Sea (MEDS-Iskenderun; N = 50, 22.84 ± 0.16 cm) in Türkiye (Fig. 1). Specimens visually in good condition were frozen soon after collection. The freezing process aims to reduce bacterial growth and reaction rates of enzymes by converting the water in the fish body into ice crystals, which also extends the rigor mortis period (Gram & Huss 1996). In addition, freezing samples causes differences in morphometric measurements compared to fresh samples (Wessels et al. 2010). For this reason, a standard method should be used in studies. Either all samples should be frozen and thawed, or fresh samples should be used. In this study, since the sampling locations are separated by a considerable distance, samples were defrosted for laboratory analysis about one month later to ensure that all fish were analyzed after a similar period of freezing. Biological examinations were performed on defrosted samples. In the laboratory, individuals were measured (total length TL, to the nearest 0.1 cm), and weighed (total weight W, to the nearest 0.01 g). Sex was determined by macroscopic examination of gonads.

2.2. Morphometric analysis

A total of 25 morphometric characters of the specimens were measured (Table 1, Fig. 2). All measurements were made point to point on the left

Table 1

Characters and abbreviations of morphometric measurements.

No	Abbreviation	Morphometric characters
1	HL	Head length
2	HD	Head depth
3	PreDD	Predorsal length
4	PostDD	Postdorsal length
5	PrePD	Prepectoral length
6	LDF	Length of dorsal fin base
7	DDF	Height of dorsal fin
8	LAF	Length of anal fin base
9	LPF	Length of pectoral fin
10	LVF	Length of pelvic fin
11	LCAUF	Length of upper caudal fin lobe
12	HCAUF	Height of caudal fin
13	ED	Eye diameter
14	InorD	Interorbital distance
15	PreorD	Preorbital distance
16	PostorD	Postorbital distance
17	InNM	Internasal distance
18	SL	Snouth length
19	DDC	Distance between dorsal and caudal fin
20	DPV	Distance between pectoral and pelvic fin
21	DVA	Distance between pelvic and anal fin
22	DAC	Distance between anal and caudal fin
23	Lcaup	Length of caudal peduncle
24	MaxBD	Maximum body depth
25	MinBD	Minimum body depth

Figure 1

Map of the sampling locations in Türkiye, (1) Marmara Sea – Bandırma, (2) Aegean Sea – Edremit, (3) Mediterranean Sea – Iskenderun.

side of each specimen using a digital caliper by the same person following Mustać and Sinovčić (2010) and Kottelat and Freyhof (2007) (± 0.001 mm).

All measurements were standardized to eliminate any size effect between the localities according to the following methodology of Elliot et al. (1995):

$$M_{adj} = M\left(\frac{L_s}{L_o}\right)^b$$

where M is the original value of the morphometric measurement, M_{adj} is the adjusted size of the measurement, L_o is the total length of the fish, and L_s is the mean of the total length of all fish. 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. The efficiency of size adjustment transformations was assessed by testing the significance of the correlation between the transformed variables and total length.

2.3. Statistical analysis

Before the analysis, normality and homogeneity of variance were determined for each data set using Kolmogorov–Smirnov and Levene's tests, respectively. In addition, the difference between female and male was determined by the *t*-test for independent samples and the Mann–Whitney *U*-test. Univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. Analysis of covariance (ANCOVA) was used to determine the effect of fish length on the morphometric measurements. In addition, data were also evaluated using Principal Component Analysis (PCA) and Discriminant Analysis (CDA). PCA helps eliminate the redundancy in the variables and isolate several independent factors for population differentiation in morphometric data (Verma & Serajuddin 2016), while CDA is used to separate stocks and estimate their differences (Box's M test, $p = 0.001$). Permutational multivariate analysis of variance (one-way PERMANOVA; Anderson 2001) based on Euclidean distance and 9999 permutations was used to compare morphometric data between the localities.

SPSS 20, Minitab 15.0, PAST 3.0 (Hammer et al. 2001) and SPSS 21.0 software and Excel were used to evaluate the data.

3. Results

None of the 25 transformed morphometric characters yielded a significant correlation with total length, and thus the allometric formula was successful in removing the size effect from the data. In addition, there were no significant differences between the sexes in terms of morphometric measurements ($p > 0.05$). Therefore, the analysis was performed for entire populations (male + female) from each sea. Univariate statistics (ANOVA) showed that 24 of the 25 measurements significantly differed between the samples to a varying degree ($p < 0.001$; Table 2).

The first two principal components (PCs) accounted for 100% of the total variance (82.34% for PC1, eigenvalue = 47.5252; 17.67% for PC2, eigenvalue = 10.1907).

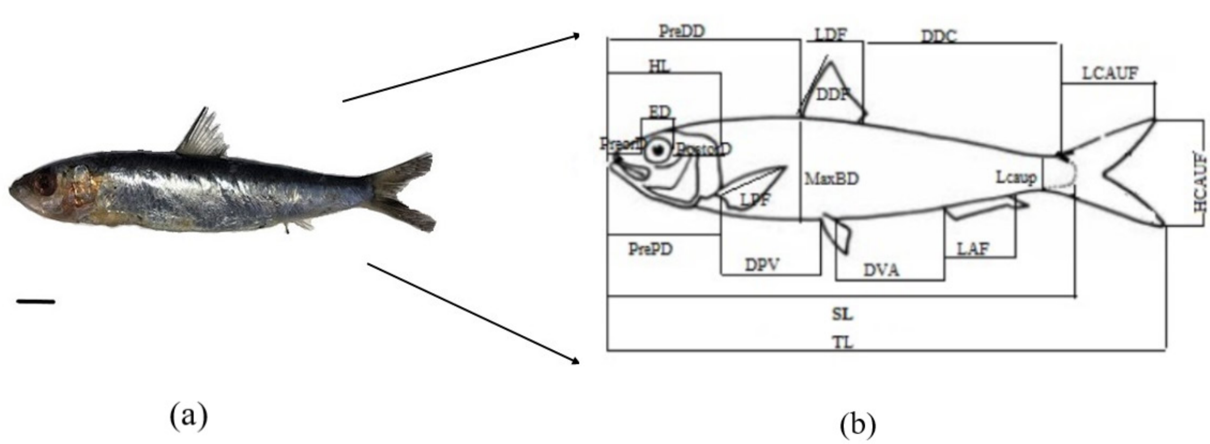

Figure 2

(a) Original photograph of the European sardine (the bar represents 1 cm). **(b)** Diagram of *Sardina pilchardus* showing several morphometric measurements; the illustration was edited based on Silva (2003).

Table 2

Univariate statistics (ANOVA) testing differences between samples from all morphometric measurements.

Character	Wilks' lambda	F	Significance
HL	0.671	37.011	0.000
HD	0.696	32.952	0.000
PreDD	0.932	5.543	0.005
PostDD	0.701	32.224	0.000
PrePD	0.753	24.703	0.000
LDF	0.943	4.600	0.011
DDF	0.998	0.140	0.870
LAF	0.598	50.744	0.000
LPF	0.651	40.535	0.000
LVP	0.745	25.794	0.000
LCAUF	0.648	41.036	0.000
HCAUF	0.630	44.401	0.000
ED	0.753	24.725	0.000
InorD	0.946	4.302	0.015
PreorD	0.853	13.050	0.000
PostorD	0.605	49.341	0.000
InNM	0.693	33.495	0.000
SL	0.461	88.119	0.000
DDC	0.894	8.998	0.000
DPV	0.958	3.334	0.038
DVA	0.541	63.949	0.000
DAC	0.562	58.957	0.000
Lcaup	0.796	19.391	0.000
MaxBD	0.467	86.340	0.000
MinBD	0.923	6.263	0.002

Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

According to the results of CDA, HL, HD, PostDD, PrePD, LAF, LVP, HCAUF, ED, PostorD, SL, DVA, and MaxBD were found to be significant in discriminating between the European sardine stocks in Türkiye ($p < 0.001$). The first two canonical discriminant functions were used in the analysis. The values of Wilks' λ range from zero to one. The closer Wilks' λ is to zero, the better the discriminating power of the CDA (Table 3). Wilks' λ test of discriminant function analysis showed significant differences in the morphometric measurements of all populations ($p < 0.001$).

The plot of DF1 and DF2 shows a clear between-stock differentiation (Fig. 3). The first DF accounted for 64.3% and the second DF accounted for 35.7% of the total variance, Morphometric characters revealed that species could be differentiated from each other by 87.7% of the total between groups variability. All samples were clearly separated from each other in the discriminant space, suggesting that there was no strong intermingling between the populations (Table 4). In addition, PERMANOVA showed a significant difference between the studied localities (F = 34.57; $p = 0.0001$).

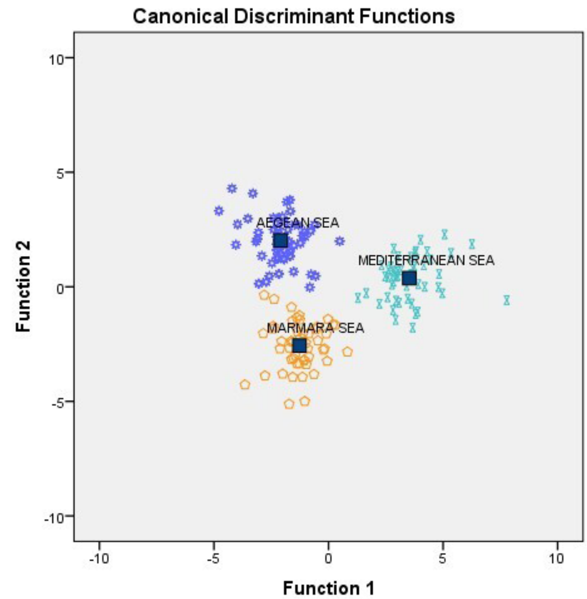

Figure 3

Discriminant analysis (CDA) scores for the classification of *S. pilchardus* by sampling area based on morphometrics.

Table 3

Results of Wilks' lambda (λ) and eigenvalue scores according to CDA.

Function	Eigenvalue	% of variance	Wilks' lambda	Chi-square	P
1	5.183[a]	64.3	0.042	462.162	0.000
2	2.875[a]	35.7	0.258	215.000	0.000

a - The analysis used the first two canonical discriminant functions

Table 4

Classification matrix results for *S. pilchardus* based on body morphometrics.

Localities		Predicted Group Membership			Total
		AS	MS	MEDS	
Original count	AS	47	8	2	54
	MS	6	41	1	50
	MEDS	1	1	47	50
%	AS	87	16	4	100
	MS	11	82	2	100
	MEDS	2	2	94	100

4. Discussion

The results obtained in this study demonstrated that there are significant phenotypic differences between the three stocks of *S. pilchardus* in Türkiye. After reviewing the literature, it is apparent that there are many studies using multivariate analysis (PCA, CDA) to analyze stocks of different fish species (Turan et al. 2006; Baibai et al. 2012; Khan et al. 2013; Vatandoust et al. 2015; Siddik et al. 2016; Ozpicak & Polat 2017; Vasconcelos et al. 2018; Hanif et al. 2019; Ghozzi et al. 2022; Mahfuj et al. 2023; Neves et al. 2023). However, there are no studies on stock discrimination using traditional morphometrics of *S. pilchardus* from the seas of Türkiye.

Analysis of fish stock structure is a useful method for managing naturally occurring populations. The isolation of a population within its natural habitat results in significant morphological variation within and between groups (Mahfuj et al. 2023). In this regard, AnvariFar et al. (2011) concluded that the Shahid-Rajaei dam on the Tajan River has probably created two morphologically different populations of *C. c. gracilis* upstream and downstream of the dam. Khan and Nazir (2019) investigated the stock structure of *Sperata aor* on the basis of morphometric characters, using the truss network. In addition, Ozpicak and Polat (2019) used traditional morphometrics to reveal morphological differences between *Barbus tauricus* populations from six coastal streams of Türkiye. It is sometimes difficult to explain the causes of morphological differences between populations (Cadrin 2000), but it is assumed that these differences may be genetically based or be the result of phenotypic plasticity in response to local environmental conditions (Murta 2000). Particular importance is attributed to environmental conditions prevailing in the early stages of development, when the phenotype of an individual is more susceptible to environmental impact (Pinheiro et al. 2005). In addition, according to Robinson and Wilson (1996), stock differentiation may result from genetic differences between stocks, which are associated with unique aquatic environments, such as fluctuations in temperature, salinity, turbidity, current patterns, and alkalinity (Mir et al. 2013; Miyan et al. 2016; Hanif et al. 2019). Nevertheless, identical environmental and habitat factors may account for stock similarities (Mahfuj et al. 2023).

The distinction between the samples may indicate a relationship between the extent of phenotypic heterogeneity and geographic distance, demonstrating minimal intermingling between the stocks of the Aegean Sea and the Sea of Marmara in this study. The Mediterranean stock is significantly separated from those of the Sea of Marmara and the Aegean Sea. The morphometric data obtained in the present study revealed that there was a remarkable difference in the size of sardines, especially in the length and weight of those collected from the Mediterranean Sea (22.84 ± 0.16 cm, 101.51 ± 2.41 g) compared to the samples collected from the Sea of Marmara (13.01 ± 0.1 cm, 14.64 ± 0.4 g) and the Aegean Sea (12.88 ± 0.12 cm, 15.21 ± 0.45 g). According to Sarmaşık et al. (2008), sardines harvested from the northern coasts, such as İstanbul and Bandırma, are smaller than those from the Mediterranean Sea. Sarmaşık et al. (2008) reported similar results from the Turkish seas. This phenomenon has already been documented (Cihangir 1996; FAO 1999), although it is still unclear whether it pertained to isolated communities occupying small areas along the country's coasts (Sarmaşık et al. 2008). This could simply be the result of temperature effects on growth rates (hot Mediterranean waters versus cooler northern coastal locations), but size differences could also be affected by the genetic background of different populations. In addition, the Mediterranean Sea is considered an oligotrophic system, less productive and generally warmer than the other areas (Stambler 2014). The European sardine has a high dispersal capacity (Garrido et al. 2015a; Santos et al. 2018; Silva et al. 2019), as well as variable population dynamics, which is mostly related to climatic variations (Silva et al. 2008; Garrido et al. 2017).

Furthermore, these morphological differences may be solely related to body shape variation and not to size effects, which were successfully accounted for by the allometric transformation. The allometric transformation was also used in this study. Using the allometric transformation, ANOVA and multivariate analysis, the size effect was successfully eliminated in the current study, and the body shape variation was found to be the cause of significant differences between the populations. The differences in the mean shape of the European sardine between the three stocks in Türkiye may be due to differences in environmental conditions rather than genetic variability. In fact, fish are more likely to exhibit morphological differences induced by environmental variations (Allendorf et al. 1987; Wimberger 1992), and this phenotypic plasticity allows them to adapt to prevailing environmental conditions by modifying their morphology, physiology, behavior or reproductive biology or survival that mitigate the effects of environmental change (Stearns 1983; Meyer 1987; Turan et al. 2004). Such phenotypic adaptations are not always the result of genetic variations (Ihssen et al. 1981; Clayton 1981; Allendorf et al. 1987).

The analysis of variance revealed significant phenotypic variation between the three populations (Tables 2 & 4). CDA can be a useful method for distinguishing between different stocks of the same species (Karakousis et al. 1991; AnvariFar et al. 2010;

Khan and Naazir 2019). Almost all 25 morphometric variables in this study were found to be significant. In addition, CDA showed that 12 morphometric measurements are critical for differentiating the sardine populations in Türkiye. In the present study, an overall classification success rate of 87.7% was achieved using 12 morphometric parameters. HL, HD and SL are particularly important in differentiating sardine stocks. It is reported that environmental factors are likely to affect anatomical structures in the head region, causing morphometric variability (Bouton et al. 2002). Morphological variations in the head region are considered to be due to differences in feeding regimes (Gatz 1979) and water quality parameters (Khan et al. 2013). Similarly, Mounir et al. (2019) investigated differences between phenotypic *S. pilchardus* stocks from the Moroccan Atlantic coast using the truss network and indicated that head measurements are important for stock discrimination and concluded that differences in the mean shape of sardines between the three stocks off the Moroccan Atlantic coast could be due to differences in environmental conditions rather than genetic variability. Khan and Nazir (2019) also indicated that morphometric variation responsible for stock separation in *Sperata aor* was observed mainly in the head region. Silva (2003) investigated morphometric variation among sardine populations from the NE Atlantic and the Western Mediterranean Sea and observed two morphological types, with geographic coherence in the NE Atlantic and the Mediterranean. Nevertheless, this differentiation was not found by Silva et al. (2012). However, Mustać and Sinovćič (2010) indicate homogeneity of sardine populations in the Zadar fishing area of the Adriatic Sea. Baibai et al. (2012) defined two different stocks using both morphometric and molecular methods on the North Atlantic coast and, as in this study, head-to-body ratios were found relevant to the identification of two morphotypes. In some cases, molecular and morphometric data may not confirm each other. Sarmaşık et al. (2008) analyzed the differences between stocks by performing molecular analysis of sardines sampled from the Aegean Sea, the Mediterranean Sea and the Sea of Marmara in Türkiye and reported that based on the results obtained from molecular analysis (mitochondrial DNA, cyt b), there are no isolated populations or heterogeneity among sardines occurring off the coasts of Türkiye. Despite the large differences in size between sampling locations observed by Sarmaşık et al. (2008), phylogenetic analysis did not reveal any intrapopulation genetic diversity. According to the results of the present study, geographic isolation and habitat differences are considered to be the main factors contributing to different growth rates and morphological differences between disjunct populations of *S. pilchardus*. Furthermore, previous studies have shown that biogeography, evolution, and long-term climate change are among the primary factors driving genetic diversity and morphological variation within a species (Hewitt 1996; Avise 2000). The results obtained in this study provide evidence that ecological differences are reflected in morphology, but are not yet at the level of genetic differentiation. In addition, Tinti et al. (2002) suggested that there is a lack of genetic heterogeneity among local sardine stocks, suggesting that there may be a large population of sardines whose boundaries are wider than the limited area studied by different researchers.

In this study, we used body morphometric approaches to detect morphological differences between sardine stocks from the seas of Türkiye, and the results indicate that there may be variation in morphological characteristics in different stocks. The study shows that different populations occur in selected habitats, which should be considered separately for species management and conservation. In addition, we would like to emphasize the necessity of using additional stock discrimination methods. Policy makers and fisheries agencies can use these new findings to improve the management of the European sardine in Türkiye by incorporating them into predictive models. However, more information is still needed, as well as a multidisciplinary and comprehensive approach to adopt comprehensible and responsible fisheries management.

Credit authorship contribution statement

Melek Ozpicak: Conceptualization; Data Curation; Investigation; Methodology; Resources; Software; Validation; Writing – Original Draft; Writing – Review and Editing.
Semra Saygin: Investigation; Methodology; Resources; Software; Validation; Writing – Original Draft; Writing – Review and Editing.

Statements & Declarations

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

The work raises no ethical issues. All fish examined were from commercial fishing activities and none of them were killed for the specific purpose of the study.

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