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*-RESEARCH ARTICLE-*

# Otolith Shape Analysis of Anchovy ( *Engraulis encrasicolus*) in the Black, Marmara and Aegean seas

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## Abstract

Otolith shape analysis is widely used for fish species identification and stock classification. The morphological structure of anchovy populations was examined with otolith characters using Truss network system. A total of 300 anchovy specimens were collected by commercial fishing vessels from six fishing areas, three from the Black Sea (Trabzon, Sinop, Istanbul), one from the Marmara Sea (Bandırma Gulf) and two from the Aegean Sea (Edremit Gulf, Izmir Gulf) between November 2001 and January 2002. Our data were subjected to univariate statistics of variance (ANOVA) and discriminant function analysis (DFA) by using SPSS 21 software version. Plotting DF1 and DF2 revealed that the Marmara stock was clearly separated from others in the discriminant space. The proportion of specimens correctly classified into their original group was the highest (70%) for the Marmara Sea samples. The most important discriminative otolith characters in distinguishing between the groups for the first and second discriminant functions were otolith width and otolith length. The Marmara Sea is the passageway between the Black Sea and Aegean Sea, and currents or water masses play an important role in its environmental conditions (e.g. temperature, salinity, food). Significant difference in the Marmara sea population may be attributed to geographical and environmental conditions suggesting separate management strategies for the resource sustainability.

**Keywords:**
*Engraulis encrasicolus*, anchovy, otolith shape analysis, stock discrimination.
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## Introduction

Stock structure analysis is of primary importance in developing an optimal strategy for its management. Various stock identification techniques have been employed to elucidate the temporal and spatial discreteness of fish stocks (Ihssen et al., 1981; Maclean & Evans, 1981; Nelson et al., 1989; Pawson & Jennings, 1996; Ayvazian et al., 2004). In recent years, otolith shape analyses have been shown to be promising tools for stock identification (Campana & Casselman, 1993; Turan, 1999; Begg & Brown, 2000; Tuset et al., 2003).Genetic methods may not be sensitive enough to detect stock structure because of high gene flow, otolith shape analysis might be a useful tool to identify stock structures as its geographical variation may be related to phenotypic local adaptation. Therefore, it can be considered to be a tool for species and stock discrimination (Cardinale et al., 2004).

Stock identification by truss network analysis is a practically useful and an effective strategy for the description of the body shape in comparison to the traditional morphometric method (Cadrin, 2005). It is effectively used to discriminate the stocks and differentiate between the population's shapes (Stratuss & Bookstein 1982).

Engraulis encrasicolus, is a small pelagic coastal marine fish largely spread from the North Sea to central Africa, including the entire Mediterranean and the Black and Azov Seas (Whitehead et al., 1988). As a consequence of its broad distribution and the existence of oceanographic barriers, the species may be composed of multiple disjunct populations. There have been a number of population structure analyses of *E. encrasicolus* carried out in Mediterranean and Atlantic waters which report morphometric and genetic differences between populations (Spanakis et al., 1989; Bembo et al., 1996, Magoulas et al., 1996; Pla et al., 1996; Tudela, 1999; Bouchenak-Khelladi et al., 2008; Sanz et al., 2008; Kristoffersen & Magoulas, 2008). Besides, in many studies otolith shape evaluated based on morphometric measurements (Russ 1990; Tuset et al., 2006; Zengin et al., 2015). The aim of this study is to characterize the stocks of anchovy in Turkish waters by using truss network and otolith shape indices.

## Material and Methods

A total of 300 anchovy specimens were collected by commercial fishing vessels from six fishing areas, three from the Black Sea (Trabzon, Sinop, Istanbul), one from the Marmara Sea (Bandırma Gulf) and two from the Aegean Sea (Edremit Gulf, Izmir Gulf) between November 2001 and January 2002 (Table 1; Figure 1).

Table 1. Sampling details of *E. encrasicolus* used in this study.

| Sampling area | Abbreviation | Sample size | Sex (M/F) | Mean STL | Range of STL |
|---|---|---|---|---|---|
| Eastern Black Sea (Trabzon) | BS1 | 50 | 28/22 | 10.48±0.07 | 9.45-11.7 |
| Central Black Sea (Sinop) | BS2 | 50 | 16/34 | 10.04±0.09 | 8.75-11.35 |
| Western Black Sea (Istanbul) | BS3 | 50 | 11/39 | 10.28±0.06 | 9.35-11.05 |
| Marmara Sea (Bandırma) | MS | 50 | 43/7 | 11.34±0.06 | 10.5-12.1 |
| Northern Aegean Sea (Edremit) | AS1 | 50 | 16/34 | 10.34±0.05 | 9.5-11.2 |
| Aegean Sea (İzmir) | AS2 | 50 | 18/32 | 10.14±0.06 | 9.15-11.45 |

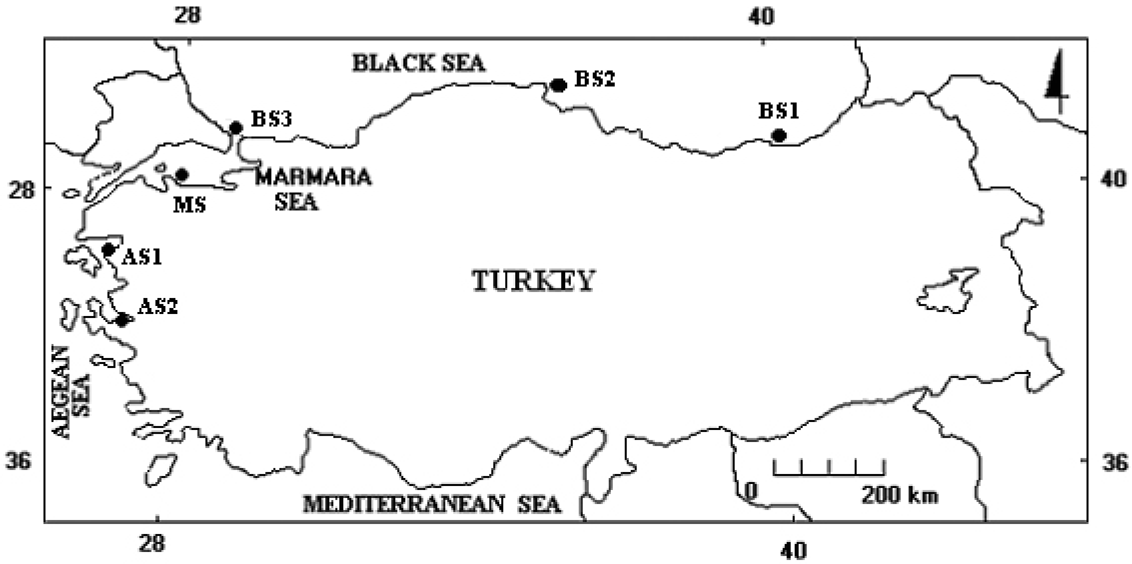


Figure 1. Sampling locations of anchovy. Abbreviations of the locations are given in Table 1.

Following the capture, samples were placed individually into plastic bags and were kept deep-frozen (-20 °C) until transportation to the laboratory. Standard length was recorded to the nearest 0.1 cm and both sagittal otoliths were removed and cleaned in water before drying and storing in envelopes. To maintain consistency and avoid asymmetric effects, only the left sagittal otolith was used for otolith shape analysis, whereas the right otolith was stored as a replacement sample( Legua et al., 2013).

The weight of the undamaged and cleaned otoliths was measured on Mettler analytical balance (to the nearest 0.01 mg). Morphometric data were [otolith length (OL), otolith width (OW)] collected using the Truss network system (Figure 2) and binocular microscope.

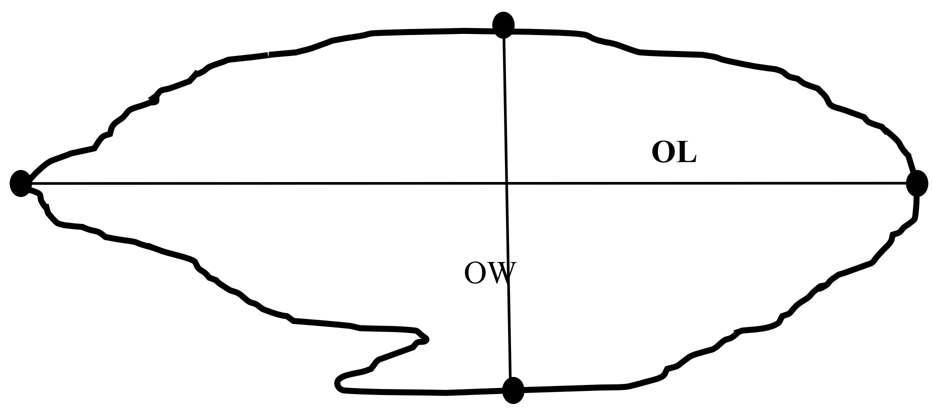


Figure 2. Locations of the landmarks defining the truss network on anchovy otolith.

Then the our data were subjected to univariate statistics of variance (ANOVA) and discriminant function analysis (DFA) by using SPSS 21 software version. One-way analysis of variance (ANOVA) was performed for the comparison of the morphometric differences between the two sexes. Discriminant function analysis (DFA) was used to determine the dissimilarity between populations.

**Results**

Plotting DF1 and DF2 revealed that the Marmara stock was clearly separated from others in the discriminant space (Figure 3).

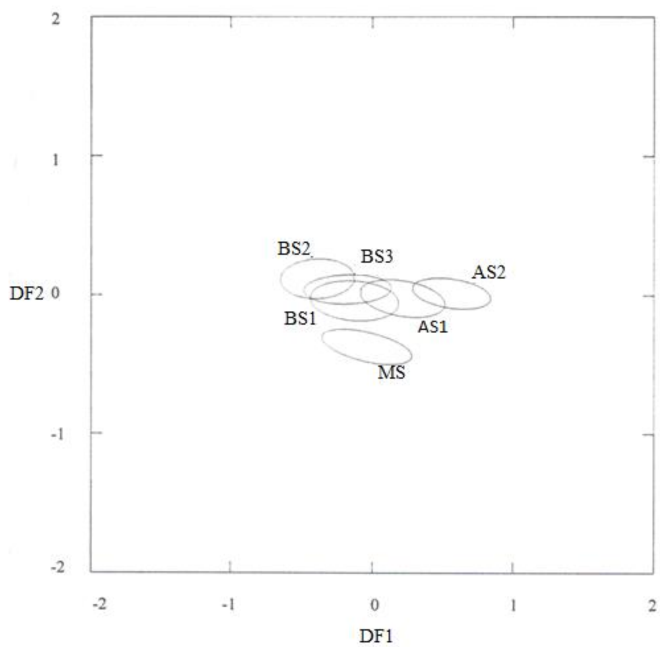


Figure 3. Discriminant function analysis plot with 95% confidence ellipses for otolith shape analysis.

The most important discriminative otolith characters in distinguishing between the groups for the first and second discriminant functions were otolith width and otolith length (Table 2).

Table 2. Results of discriminant function analysis (DFA). (variables ordered by activity degrees in distinguishing of populations)

| | Function | | |
|---|---|---|---|
| Characters | DF1 | DF2 | DF3 |
| Otolith width | 0.868 | 0.138 | 0.478 |
| Otolith length | 0.809 | 0.402 | 0.429 |
| Otolith weight | 0.530 | 0.839 | 0.127 |

Using these otolith characters each specimen could be classified correctly to the original populations with an accuracy of 35% (Table 3). The proportion of those correctly classified into their original group was highest (70%) for the Marmara sea sample (MS).

Table3. Correct classification showing the percentage of specimens classified in each group

| Samples | Group | | | | | |
|---|---|---|---|---|---|---|
| | BS1 | BS2 | BS3 | MS | AS1 | AS2 |
| BS1 | **6** | 34 | 8 | 14 | 28 | 10 |
| BS2 | 2 | **52** | 4 | 8 | 10 | 24 |
| BS3 | 4 | 40 | **8** | 10 | 20 | 18 |
| MS | 4 | 6 | 14 | **70** | 2 | 4 |
| AS1 | 4 | 14 | 6 | 2 | **62** | 12 |
| AS2 | 2 | 24 | 6 | 8 | 48 | **12** |

## Discussion

The results obtained from otolith characters in this work indicate the existence of morphologically differentiated groups of *E. encrasicolus* in Turkish territorial waters. Marmara Sea (MS) sample exhibited a marked separation from all others for otolith characters. The Marmara Sea is the passageway between the Black Sea and Aegean Sea, and currents or water masses play an important role in its environmental conditions (e.g. temperature, salinity, food). Most authors agree that environmental conditions play the largest part in determining morphological variation (Winans, 1984). Hence the variation observed in Marmara Sea samples (MS) may be attributable to the productivity and temperature differences within this sea, presumably representing growth and development in contrasting waters. Environmental factors such as sea temperature and food avail-ability have been linked to spatial variation in fish growth rates(Munk et al., 1991; Campana & Casselman, 1993; Gallegoet al., 1999; Bailey & Heath, 2001; Fox et al., 2003). Variation in growth rate produces corresponding variation in otolith microstructure and shape (Gauldie & Nelson, 1990), due to the proportional relationship between otolith growth and somatic growth (Campana & Neilson, 1985; Burke et al., 2008).

Otolith shape analysis is widely used for fish species identification and stock classification. The morphological structure of anchovy populations was examined with otolith characters using Truss network system. Significant difference in the Marmara sea population may be attributed to geographical and environmental conditions suggesting separate management strategies for the resource sustainability. Therefore, further study can be done on the Sea of Marmara to investigate growth patterns and environmental effects on otolith shape. However, future studies based on the genetic markers and biochemical methods can be used to validate the findings of this study.

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