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# Morphometric analysis of anchovy (*Engraulis encrasicolus* Linnaeus, 1758) otoliths in Georgia and Marmara Seas

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**Abstract**

The objective of the study was to examine whether there is a geographical differentiation between the anchovy stocks as well as to carry out otolith morphology and shape analyses work on the otoliths of anchovies caught in Georgia and Marmara. Otoliths obtained from anchovies (*Engraulis encrasicolus*) caught at two different regions (Georgia and Marmara Sea) were used for otolith morphology and shape analysis in the fishing season of 2011-2012. LAS Image Analysis 3.8 software in MZ75 Leica imaging system was used for biometric measurements and shape analyses of the otoliths. The average lengths of the otoliths were measured to be 2.94±0.263 mm for Georgia anchovy and 2.74±0.123 mm for Marmara anchovy. Additionally, the average widths of the otoliths were measured to be 1.44±0.142 mm and 1.31±0.579 mm for anchovy from Georgia and Marmara, respectively. The shape factor in the otolith shape analyses was measured to be $0.49 \leq F_F \leq 0.74$ for the Georgia anchovy and $0.55 \leq F_F \leq 0.70$ for the Marmara anchovy. The roundness factor for Georgia anchovy was determined to be $1.26 \leq R_D \leq 1.97$ and for the Marmara anchovy it was $1.33 \leq R_D \leq 1.69$. The length/width ratio was determined to be $1.81 \leq A_R \leq 2.28$ for Georgia anchovy and between $1.90 \leq A_R \leq 2.37$ for Marmara anchovy. According to the statistical analyses carried out in the two regions. $A_R$ and $F_F$ values were calculated to be statistically significant ($p<0.05$).

**Keywords**: *Engraulis encrasicolus*, Otolith, Morphometric measurements, Shape analyses, AR, FF, and RD

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## Introduction

Otoliths are found in bony fish and known as ear stones. The otoliths are found inside the inner ear. Otoliths play important roles in hearing, attainment of balance as well as detection of weight and gravity in fish (Campana and Neilson, 1985).

The growth of an otolith occurs when an element or compound in the environment piles up on top of one another on the growth surface of otoliths. Since these elements stay in otoliths continuously and since the growth of an otolith starts before hatching and continues until death, the whole life of a fish is recorded in the otolith. In short, otoliths are black boxes for fish. In this case, otoliths express the water composition in which the fish live as well as its temperature. The increase in otolith growth (either daily or annually) is a perfect tool for the chronological recording of the environmental conditions that the fish have been subject to until today (Campana and Neilson, 1985).

In addition to otolith chemistry; the temperature status of the environment in which the fish live, the determination of anadromous fish, determination of migration paths, determination of age and the use as natural brand or metabolic indicator in the differentiation of stocks are important factors in putting forth the life of fish. Otolith shape is frequently used to determine population or stocks in the past and today (Castonguay *et al*., 1991; Campana and Casselman, 1993; Begg and Brown, 2000; De Vries *et al*., 2002; Tuset *et al*., 2003; Stransky *et al*., 2008).

Anchovies are the subject of this study and they are pelagic species the reproduction and feeding areas of which are different locations in the Black Sea. However, there are various forms for which the wintering areas coincide during the fishing period and these forms create different stocks that are independent of each other (Gücü and Aydın, 2015). Previous studies indicate that the growth ratios (The growth ratio of Azak Anchovy is smaller than that of the Black Sea Anchovy) and otolith indexes (the otolith length/width ratio of the Black Sea anchovy) of these stocks are different (Chashchin, 1996). Egg and larvae surveys carried out put forth the possibility of the existence of a local stock (Niermann *et al*., 1994; Gücü and Aydın, 2015). Studies carried out on the differentiation of anchovy stocks have mostly examined the meristic properties and genetic structure of the fish (Junquera and Perez-Gándaras, 1993; Aka, 2003).

A lot of studies have been done on Anchovy otoliths. Otolith shape analyses and dimensions of the anchovy, *Engraulis encrasicolus* L., in the Black and Marmara Seas (Zengin *et al.*, 2015). Morphometric structuring of the anchovy (*Engraulis encrasicolus* L.1758) in the Black, Aegean and Northeastern Mediterranean Seas (Turan *et al*., 2004). Morphologic and allozyme analyses of european anchovy (*E. encrasicolus*) in the Black, Marmara and Aegean Seas (Erdoğan *et al*., 2009).

However, there is no precedent in the comparative form between different regions, such as Georgia.

The objective of the study is to examine whether there is a geographical differentiation between the anchovy otolith morphology and shape analyses work on the otoliths of anchovies caught in Georgia and Marmara.

## Materials and methods

Otoliths of anchovies caught from two different regions Georgia (5 km west of Poti City) and Marmara (10 km south of İstanbul) have been used for otolith morphology and shape analyses (Fig. 1). The anchovies used for analyses were carefully selected in the same length group and age interval. Required morphometric measurements were taken from 51 anchovies in Georgia with an average length of 11.71±1.551 cm and an average weight of 9.80±3.14 g during the fishing season of 2011-2012. Marmara anchovies were in the similar length group. The average lengths of the anchovy samples from Marmara were measured to be 11.33±0.520 cm and the average weights were 9.73±1.348 g (N=51).

**Figure 1: Marmara Region (10 km south of İstanbul) and Georgia coasts (5 km west of Poti City) from where the anchovies were sampled.**

LAS Image Analysis 3.8 software in the MZ75 Leica imaging system was used for biometric measurements and shape analyses carried out on otoliths. The measurements acquired from the otoliths are given in Fig 2. Right sagitta otoliths were used for measurements.

According to Ponton (2006), maximum otolith length (LO, mm), otolith width (OW, mm), area (A, mm$^2$), perimeter (P, mm) measurements are taken for morphometric analyses which are included in the otolith shape definition. Form factor, roundness and length ratio (Zorica *et al.*, 2010) were calculated for otolith shape definition using the data acquired via measurements.

($F_F$) Formfactor= $4\pi \times Area \div Perimetre^{2}$

($R_D$) Roudness= $4\pi \times Area \div \pi Max\ R^2$

($A_R$) Length ratio = Length÷width

$R^2$= Diameter$^2$

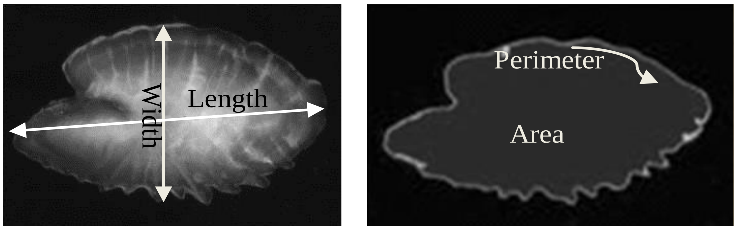


**Figure 2: Measurements of the Georgian anchovy otoliths taken via LAS Image Analysis 3.8.**

LAS Image Analysis 3.8 software was used to acquire the data and Excel, Primer 5.0 and Statistica 8.0 package software programme was used for data analyses.

## Results

Correlation coefficient was determined to be greater for Georgia otoliths during the regression relationship carried out between the length-width measurements for the otoliths obtained from anchovies caught in the Georgia border and the Marmara region (Figs. 3, 4).

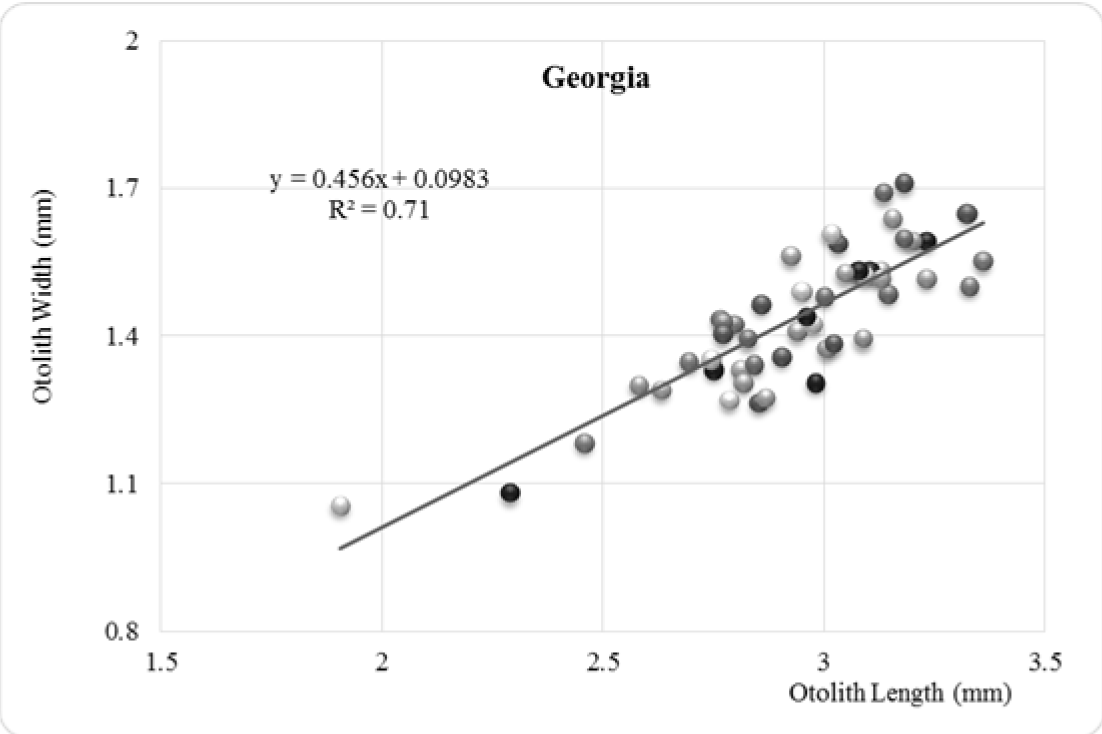


**Figure 3: The length-width measurements for ancovy otoliths (Georgia).**

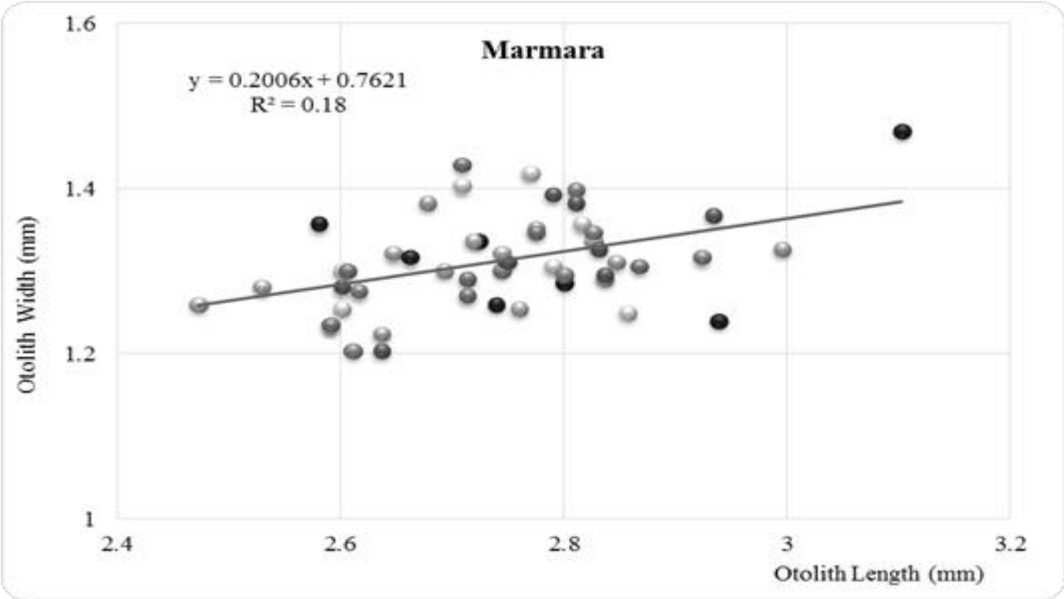


**Figure 4: The length-width measurements for ancovy otoliths (Marmara).**

In this current study, it was observed that average otolith length of Georgian anchovies was 2.94±0.263 mm and Marmara anchovies was 2.74±0.123 mm. Average otolith widths were 1.44±0.142 mm and 1.31±0.579 mm for Georgian and Marmara anchovies, respectively whereas otolith area varied between 1.42 and 3.76 mm² for Georgian anchovies and between 2.16 and 3.11mm² for Marmara anchovies.

Form factor acquired from otolith shape analyses was $0.49 \leq F_F \leq 0.74$ and $0.55 \leq F_F \leq 0.70$ for Georgian and Marmara anchovies, respectively. The roundness factor was found to be $26 \leq R_D \leq 1.97$ for Georgian anchovy and $1.33 \leq R_D \leq 1.69$ for Marmara anchovy whereas the length/width ratio were $1.,81 \leq A_R \leq 2,28$ for Georgia anchovy and $1.90 \leq A_R \leq 2,37$ for Marmara anchovy (Table 1 ), (Figs. 5,6.).

**Table 1: Morphometric measurements of otoliths (otolith length: OL, otolith width: OW, area: A, perimeter: P and shape defining factors (form factor: $F_F$, roundness: $R_D$, length-width ratio: $A_R$) (min-max, average and sd values).**

| | n | OL(mm) | OW(mm) | A(mm²) | P(mm) | $F_F$ | $R_D$ | $A_R$ |
|---|---|---|---|---|---|---|---|---|
| Georgia | 51 | 2.94 ± 0.263 | 1.44 ± 0.142 | 2.95 ± 0.501 | 7.61 ± 0.802 | 0.64 ± 0.045 | 1.48 ± 0.110 | 2.05 ± 0.111 |
| | | (1.90 – 3.36) | (1.05 – 1.71) | (1.42 - 3,76) | (4.91 – 9.54) | (0.49 – 0.74) | (1.26 – 1.97) | (1.81 – 2.28) |
| Marmara | 51 | 2.74 ± 0.123 | 1.31 ± 0.579 | 2.49 ± 0.173 | 7.04 ± 0.332 | 0.63 ± 0.032 | 1.49 ± 0.076 | 2.09 ± 0.010 |
| | | (2.47 – 3.10) | (1.20 – 1.47) | (2.16 – 3.11) | (6.48 – 8.29) | (0.55 – 0.70) | (1.33 – 1.69) | (1.90 – 2.37) |

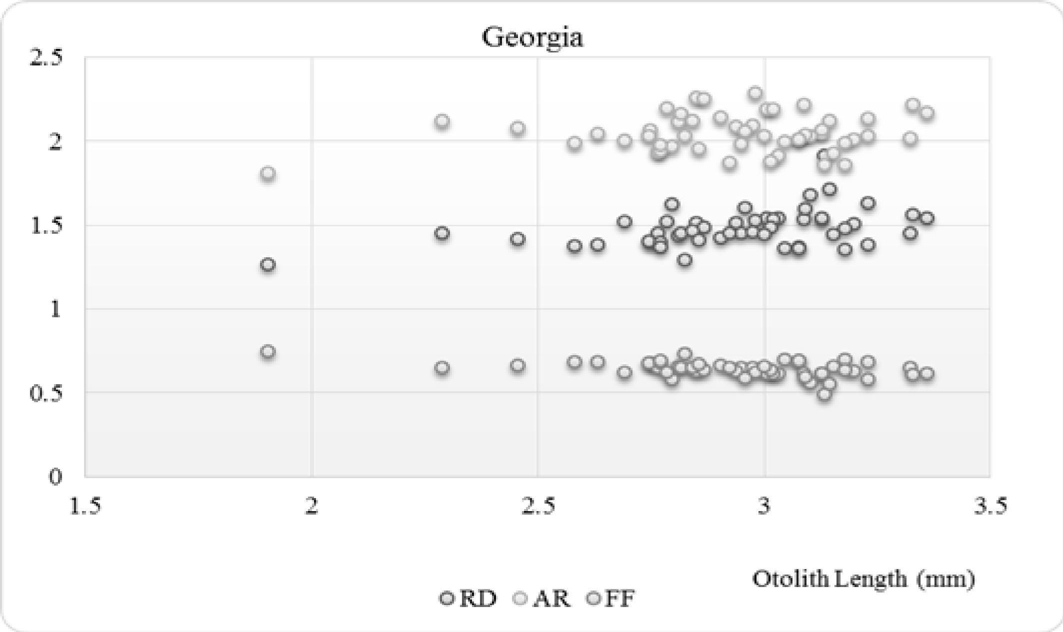


**Figure 5: Relationship between otolith length and otolith shape factors (form factor: $F_F$, roundness: $R_D$, length-width ratio: $A_R$) for the Georgian anchovy.**

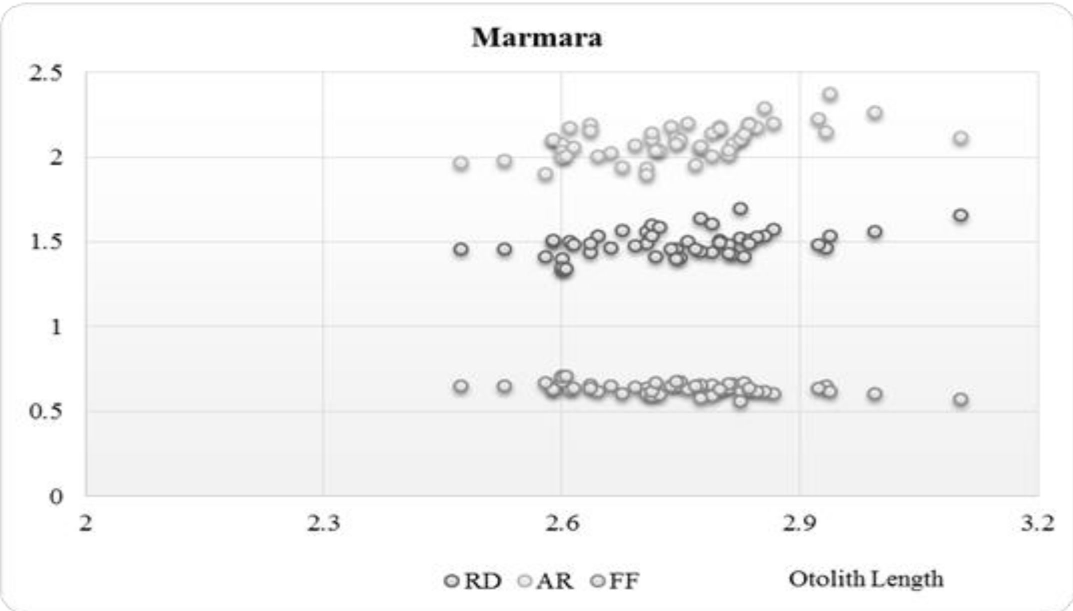


**Figure 6: Relationship between otolith length and otolith shape factors (form factor: $F_F$, roundness: $R_D$, length-width ratio: $A_R$) for the Marmara anchovy.**

Correlation values were determined to be low as a result of the analyses carried out between the otolith shape factors and otolith lengths on two stocks that were thought to be different and the highest length/width ratio was determined for Marmara anchovy as 0.55 (Table 2). The roundness factor was determined to be insignificant during the analysis (t–test) carried out on the shape factors between groups (Georgia-Marmara anchovy) whereas it was determined at $p<0.05$ between otolith length/width ratio and form factors (Table 2). The PCA analyses carried out also put forth the difference between the anchovy (Georgia-Marmara) otoliths (Fig. 7).

**Table 2: Correlation value for the otolith length and shape factors (form factor: $F_F$, roundness: $R_D$, length-width ratio: $A_R$) of both regions.**

| Otolith shape factors | $F_F$ | | $R_D$ | | $A_R$ | |
|---|---|---|---|---|---|---|
| | r | P | r | P | r | P |
| Georgia anchovy | 0.45 | <0.001 | 0.43 | <0.001 | 0.16 | >0.001 |
| Marmara anchovy | 0.43 | <0.001 | 0.43 | >0.001 | 0.55 | >0.001 |
| Georgia-Marmara anchovy (t-test) | <0.05 | | >0.05 | | <0.05 | |

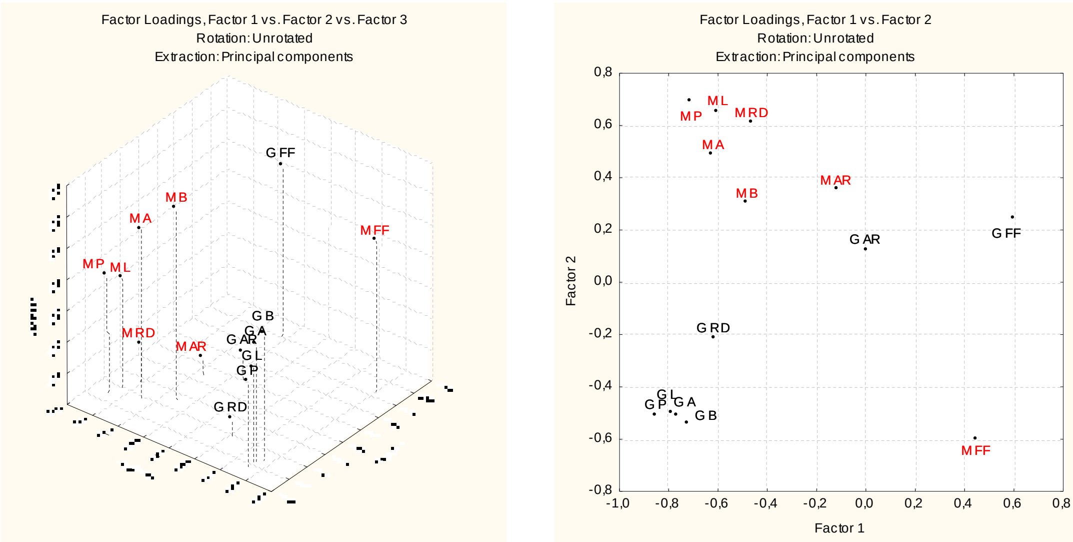


**Figure 7: PCA analysis on Marmara and Georgia region anchovy otoliths and its 3D and 2D view. (M: Marmara, G: Georgia, L: otolith length, B: otolith width, A: area, P: perimeter, $F_F$: form factor, $R_D$: roundness, $A_R$: length-width ratio).**

## Discussion

Regional similarities and differences of the morphometric analyses carried out for anchovy otoliths from Marmara and Georgia regions have been compared in this study. It has been put forth in otolith morphology studies used to determine fish stocks that length, area and perimeter measurements are the most important parameters. Whereas other similar studies have stated that weight and morphometric measurements are important as well (Bolles and Begg, 2000; Torres *et al*., 2000; De Vries *et al*., 2002; Cardinale *et al*., 2004; Tuset *et al*., 2006; Jónsdóttir *et al*., 2006). Torres *et al*. (2000) carried out studies on the morphology (size and shape) of saccular otolith by means of image analysis on three species of the genus *Merluccius*. As a result of the study, the morphometric analysis of sagittae otoliths acted as a clear diagnostic tool to differentiate individuals from different geographical distribution areas.

Even though studies have been carried out on otoliths regarding the stock differentiation of fish species, it has been observed that the number of studies which showed differences among the anchovy stocks is not enough to understand the majority.

Previous studies on fish stocks have put forth that the growth ratio for the Azak anchovy is smaller than that of the Black Sea anchovy. It is also shown that the otolith indexes (Otolith length/width ratio for Black Sea anchovy is 2.15 whereas that for Azak anchovy is 1.96) are different (Chashchin, 1996). Chashchin (1996) have put forth in the study carried out on otolith length/width ratio that whereas the $A_R$ value for some of the samples reflects Azak stock with the value of 1.96, it was stated to be Black Sea anchovy stock with a ratio of 2.15. Zorica *et al.* (2010) have determined the otolith shape factors for anchovies (*E. encrasicolus*) in the Adriatic as $F_F$=0.744, $R_D$=0.758 and $A_R$=0.685 during the study carried out in 2010.

The otolith length/width ratio for Georgia anchovy was determined to be $1.81 \leq A_R \leq 2.28$ in this study and for Marmara anchovy it was determined to be $1.90 \leq A_R \leq 2.37$. When previously reported studies were compared to this study for $A_R$ values, the results were similar with those determined in the study by Chashchin (1996), but the values obtained were greater than the results of Zorica *et al*. (2010).

Salas and Lenfant (2007) stated that otolith shape in the European anchovy can be used as a potential marker for stock discrimination of species. Differences observed at the end of this study suggest that the European anchovies in the Bay of Biscay are members of at least three different cohorts. The biological significance of these distinctions and their possible implications for management of the European anchovy fishery, however, are not immediately apparent. Whether this means that the European anchovy in the Bay of Biscay should be managed every year as separate stocks remains disputable. In general, the temporal differences in otolith shape in *E. encrasicolus* could be the result of both environmental and genetic effects although the latter cannot be inferred from this study. Daryaei *et al.* (2013) have carried out a study on the anchovy (*Encrasicholina punctifer*) stocks in Basra bay and Sea of Amman examining the stock structure via otolith image analysis and mathematical equalities. As a result, they have stated that when there is no statistically significant difference between shape analyses, the stocks in the two regions will probably be the same.

Regional differences and similarities were examined using morphometric and shape analysis results acquired in this study and to this end t- test and PCA analysis have been carried out. The statistically significant difference between $A_R$ and $F_F$ values acquired as a result of the study led us to think that the anchovy stocks in Georgia and Marmara can be different. The results of the all-purpose morphological analyses carried out on anchovy otoliths will contribute to the studies that will be carried out in the future.

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