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Using Scanning Electron Microscopy and Length-Otolith Size Relationship for Otolith Morphological Description of *Capoeta banarescui* Turan et al., 2006 and *Squalius cephalus* (L., 1758) (Actinopterygii: Cyprinidae) from Turkey

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Abstract: The aims of this study were to: 1) identify unstudied otolith morphometry and 2) determine the relationship between otolith size-fish length in *Capoeta banarescui* and *Squalius cephalus.* A total of 346 samples were caught from the Lower Melet River and Çamlıdere Dam Lake. Fork length (FL) of the samples was measured. Asteriscus otolith pairs were removed from each individual. Otolith variables such as weight, length, width, area, and perimeter were measured for undamaged and clean otolith pairs. Differences between right and left otoliths were tested using a paired t-test. The difference between right and left otoliths was not statistically significant (P>0.05) for otolith weight (WO), length (OL) and width (OW) measurements for both species. Shape indices, such as form factor, roundness, aspect ratio, circularity, rectangularity and ellipticity, were calculated using otolith measurements. Additionally, otolith morphologic features were examined using Scanning Electron Microscopy (SEM). According to SEM, otolith surface and margin were different for *C. banarescui* and *S. cephalus*. The highest correlation of fork length-otolith dimensions were calculated as OL = 0.1179FL+0.5389 (r^2=0.88) for *C. banarescui* and WO = 0.0005FL-0.0071 (r^2=0.81) for *S. cephalus*. Otolith length was more suitable than otolith weight for predicting of *C. banarescui,* while otolith weight was the best predictor of *S. cephalus.*

Key words: Morphology; biometry; SEM; *Capoeta banarescui*; *Squalius cephalus*

Introduction

The otoliths are three calcareous structures located in the inner ear of most teleost fishes. They are named sagitta, asteriscus and lapillus, based on the location in the ear. In most marine bony fishes, while the sagittae are the biggest pair of otoliths, the lapillii are the smallest, while in Cypriniformes the asteriscii are the largest otoliths, the sagittae are the smallest (Campana 2004).

The otolith is one of the most important bony structures to understand the fish life and population structures for the ichthyologists (Chilton & Beamish 1982). Otolith morphology and morphometry studies are very limited, despite the potential use of otoliths in population dynamics, estimation of growth parameters and age determination (Tuset et al. 2008). Otoliths are bony structures which are commonly used in morphological and taxonomical studies because of their dimensions, morphological diversity, chemical composition and ease of collection.

The estimated relationship between fish length and otolith biometry can be used to determine fish length during development based on otolith morphometry. The otoliths can remain undigested for long periods in carnivore fish stomach and is possible to estimate prey fish size based on otoliths found in the stomach (Bostanci 2009). Various studies used otolith analyses (Tuset et al. 2003, Campana 2004,

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Ponton 2006, Tuset et al. 2008, Bostanci 2009, Zorica et al. 2010, Skeljo & Ferri 2012, Bostanci et al. 2015, 2016a, Yilmaz et al. 2015, Yoraz 2015, Yazicioğlu et al. 2017, Zengin et al. 2017).

Turkey has a very rich freshwater fish fauna because of the great number of water bodies such as rivers, lakes and reservoirs. Turan et al. (2006) have determined some metric and meristic characteristics of *Capoeta banarescui* Turan, Kottelat, Ekmekçi & Imamoglu, 2006. However, otolith biometry and morphology of the species are largely unknown. Although *Squalius cephalus* (L., 1758) is widely a distributed cyprinid species in European freshwaters (Froese & Pauly 2017), there is no detailed information about shape indices and otolith morphology of the species.

The aim of the present study is to explore unstudied otolith biometry and morphology using SEM and image analysis system and to determine the relationships between fork length and otolith variables (weight, length and width) of *C. banarescui* and *S. cephalus*.

Materials and Methods

All specimens of *C. banarescui* and *S. cephalus* were caught using fishing cast nets with different mesh sizes and a Samus-725mp brand shocker from the Lower Melet River (40º18'-41º08'N - 36º52'-38º12'E) and the Çamlıdere Dam Lake (40°24'1.16"N - 32°21'30.66"E), Turkey. Each fish was cleaned from external materials. For each fish, fork length (FL) was measured to the nearest 0.1 cm using digital calliper. Otolith pairs were removed from each individual. Undamaged and clean pairs were weighed to the nearest 0.0001 g on Precisa XB220A brand balance. Otolith length (OL) (mm), otolith width (OW) (mm), otolith area (A) (mm^2) and otolith perimeter (P) (mm) were measured using Leica S8APO brand microscope with a computer-connected camera system for the selected otolith pairs (Fig. 1).

Differences between right and left otoliths were tested using a paired t-test. The shape indices such as form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R) and ellipticity (E) were calculated following Tuset et al. (2003) and Ponton (2006); see Table 1.

Before scanning, the otolith pairs were attached to a stub using double-sided carbon tape. The immobilised otoliths were coated with 13.5 nm gold and were analysed using SEM (JMS-6060LV brand microscope) at 5.0 KV in the laboratory of the Biology Department at the Ondokuz Mayıs University (Figs. 2, 3).

Relationships between fork length and otolith weight, OL and OW were estimated using linear regression models. MINITAB 16.0 program was used for all statistical analyses.

Results

A total of 346 specimens were examined in the current study (*C. banarecui*, n=247; *S. cephalus*, n=99). Captured samples were transported to the

Table 1. Definition of shape indices used in the present study.

Shape indices	Formula
Form factor (FF)	$FF= 4 \pi A P^{-2}$
Roundness (RD)	$RD= 4 A (\pi OL^2)^{-1}$
Aspect ratio (AR)	$AR= OL\ OW^{-1}$
Circularity (C)	$C= P^2 A^{-1}$
Rectangularity (R)	$R= A (OL\ OW)^{-1}$
Ellipticity (E)	$E= (OL – OW) (OL+OW)^{-1}$

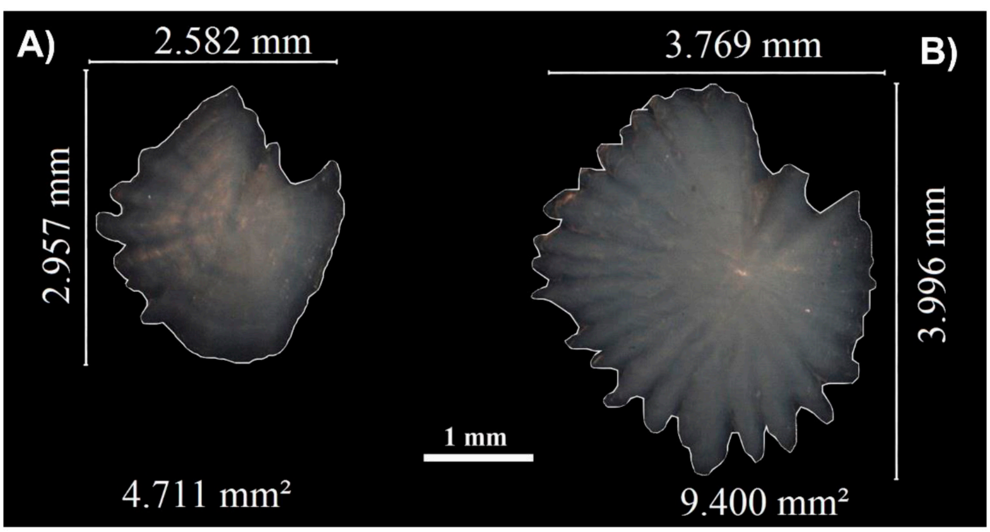

Fig. 1. Length, width, area dimensions of *Capoeta banarescui* (A), *Squalius cephalus* (B)

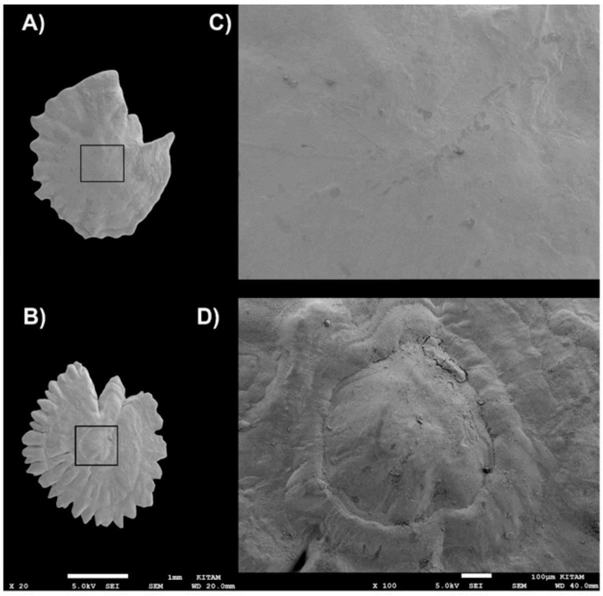

Fig. 2. SEM images of *Capoeta banarescui* otoliths' distal surfaces (A, C) and *Squalius cephalus* otoliths' distal surfaces (B, D).

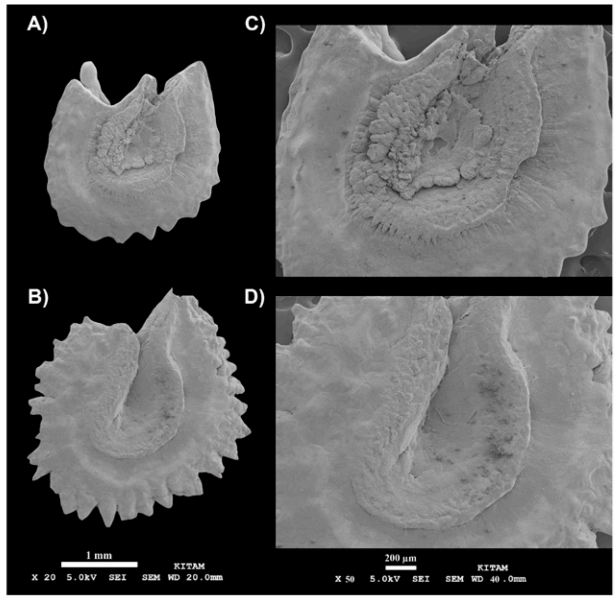

Fig. 3. SEM images of *Capoeta banarescui* otoliths' proximal surfaces (A, C) and *Squalius cephalus* otoliths' proximal surfaces (B, D).

Hydrobiology Laboratory at the Ordu University. Fork length was determined 7.2-20.0 cm for *C. banarecui* and 20.1-35.3 cm for *S. cephalus*.

When the right and left otoliths were evaluated separately by using the paired-t test for both species, the difference between them was not statistically significant (P>0.05) for otolith weight, length and width (Fig. 1). For this reason, the right otolith was preferred for all further analyses. WO, OL, OW, A and P mean values were determined and presented for *C. banarescui* (Fig. 1A) and for *S. cephalus* (Fig. 1B) in Table 2. The right otolith weight values were between 0.0006-0.0023 g and 0.0030-0.0116 g for *C. banarescui* and *S. cephalus*, respectively (Table 2).

Based on the right otolith measurements, FF, RD, AR, C, R and E were calculated and presented for both species (Table 3). The form factor is an important shape index because it allows estimating the surface area irregularity. When its mean value is 1.0, it is a perfect circle. It is called irregular when the value is < 1.0. Moreover, circularity and roundness also give information about features of the perfect circle of the otoliths (Tuset et al. 2003). Otolith length is very influential on shape indices. E. g., while the otolith length increases, form factor, circularity and roundness values decrease in marine species (Zorica et al. 2010). In many studies, the otolith shape indices were calculated for both freshwater and marine species (Gümüş & Kurt 2009, Bostanci et al. 2015, 2016a, Kontaş et al. 2016, Yedier et al. 2016).

Distal and proximal surfaces morphologies of otoliths of *C. banarescui* and *S. cephalus* were examined using the scanning electron microscope (SEM; Figs. 2, 3) for the first time. Otoliths of *C. banarescui* are thin and very fragile, generally discoidal with crenate margins (Fig. 2A), with smooth and entirely convex distal surface (Fig. 2C). Their proximal side is also with smooth surface and is entirely concave in *C. banarescui* (Fig. 3A). The well-defined rostrum and antirostrum were detected for *C. banarescui*. The sulcus acusticus is round, with curved terminal end and it is presented in detail (Fig. 3A, 3C).

Otoliths of *S. cephalus* are thick, with serrate margins (Fig. 2B). While the otolith distal side has undulated surface and is entirely convex (Fig. 2D), the proximal side has irregular surface and is entirely concave (Fig. 3B). Sulcus acusticus is also round with curved terminal end for *S. cephalus* (Fig. 3B, 3D). When the otoliths were compared in detail according to their margins, crenate and serrate margins were determined for *C. banarescui* and *S. cephalus*, respectively. The sulcus acusticus is more prominent in *S. cephalus* than in *C. banarescui.* The electron microscope images were successfully used to determine otolith morphology of *C. banarescui* and *S. cephalus*. The results are correlated with previous studies on *Alburnus chalcoides, A. escherichii, A. mossulensis* and *A. tarichi* (see Bostanci et al. 2015), *Engraulis encrasicolus*, *Merlangius merlangus*, *Mullus barbatus, Psetta maxima* and *Sprattus sprattus* (see Yoraz 2015). In the current study, otolith morphologic features, biometry and their SEM images were presented for the first time in *C. banarescui* and *S. cephalus*.

Table 2. Descriptive statistics of the otolith measurements for *Capoeta banarescui* and *Squalius cephalus* (SE, standard error; SD, standard deviation).

Species	Variables	Mean	SE	SD	Min.	Max.
Capoeta banarescui	Otolith Weight (WO) (g)	0.0012	0.000021	0.000325	0.0006	0.0023
	Otolith Length (OL) (mm)	2.1522	0.0189	0.2909	1.4480	2.9530
	Otolith Width (OW) (mm)	1.8782	0.0154	0.2373	1.2840	2.5250
	Otolith Area (A) (mm^2)	2.6149	0.0403	0.6205	1.2650	4.0070
	Otolith Perimeter (P) (mm)	7.0021	0.0740	1.1388	4.3980	9.9650
Squalius cephalus	Otolith Weight (WO) (g)	0.0054	0.000216	0.001634	0.0030	0.0116
	Otolith Length (OL) (mm)	3.6384	0.0571	0.4308	3.0370	5.1650
	Otolith Width (OW) (mm)	3.1681	0.0386	0.2916	2.7240	4.0870
	Otolith Area (A) (mm^2)	7.3900	0.1960	1.4790	5.3790	12.727
	Otolith Perimeter (P) (mm)	15.207	0.2730	2.0640	11.891	21.500

Table 3. Descriptive statistics of six shape indices of otoliths for *Capoeta banarescui* and *Squalius cephalus* (SE, standard error; SD, standard deviation)

Species	Variables	Mean	SE	SD	Min.	Max.
Capoeta banarescui	Form Factor (FF)	0.6716	0.00454	0.06989	0.4838	0.8591
	Roundness (R)	0.7129	0.00301	0.04632	0.5775	0.8303
	Aspect Ratio (AR)	1.1464	0.00432	0.06660	1.0087	1.3985
	Circularity (C)	18.916	0.13500	2.07900	14.620	25.961
	Rectangularity (R)	0.6396	0.00158	0.02431	0.5510	0.7116
	Ellipticity (E)	0.0673	0.00184	0.02830	0.0043	0.1662
Squalius cephalus	Form Factor (FF)	0.4052	0.00775	0.05850	0.3074	0.5414
	Roundness (R)	0.7091	0.00649	0.04898	0.5629	0.7948
	Aspect Ratio (AR)	1.1475	0.00851	0.06420	1.0272	1.3442
	Circularity (C)	31.638	0.60700	4.58500	23.198	40.855
	Rectangularity (R)	0.6365	0.00243	0.01838	0.5940	0.6674
	Ellipticity (E)	0.0679	0.00367	0.02770	0.0134	0.1468

The relationship between fish length and otolith biometry for *C. banarescui* is presented in Table 4. Otolith length, width and weight were used in regression calculations to relate fish length and otolith biometry. High regression coefficient (r^2) suggests the growth of bony structure have continued proportionally with body growth. This was not the case for *C. banarescui*. When the equation of the relationship between fish length and otolith biometry was calculated, the least value of the coefficient of regression was attained for otolith weight (Table 4). The highest was the strength of the fork length-otolith length relationship (r^2=0.88). This result indicates that otolith length and width should be used rather than the otolith weight for estimating fish length. In addition, similar results were obtained for one of the cyprinid fish, *B. tauricus*: the relationship between fish length and otolith weight (r^2=0.65) is lower than the other relationships between otolith length (r^2=0.80) (Kontaş & Bostanci 2015). Moreover, otolith width was determined as the strongest indicator for *A. boyeri* population inhabiting Hirfanlı Dam Lake, Turkey (Bostanci et al. 2016b). On the other hand, for *S. cephalus* the highest coefficient value was that for fork length-otolith weight relationship and it was determined for all individuals (r^2=0.81). The lowest coefficient value was determined for all individuals (r^2=0.75) for the fork length-otolith width relationship. The relationships between otolith size (length and width) and body length for five cyprinid fish species, *Abramis brama*, *Blicca bjoerkna*, *Carassius gibelio*, *Chondrostoma regium* and *Scardinius erythrophthalmus*, have been studied for the Lake Ladik (Turkey) and all relationships were highly significant (Yilmaz et al. 2015).

Discussion

Otolith biometry can change from species to species and the presence of this difference should be studied for marine and freshwater fish species. Otolith morphometry and morphology can be useful for species identification and stock discrimination (Campana 2004, Tracey et al. 2006, Dou et al. 2012, Bostanci et al. 2015, Zengin et al. 2015). The otolith variability, such as otolith measurements and shape indices, seem to be associated with some biotic and abiotic factors such as genetic, ontogenetic and environmental factors (water temperature, dissolved oxygen, pH and salinity), as well as biological factors (growth, feeding, sex, population and prey-predator

Table 4. Regression equations between *Capoeta banarescui* and *Squalius cephalus* fork length and otolith variables for all individuals. WO, otolith weight (g); OL, otolith length (mm); OW, otolith width (mm); FL, fork length (cm); r^2, coefficient of regression.

Species		Regression Equation	r^2	Number of fish (n)
Capoeta banarescui	WO	WO = 0.0001FL-0.0002	0.55	247
	OL	OL = 0.1179FL+0.5389	0.88	247
	OW	OW = 0.0917FL+0.6281	0.83	247
Squalius cephalus	WO	WO =0.0005FL-0.0071	0.81	99
	OL	OL = 0.1245FL+0.4822	0.76	99
	OW	OW = 0.084FL+1.0378	0.75	99

relationship) (Campana 2004, Cardinale et al. 2004). Therefore, otolith size and shape should be taken into consideration in fisheries, because they can be inter-intra species specific. In addition, Tuset et al. (2008) reported that otolith shape and size vary among fish species and information about otolith morphometry can provide the characterisation of some ichthyological taxa.

The current study indicates that *C. banarescui* and *S. cephalus* fork length can be obtained reliably from otolith biometry variables, such as length and width; however, otolith weight can be a more reliable character for different fish species. The specific equations we present here can be useful for different studies on population dynamics, food and feeding, yield estimates and even palaeontology. Further studies are needed to examine the otolith morphology for other freshwater and marine species.

References

Bostanci D. 2009. Otolith biometry – body length relationships in four fish species (chub, pikeperch, crucian carp, and common carp). Journal of Freshwater Ecology 24: 619-624.

Bostanci D., Polat N., Kurucu G., Yedier S., Kontaş S. & Darçin M. 2015. Using otolith shape and morphometry to identify four *Alburnus* species (*A. chalcoides, A. escherichii, A. mossulensis* and *A. tarichi*) in Turkish inland waters. Journal of Applied Ichthyology 31: 1013-1022.

Bostanci D., Yilmaz M., Yedier S., Kurucu G., Kontaş S., Darçin M. & Polat N. 2016a. Sagittal Otolith Morphology of Sharpsnout Seabream *Diplodus puntazzo* (Walbaum, 1792) in the Aegean Sea. International Journal of Morphology 34 (2): 484-488.

Bostanci D., Yedier S., Kontaş S., Kurucu G. & Polat N. 2016b. Relationship Between Total Length and Otolith Size in *Atherina boyeri* Risso, 1810 From Hirfanlı Dam Lake (Kırşehir, Turkey). FABA 2016: International Symposium on Fisheries and Aquatic Sciences. Antalya, Turkey: 3-5 November 2016.

Campana S. E. 2004. Photographic atlas of fish otoliths of the Northwest Atlantic Ocean. Ontario: NRC Research Press, 284 p.

Cardinale M., Doering-Arjes P., Kastowsky M. & Mosegaard H. 2004. Effects of sex, stock, and environment on the shape of knowing-age Atlantic cod (*Gadus morhua*) otolith. Canadian Journal of Fisheries and Aquatic Sciences 61 (2): 158-167.

Chilton D. E. & Beamish R. J CHILTON D. E. & BEAMISH R. J. 1982. Age determination methods for fishes studied by the Groundfish program at the Pacific Biological Station. – Canadian Special Publication of Fisheries and Aquatic Sciences, 60: 102pp.

Dou S. Z., Yu X. & Cao L. 2012. Otolith shape analysis and its application in fish stock discrimination: a case study. – Oceanologia et Limnologia Sinica, 43: 702-712.

Froese R. & Pauly D. 2017. FishBase. World Wide Web electronic publication. www.fishbase.org version (Accessed on: 25 Jan 2017).

Gümüş A. & Kurt A. 2009. Age structure and growth by otolith interpretation of *Neogobius melanostomus* (Gobiidae) from Southern Black Sea. Cybium 33 (1): 29-37.

Kontaş S. & Bostanci D. 2015. Morphological and Biometrical Characteristics on Otolith of *Barbus tauricus* Kessler, 1877 on Light and Scanning Electron Microscope. International Journal of Morphology 33 (4): 1381-1386.

Kontaş S., Yedier S., Bostanci D. & Polat N. 2016. *Cyprinion macrostomum* Heckel, 1843 Otoliths and Scales Morphologies Inhabiting Kangal Balıklı Çermik Thermal Spring (Sivas, Turkey). International Conference on Advances in Natural and Applied Sciences. Antalya, Turkey: 21-23 April 2016.

Ponton D. 2006. Is geometric morphometrics efficient for comparing otolith shape of different fish species? Journal of Morphology 267: 750-757.

Skeljo F. & Ferri J. 2012. The use of otolith shape and morphometry for identification and size-estimation of five wrasse species in predator-prey studies. Journal of Applied Ichthyology 28 (4): 524-530.

Tracey S. R., Lyle J. M. & Duhamel G. 2006. Application of elliptic Fourier analysis of otolith form as a tool for stock identification. Fisheries Research 77: 138-147.

Turan D., Kottelat M., Ekmekçi F. G. & Imamoğlu H. O. 2006. A review of *Capoeta tinca*, with descriptions of two new species from Turkey (Teleostei: Cyprinidae). Revue Suisse de Zoologie 113 (2): 421-436.

Tuset V. M., Lombarte A., Gonzalez J. A., Pertusa J. F. & Lorente M. 2003. Comparative morphology of the sagittal otolith in *Serranus* spp. Journal of Fish Biology 63 (6): 1491-1504.

Tuset V. M., Lombarte A. & Assis C. A. 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. Scientia Marina 72 (1): 7-198.

Yazicioğlu O., Yilmaz S., Erbaşaran M., Uğurlu S. & Polat N. 2017. Bony structure dimensions-fish length relationships of pike (*Esox lucius* L., 1758) in Lake Ladik (Samsun, Turkey).

North-Western Journal of Zoology 13 (1): 149-153.

Yedier S., Kontaş S., Bostanci D. & Polat N. 2016. Otolith and scale morphologies of doctor fish (*Garra rufa*) inhabiting Kangal Balıklı Çermik thermal spring (Sivas, Turkey). Iranian Journal of Fisheries Sciences 15 (4): 1593-1608.

Yilmaz S., Yazicioğlu O., Yazici R. & Polat N. 2015. Relationships between fish length and otolith size for five cyprinid species from Lake Ladik, Samsun, Turkey. Turkish Journal of Zoology 39: 438-446.

Yoraz A. 2015. Morphometric Description and Visual Analysis of the Annulus Formation in Sagittal Otoliths of Some Pelagic and Demersal Fish Species in the Middle Black Sea Region Fishery. MSc. Thesis. Samsun, Institute for Graduate Studies in Science and Technology, Department of Biology, Ondokuz Mayıs University, 407 p. (in Turkish).

Zengin M., Saygin S. & Polat N. 2015. Otolith Shape Analyses and Dimensions of the Anchovy *Engraulis encrasicolus* L. in the Black and Marmara Seas. Sains Malaysiana 44 (5): 657-662.

Zengin M., Saygin S. & Polat N. 2017. Relationships between otolith size and total length of bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) in Black Sea (Turkey). North-Western Journal of Zoology 13 (1): 169-171.

Zorica B., Sinovcic G. & Kec V. C. 2010. Preliminary data on the study of otolith morphology of five pelagic fish species from the Adriatic Sea (Croatia). Acta Adriatica 51 (1): 89-96.

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