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Comparison of otolith morphology of invasive big-scale sand smelt (*Atherina boyeri)* from natural and artificial lakes in Turkey

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Abstract

In this study, otolith morphology and otolith contour from big-scale sand smelt, *Atherina boyeri* were analyzed in Lake Eğirdir and Lake İznik (natural lakes), and Hirfanlı Dam Lake (artificial lake). The otolith contour and sixteen otolith morphological characters such as width, depth, shape, sulcus acusticus shape, ostium, cauda, rostrum size-shape, and thickness, antirostrum size-shape, and thickness, anterior and posterior regions, mesial and lateral surfaces were successfully investigated for three total length groups of *A. boyeri* inhabiting the lakes. Intraspecific variation in rostrum shape, rostrum size, rostrum thickness, antirostrum, anterior region, mesial and lateral surfaces were observed for the three total length groups of three *A. boyeri* populations. In the three different lakes, saccular otolith surface morphology of *A. boyeri* was detected such as a flattened surface for Lake Eğirdir, rough surface for Lake İznik, and relatively smooth surface for Hirfanlı Dam Lake using the Scanning Electron Microscope. The otolith contours showed noticeable differences in the three total length groups of *A. boyeri* from the different lakes. Intraspecific variability of the otolith characters and morphology consisting of the otolith surface morphology and otolith contour were presented and compared for the three total length groups of the *A. boyeri* from the different lakes.

Keywords: Morphology, Intraspecific, *Atherina boyeri*, Invasive, Natural and artificial lakes

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Introduction

Non-native invasive species are one of the main global threats to marine and freshwater biodiversity. They are capable of surviving in a variety of different locations, habitats, and conditions because of their unique characteristics to reproduce, to grow, to avoid predators, and to resist disease. The species have high growth and reproduction rates and are well adapted to the local climate, habitat, and land-use patterns (Richardson and Neave, 2009). Correctly identifying invasive species is important to the management of the marine and freshwater habitats. Otoliths are generally correlated with ambient water conditions such as trace element concentrations, temperature, and salinity. The otoliths provide a permanent record reflecting the environmental and ecological conditions experienced by a fish throughout their life. In fisheries science, otoliths are commonly used for the relationships between otolith dimensions and fish length (Bostanci *et al.*, 2017; Yazicioğlu *et al.*, 2017). They are also important biological and taxonomical archives of fish populations in marine and freshwater ecosystems (Tuset *et al.*, 2003).

Otoliths have diverse morphology and contour types with considerable intra and inter-specific variations in marine and freshwater fish. Generally, the use of otolith morphological characteristics can be grouped into three categories. The first category comprises one-dimensional external and internal linear measurements such as nucleus length, otolith length, hyaline bandwidth, and otolith width (Begg and Brown, 2000). The second category includes two-dimensional size measurements such as area and perimeter, and shape indices such as form factor, ellipticity, circularity, roundness, aspect ratio, and rectangularity (Tuset *et al.,* 2003; Ponton, 2006). A third morphological technique examines otolith contours (Hüssy *et al.,* 2016).

Differences in morphological characteristics of otoliths are commonly used in fisheries studies, and also published as indices for specific discrimination and identification of freshwater and marine fish species/ populations. Therefore, numerous otolith atlases and identification guides are published based on this information (Tuset *et al.,* 2008). Previous studies have also shown that otolith morphological characters can be varied in the same species from different localities, habitats, and populations (Bostanci *et al.,* 2015). Several biotic and abiotic factors such as diet, habitat, and genetic differences can influence the otolith morphology (Tuset *et al.,* 2015).

Atherina, a genus known as the silversides in the Atherinidae family, is represented by 5 species worldwide (Froese and Pauly, 2017). Big-scale sand smelt *A. boyeri* Risso, 1810 is a small, short-lived, pelagic fish species commonly found in the Mediterranean, northeast Atlantic, and Turkish inland waters (Froese and Pauly, 2017). *A. boyeri* is uniquely able to tolerate great ranges in salinity concentrations, enabling this species to persist in the

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marine, brackish, and freshwaters. In Turkey, *A. boyeri* is accidentally introduced often to rivers, lakes, ponds, canals, and reservoirs, and it becomes dominant in inland waters because of its high adaptation and reproduction rates (Küçük *et al.,* 2006). Although *A. boyeri* was firstly reported in Sapanca Lake (Turkey), this species widely spread throughout Turkey (Küçük *et al.,* 2012). *A. boyeri* is one of the most commercially–important, invasive fish species in the region (Gençoğlu and Ekmekçi, 2016).

Otolith morphology analysis is still limited for many non-indigenous fish species such as *A. boyeri.* Given the range of expansion of this species to marine and freshwaters, we hypothesize there are differences in otolith morphology among the populations. The main objective of the current study is to examine intraspecific differences in sagittal otolith patterns using the otolith morphology, shape, contour shape, and otolith surface morphology of an invasive *A. boyeri* from natural and artificial lakes in Turkey.

Materials and Methods

All fish samples were collected using fishing nets with different mesh sizes by local commercial fishermen in three lakes: (*****) Lake Eğirdir 38°03′24″N-30°51′58″E (Isparta, Turkey), (******) Lake İznik 40.43°N-29.52°E (Bursa, Turkey), (*******) Hirfanlı Dam Lake 39°16′20″N-33°31′08″E (Kırşehir, Turkey) (Fig. 1). *Atherina boyeri* samples were measured to the nearest 0.01 mm for total length (TL) and weighed to the nearest 0.01 g. Right and left sagittal otoliths were removed, cleaned, and categorized based on the total length of the fish. Total length groups are; I (TL≤70.0 mm), II (TL 70.1–90 mm), and III (TL>90 mm). Otolith pairs were weighed to the nearest 0.0001 g. Otolith length (OL in mm), width (OW in mm), area (OA in mm^2) and perimeter (OP in mm) were measured using Leica S8APO brand microscope with Leica Application Suite software (Ver. 3.7.0, Leica Microsystems Limited, Heerbrugg, Switzerland).

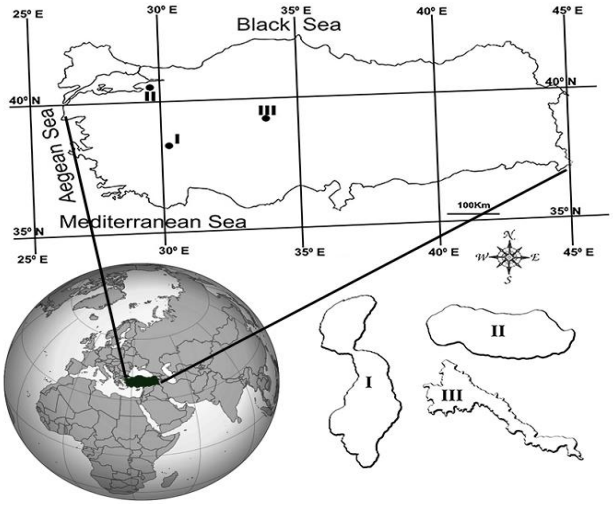

Figure 1: Study area map with sampling locations. I Lake Eğirdir, II Lake İznik, III Hirfanlı Dam Lake.

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Distal and mesial surfaces were photographed for each left otolith. Sixteen otolith metrics such as width, depth, shape, sulcus acusticus, rostrum size and shape, rostrum thickness, antirostrum, anterior, and posterior regions, mesial and lateral surfaces were determined for each TL group. SHAPE software (Ver. 1.3) program was used to extract the contours of the *A. boyeri* otolith outline (Iwata and Ukai, 2002) for the three TL groups. Otoliths were analyzed using SEM at 5.0 kV in order to determine distal otolith surface characteristics in the three *A. boyeri* populations.

Fish length, weight, and otolith morphometric data were tested using the Kolmogorov–Smirnov test for normality and their homogeneity of variance was analyzed using Levene's test. The right and left otoliths were compared and the differences between variables of the pairs were investigated with paired t-test for all samples. Statistical analyses were performed using MINITAB 17.0 software program.

Results

Investigation of morphological sagittal otolith characteristics such as depth, width, shape, sulcus acusticus shape, cauda, ostium, rostrum size-shape, and thickness, antirostrum size-shape, and thickness, mesial and lateral surfaces, anterior-posterior regions is useful in the discrimination of *A. boyeri* species in different freshwater habitats. *A. boyeri* ranged in total length and weight from 58.98-95.0 mm and 1.35-5.39 g, respectively for Lake Eğirdir (n=174), from 63.67-103.63 mm and 1.53-6.05 g, respectively for Lake İznik (n=58), and 41.0-99.0 mm and 0.8-5.4 g, respectively for Hirfanlı Dam Lake (n=124). The weight, length, width, area, and perimeter of undamaged otolith pairs were examined. Statistical analyses revealed a normal distribution (Kolmogorov–Smirnov test, $p>0.05$) and homogeneity of variances were not significant (Levene's test, $p>0.05$).

Table 1: Descriptive statistics (Mean±Standard Error, Min-Max) of left sagittal otolith measurements of *Atherina boyeri* from the Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake.

Otolith Measurements	Lake Eğirdir			Lake İznik			Hirfanlı Dam Lake		
	Group I TL ≤ 70.0 mm	Group II TL 70.1-90 mm	Group III TL > 90 mm	Group I TL ≤ 70.0 mm	Group II TL 70.1-90 mm	Group III TL > 90 mm	Group I TL ≤ 70.0 mm	Group II TL 70.1-90 mm	Group III TL > 90 mm
OWe	0.0016±0.00023 0.0011-0.0022	0.0026±0.00003 0.0017-0.0040	0.0035±0.00017 0.0029-0.0043	0.0011±0.00003 0.0010-0.0015	0.0015±0.00011 0.0010-0.0035	0.0038±0.00012 0.0032-0.0044	0.0011±0.00003 0.0003-0.0020	0.0029±0.00046 0.0017-0.0045	0.0051±0.00010 0.0050-0.0052
OL	2.302±0.0887 2.113 - 2.524	2.742±0.0130 2.345 - 3.175	3.123±0.0704 2.952 - 3.657	2.125±0.0259 1.935 - 2.318	2.380±0.0396 2.051 - 2.961	3.080±0.0325 2.891 - 3.192	2.084±0.0236 1.512 - 2.682	2.809±0.136 2.424 - 3.224	3.395±0.0145 3.381 - 3.410
OW	1.562±0.0704 1.409 - 1.703	1.788±0.0094 0.989 - 2.024	1.935±0.0375 1.730 - 2.104	1.456±0.0175 1.329 - 1.596	1.613± 0.0214 1.427 - 1.944	2.044±0.0462 1.819 - 2.167	1.383±0.0150 1.027 - 1.698	1.828±0.0710 1.569 - 2.040	2.290±0.0210 2.269 - 2.311
OA	2.598±0.228 2.089 - 3.137	3.560±0.0319 2.143 - 4.536	4.268±0.155 3.309 - 4.806	2.240±0.0440 1.916 - 2.517	2.765±0.0819 2.139 - 4.124	4.627±0.131 3.988 - 5.054	2.096±0.0449 1.117 - 3.084	3.791±0.344 2.880 - 5.030	5.432±0.0485 5.384 - 5.481
OP	6.192±0.253 5.656 - 6.814	7.387±0.0337 6.506 - 8.560	8.122±0.163 6.995 - 8.486	6.146±0.0708 5.665 - 6.748	6.835±0.0941 5.984 - 8.171	8.539±0.137 7.835 - 9.050	5.507±0.0612 4.032 - 6.969	7.470±0.359 6.397 - 8.618	9.090±0.116 8.973 - 9.206

(OWe, Otolith weight; OL, Otolith length; OW, Otolith width; OA, Otolith area; OP, Otolith perimeter; TL, Total length).

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Differences between right and left otoliths were not significant (paired t-test, $p>0.05$). Therefore, left otoliths were preferred for further calculations. Each of the TL groups included *A. boyeri* samples from the Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake (Table 1).

Left otoliths morphometric characteristics (weight, length, width, area, and perimeter), mean, standard error, minimum, and maximum values were presented for the three TL groups of *A. boyeri* in the lakes (Table 1).

Common otolith morphological features were used to determine *A. boyeri* sagittal otolith morphology (Fig. 2).

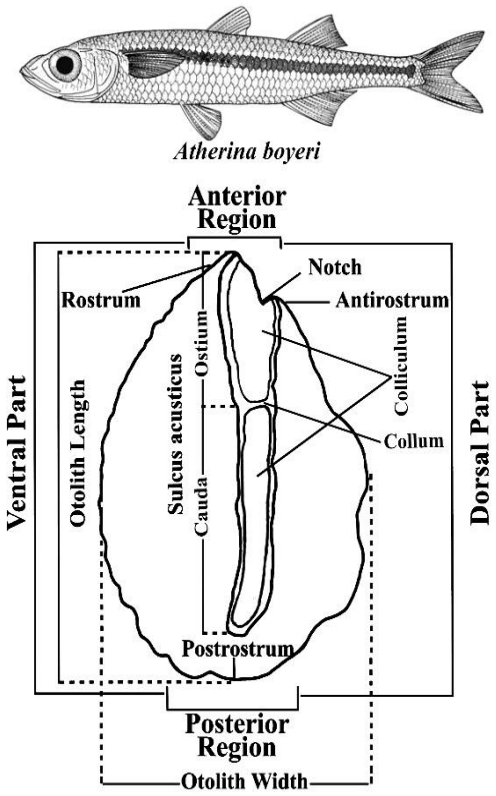

Figure 2: *Atherina boyeri* image and definition of sagittal otolith morphological and morphometric features.

General images of distal otolith surfaces were evaluated for the three TL groups of *A. boyeri* populations in the three different lakes (Fig. 3). Morphological otolith characteristics were variable in the lakes (Table 2).

Generally, sagittal otoliths in *A. boyeri* are elliptical with ostial and median sulcus acusticus. The sulcus acusticus type is heterosulcoid with differentiated ostium and cauda on the otolith surface.

The ostium is funnel-like and shorter than the cauda. The cauda is tubular and slightly curved (Table 2).

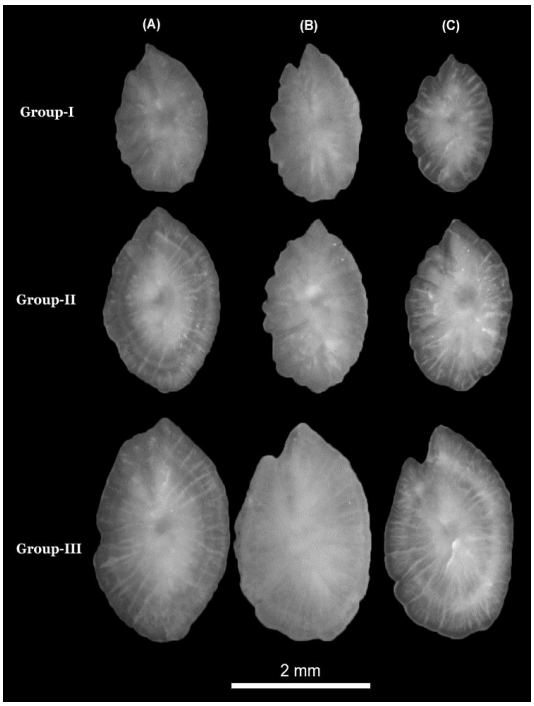

Figure 3: The left sagittal otolith lateral surface images of the total length (TL) groups of the *Atherina boyeri* from Lake Eğirdir (A), Lake İznik (B), and Hirfanlı Dam Lake (C). Group I (TL≤70.0 mm), Group II (TL 70.1-90 mm), and Group III (TL>90 mm).

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Table 2: Left sagittal otolith morphological characteristics of *Atherina boyeri* in the total length groups from Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake.

Otolith morphological characteristics	Lake Eğirdir			Lake İznik			Hirfanlı Dam Lake		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Otolith Shape	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Otolith Width	Moderately thin	Moderately thin	Moderately thin	Moderately thick	Moderately thick	Moderately thick	Moderately thin	Moderately thin	Moderately thin
Otolith Depth	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow
Rostrum Size	Short	Short	Short	Slightly short	Short	Short	Short	Short	Short
Rostrum Shape	Pointed	Pointed tip	Pointed tip	Pointed tip	Pointed	Pointed	Pointed tip	Moderately pointed	Moderately pointed
Rostrum Thickness	Thin	Thin	Thin	Thin	Thin	Broad	Thin	Thin	Thin
Antirostrum Size	Small	Absent	Absent	Board	Small	Board	Small	Absent	Board
Antirostrum Shape	Slightly pointed	-	-	Pointed tip	Slightly pointed	Pointed	Slightly pointed	-	Pointed
Antirostrum Thickness	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin
Mesial Surface	Concave	Concave	Concave	Concave	Concave	Concave	Convex	Convex	Convex
Lateral Surface	Convex	Convex	Convex	Convex	Convex	Convex	Concave	Concave	Concave
Anterior Region	Peaked	Peaked	Peaked	Double Peaked	Peaked	Double Peaked	Double Peaked	Peaked	Double Peaked
Posterior Region	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Sulcus acusticus Shape	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid	Ostial, median, Heterosulcoid
Ostium Cauda	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular	Funnel-like Tubular

Left otolith distal and mesial surface contours and left otoliths sulcus acusticus of each TL group in the lakes were analyzed using SHAPE software. Otolith contours were evaluated to determine the otolith morphology and otolith contour diversity among the three TL groups of the populations (Fig. 4). For I-TL groups, the otolith dorsal margin of the Lake Eğirdir population is less sinuate than the Lake İznik and the Hirfanlı Dam Lake populations. The otolith ventral margin is almost entire for I-TL of the three populations (Fig. 4). For II-TL groups; the dorsal margin is crenate for the Lake İznik and the Hirfanlı Dam Lake populations, however, it is entire for the Lake Eğirdir population. The ventral margin is entire for II-TL of the three populations (Fig. 4). For III-TL groups, the dorsal margin is undulated to entire for the populations, and the ventral margin is entire for III-TL groups of the fish from in the lakes (Fig. 4). Two *A. boyeri* populations (Lake İznik and the Hirfanlı Dam Lake) had a dorsal notch in the sagittal otoliths, which is absent for the Lake Eğirdir population (Fig. 4). SEM images of the left otolith distal surface of each *A. boyeri* from the Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake were prepared for comparative investigation of the otolith morphology and otolith surface morphology (Fig. 5) The left otolith distal surface morphology was identified as flattened, rough, and relatively smooth for the *A. boyeri* from Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake, respectively (Fig. 5).

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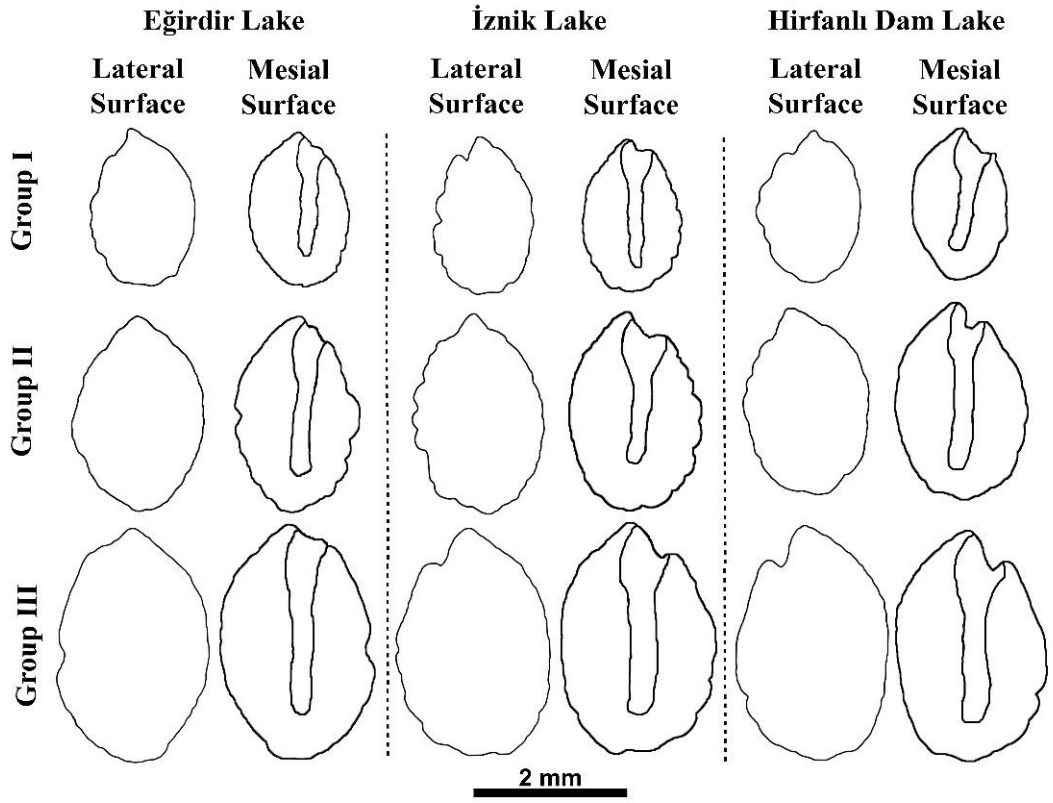

Figure 4: The left sagittal otolith lateral and mesial surfaces and with sulcus acusticus contour shapes of *Atherina boyeri* from Lake Eğirdir, Lake İznik, and Hirfanlı Dam Lake. Group I (TL≤70.0 mm), Group II (TL 70.1-90.0 mm), and Group III (TL>90.0 mm).

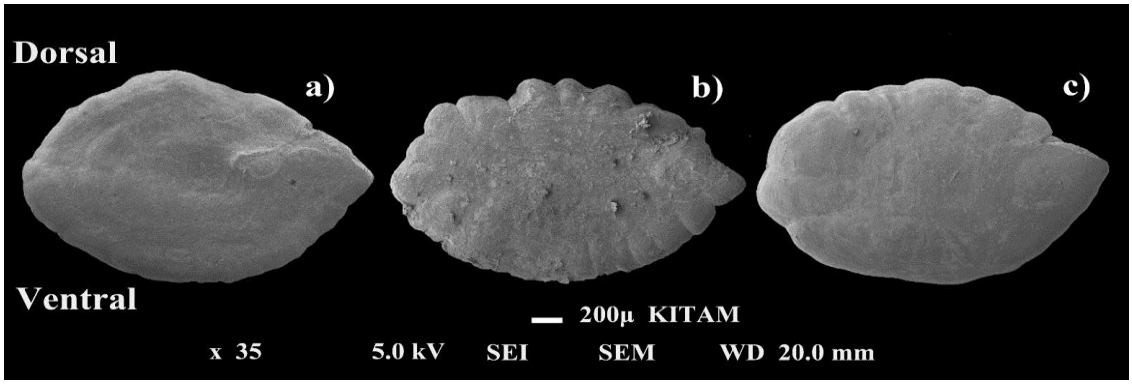

Figure 5: Lateral otolith surface SEM images of the *Atherina boyeri* from Lake Eğirdir (a), Lake İznik (b), and Hirfanlı Dam Lake (c).

Discussion

The current study demonstrates detailed differences among the sagittal otolith morphology, otolith contour, surface morphology and otolith contour wavelets in the three TL groups of the *A. boyeri*. Intraspecific variabilities have been shown among *A. boyeri* populations in the present study. Many studies have concluded that the otolith morphology is associated with more than one factor, such as the ontogenetic, and genetics of the species (Tuset *et al.*, 2003), environmental factors, habitat, temperature, seasonal variations and diet (Campana, 2001), as well as the biological and ecological behavior of fish (Tuset *et al.*, 2003). The

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environmental factors and genetic differences directly or indirectly affect the otolith character; therefore, the characters such as morphology, surface shape, and otolith contour are the unique specialties of the otoliths (Figs. 3-5). Observed differences in *A. boyeri* otolith morphological characteristics and otolith contour could be the result of environmental effects like stress, type, and quality of habitat inducing differences in food quality and quantity. SEM images are useful data to compare the three *A. boyeri* populations because the otolith surface morphology is essentially identical among the three *A. boyeri* populations. In the current study, different types of characters in the otolith surface morphology were observed such as flattened surface for the Lake Eğirdir population, rough surface for the Lake İznik population, and relatively smooth surface for the Hirfanlı Dam Lake population. As mentioned above, *A. boyeri* samples were collected in three different freshwater habitats. Ecological and environmental factors such as habitat or ecosystem complexity affect otolith shapes; therefore, this kind of differences in growth rate of *A. boyeri* may be a primary factor in the development of differences in otolith morphometry, shapes, otolith contour, and even surface shapes. The otolith characters such as elliptic shape, otolith depth, median sulcus acusticus, and rounded posterior region are not varied for both the three TL groups and *A. boyeri* populations from the three different lakes. However, several characters are varied such as the rostrum shape, rostrum size, rostrum thickness, antirostrum, antirostrum region, otolith width, mesial, and lateral surfaces of the TL groups of the three different lakes.

The present study indicated that otolith morphology, surface morphology, and otolith contour are useful tools for identifying *A. boyeri* in different lakes. The results of the current study supported that otolith morphology varies among species or population in the same species (Bostanci *et al.*, 2015; Zengin *et al.*, 2015; Yedier *et al.*, 2016). For this reason, otolith morphology and shape have been frequently used in species identification and stocks separations (Kontaş and Bostanci, 2015; Bostanci *et al.*, 2016).

The otoliths contour was also varied among the TL groups of each population. The reconstruction of the contour shapes from the average harmonics showed distinct morphological differences among the three TL groups in the populations. In the current study, the otolith contour was used for the identification of invasive *A. boyeri* in different lakes. The overall otolith contour of the three TL groups in the three populations can be reshaped by different environmental conditions; mainly in the anterior region and the dorsal part. The otolith morphology and otolith contour results are similar to results of previous studies including freshwater and marine species and populations such as *Clupea* spp. (Bird *et al.*, 1986), *Coryphaena hippurus* (Duarte-Neto *et al.*, 2008), *Aphanopus carbo* (Farias *et al.*, 2009),

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Engraulis encrasicolus (Bacha *et al.*, 2014), *Alburnus* spp. (Bostanci *et al.*, 2015), and *Sebastes* spp. (Tuset *et al.*, 2015).

Most of the morphological studies involved general external otolith morphology, but they did not include comparative otolith morphology in fisheries. The current study is one of the first comparative morphological studies involving otolith surface morphology and otolith contour of an invasive *A. boyeri* in the natural and artificial lakes. These characters were also confirmed for one of the invasive *A. boyeri* populations in different habitats. Several authors have indicated that the differences in habitat use by fish can affect the different structures of sagittal otoliths (Lombarte and Cruz, 2007, Lombarte *et al.*, 2010).

In the current study, *A. boyeri* from natural and artificial lakes had considerable differences in the otolith morphology and morphometry. This could be associated with habitat differences with biotic and abiotic factors. The present study also shows robust identification of the otolith characters in divided TL groups of three *A. boyeri* populations in the lakes. The study also points out detailed information to eliminate the lack of information in sagittal otolith morphology, otolith contour and surface morphology of an invasive fish *A. boyeri* in the different lakes.

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