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Otolith Shape and Characteristics as a Morphological Approach to the Stock Identification in *Barbus tauricus* (Cyprinidae)

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Abstract—This study is aimed to discriminate *Barbus tauricus* stocks using otolith shape indices and morphometrics. *B. tauricus* samples were obtained from some inland waters of Black Sea basin (Streams Akçay, Engiz, Terme, Karadere and Değirmenağzı), and also *Luciobarbus sp.* samples from Sakarya River were used as an outgroup. Utricular and lagenar otoliths were removed by making left and right distictions. Otolith width, length, perimeter and area were determined by Imaging Software. Power model was applied to estimate the relationships between the otolith measurements and total length. Form Factor, Circularity, Roundness, Rectangularity, Aspect Ratio and Ellipticity were used for otolith shape analyses. A standardized model was used to remove size effect on otolith measurement. Discriminant function analysis were performed to detect differences in otolith shape variations. The discriminant function analysis performed for otolith shape indices and measurements explains the intraspecific variability among localities. According to discriminant function analysis, 75.3% of *B. tauricus* stocks were correctly classified. The results of this study provide the first comprehensive data regarding the otolith shape analyses and relationship between the otolith morphometrics with total length of Crimean barbel.

Keywords: Barbus tauricus, stock discrimination, shape indices, otolith morphometry, Black Sea basin

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INTRODUCTION

Otoliths, calcareous structures located in the head of fishes, are considered as a true biological and environmental archive of fishes. These structures have been quite a while used for different kind of ichthyological studies especially taxonomy (Bourehail et al., 2015), genetic and phylogeny (Reichenbacher et al., 2007; Firidin et al., 2017), predator-prey relationships (Granadeiro and Silva, 2000), fossil studies (Gierl and Reichenbacher, 2015), chemistry (Miyan et al., 2016), age determination (Polat et al., 2005) and stock identification (Zengin et al., 2015). Natural changes or human impacts on environment have often been quantified using indices of ecological diversity (D'onghia et al., 2003). At this point, otolith shape analysis and characteristics are evaluated as a cheap, practice and time-effcient methods for determining stock differentiations and impacts of ecological differences on fish species.

The terms "stock" is an important taxonomic group that is identified as a fundamental unit for the management of fisheries resources (Vignon and Morat, 2010). Modern stock concept refers to arbitrary groups of fish with members of each group having similar life history characteristics (Begg and Waldman, 1999). Environmental variables can affect the lives of organisms and may lead to changes in morphology. For this reason, while the fish in the same stocks have similar morphology, different stocks of the same species may have some variations from the others. Otoliths show phenotypic plasticity as inter- and/or intra-specific and inter- and/or intra-populations variations. Because the use of otolith shape analysis for stock identification is based on the confounding interaction between genetically and environmentally induced differences, the quantitative measurement of otolith shape determinants calls for special consideration and further investigation. Furthermore, fish size-otolith dimensions relationships have several benefits in estimating the size of the prey. Fish size and/or weight can be functionally related to an appropriate otolith measurement (length, width or weight) and the resulting relationships can subsequently be used for size estimation (Granadeiro and Silva, 2000; Škeljo and Ferri, 2012; Kontas and Bostanci, 2015; Saygin et al., 2017; Zengin Özpiçak et al., 2018). Fish length-otolith biometry studies are base researches that can be used to determine size distributions of fish consumed by predators and stock discrimination (Harvey et al., 2000; Montanini et al., 2016; Jawad et al., 2017a; Park et al., 2018).

The genus *Barbus* which has more than 34 species all around the world is a polyphyletic group with different genetic and morphometric variations (Berrebi,

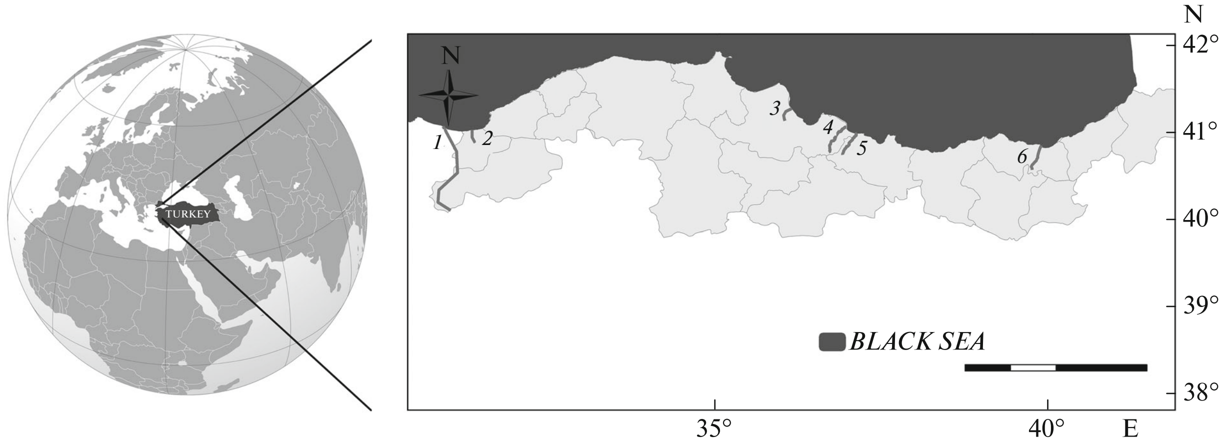

Fig. 1. Study area (*1*) Sakarya River, (*2*) Değirmenağzı Stream, (*3*) Engiz Stream, (*4*) Terme Stream, (*5*) Akçay Stream, (*6*) Karadere Stream, (■) Black Sea. Scale bar: 240 km.

1995; Casal-Lopez et al., 2015; Froese and Pauly, 2018). In Mediterrranean primary freshwater fauna, species of genera *Barbus* and *Luciobarbus* are pointed to as "model organisms" in different kind of studies especially genetical and morphological (Doadrio et al., 2002; Casal-Lopez et al., 2015; Antal et al., 2016; Turan et al., 2018; Özpicak and Polat, 2019). Barbels are bottom dwellers adapted to a variety of habitats, ranging from small mountain brooks to large and slow-flowing rivers and lakes. In all Barbel species, *Barbus tauricus* Kessler, 1877, Crimean Barbel is widely distributed in Black Sea watersheds and inhabits generally in mountain streams and also occur in lakes. In literature, there are some studies about ecology (Carosi et al., 2017), reproductive biology (Dopeikar et al., 2015), systematic (Turan et al., 2018), otolith morphometry (Kontaş and Bostanci, 2015), molecular (Tsigenopoulos et al., 2002; Ren and Mayden, 2016), phylogeny (Antal et al., 2016) and morphometry (Özpicak and Polat, 2019) of some *Barbus* species.

The aim of this study was to examine morphological variations in utricular and lagenar otoliths, reveal relationships of total length-otolith mesurements and also determine the intraspecies variation in populations of *B. tauricus,* one of the primary freshwater fishes in the Cyprinidae, sampled from five different localities in the Black Sea Region.

MATERIALS AND METHODS

Barbus tauricus samples were obtained between 2015–2017 from some inland waters of Black Sea Region (Akçay Stream (41°05′31″ N, 37°07′21″ E), Engiz Stream (41°28′55″ N, 36°02′50″ E), Terme Stream (41°09′34″ N, 36°53'28″ E), Karadere Stream (40°51′54″ N, 40° 1′10″ E), Değirmenağzı Stream (41°05′07″ N, 31°06′07″ E)) (Fig. 1) with SAMUS 725 MP electroshocker. The samples were captured from the parts of the rivers which are defined as the "Barbel Zone". In addition, *Luciobarbus* samples from Sakarya River were used as an outgroup. Totally 235 samples, 224 samples *of B. tauricus* (Akçay Stream ($n = 51$), Engiz Stream ($n = 55$), Terme Stream ($n = 55$), Karadere Stream ($n = 50$), Değirmenağzı Stream ($n = 13$)) and 11 samples of *Luciobarbus sp,* were used in analysis.

All captured fish were measured for total length (*TL*) (± 0.1 cm) and weighted (± 0.01 g). The sex was determined by macroscopic examination of the gonads. Utricular (lapillus) and lagenar (asteriscus) otoliths were removed by making left and right distinctions, cleaned and stored dry before the examination. All lapillus and asteriscus otolith pairs were photographed on the distal side with a Leica DFC295 digital camera (Fig. 2). Otolith width (*OW*), length (*OL*), perimeter (*OP*) and area (*OA*) (±0.001 mm) were determined by Imaging Software. Otolith shape indices such as aspect ratio, roundness, circularity, rectangularity, ellipticity, and form factor were calculated using the following formulas; roundness (RO) = $(4OA)/(\pi OL^2)$; circularity (C) = (OP^2/OA); form factor (FF) = $(4\pi OA)/OP^2)$; ellipticity (E) = $(OL - OW)/(OL + OW)$; rectangularity (REC) = $(OA/(OL \times OW)$ and aspect ratio (AR) = (OL/OW) (Tuset et al., 2003). Roundness and circularity provide a similarity to a perfect circle, ranging from a minimum value of 1 to a maximum of 12.57. The form factor is a mean to estimate the irregularity of a surface area from 1.0 (a perfect circle) to <1.0. Rectangularity describes the variations of length and width with respect to the area with 1.0 being a perfect square and <1 being a non-square. Ellipticity indicates the proportional change of the short and long axes from 0 (a perfect round shape) to close to 1 (Russ, 1990; Zischke et al., 2016). All the variables were tested for normality and homogeneity of variance using the Kolmogorov–Smirnov, Shapiro and Levene's test respectively. Different tests were

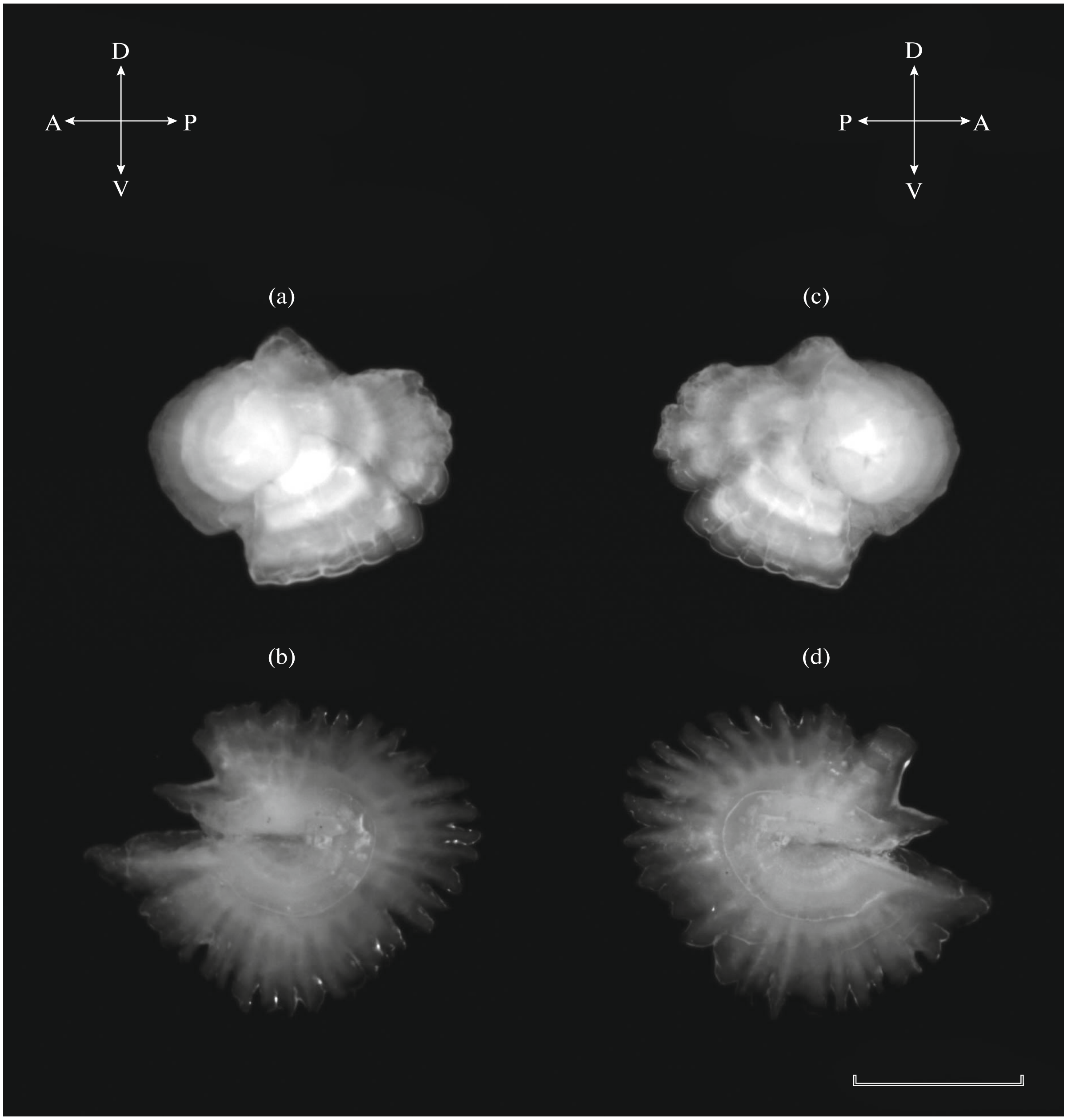

Fig. 2. Otolith pairs of *Barbus tauricus*: (a) right lapillus and (b) left asteriscus in distal side; (c) left lapillus and (d) right asteriscus in distal side (*D*, dorsal; *V*, ventral; *A*, anterior; *P*, posterior). Scale bar: 1 mm.

used depending on whether the data were normally distributed or not. Paired *t*-test, Wilcoxon test, Independent Two Sample *t* test, Mann–Whitney U-test and ANOVA-Tukey test were used in analyses. A standardized model was used to remove size effect on otolith measurement were performed by following equation (Elliot et al., 1995; Lleonart et al., 2000).

$$Zij = Yij\left(X_0/Xj\right)^{bj},$$

where *Yij* is the each orjinal measurements of the individual *j*, *Xj*, is the total length of the individual *j*, X_0 (134.04 mm) is the reference length, bj is the allometric parameter relating the dependent variable *Yi*, with the independent variable *X*, *Zij*, is the value of standardized measurement.

Power model ($y = ax^b$, where y is otolith measurement and x is fish length) were applied to estimate the relationships between the otolith measurements and *TL*. According to Harvey et al. (2000) fish length-otolith measurements relationships can be described by using a simple linear regression. However, Lleonart et al. (2000) indicated that the linear model is inappropriate for otolith and fish length relations because the linear model cannot detect shape changes and the

Table 1. Left and right otolith comparisons in terms of otolith length and otolith

Locality, Stream	*n*	*TL*, cm (min–max)	Variable	Otolith mophometrics Mean ± Std	*p*
Akçay	51	6.6–18.7	*AOL*	1.40 ± 0.37	>0.05
			AOW	1.64 ± 0.037	
			LOL	1.31 ± 0.23	
			LOW	0.98 ± 0.18	
Engiz	55	5.7–21.1	*AOL*	1.41 ± 0.28	>0.05
			AOW	1.23 ± 0.24	>0.05
			LOL	1.17 ± 0.20	<0.05*
			LOW	0.8 3± 0.14	>0.05
Terme	55	7.5–24.2	*AOL*	1.66 ± 0.30	>0.05
			AOW	1.40 ± 0.22	>0.05
			LOL	1.32 ± 0.20	<0.05*
			LOW	0.98 ± 0.17	>0.05
Karadere	50	10.8–26.1	*AOL*	1.77 ± 0.28	>0.05
			AOW	1.49 ± 0.19	
			LOL	1.47 ± 0.19	
			LOW	1.07 ± 0.13	
Değirmenağzi	13	8.7–18.1	*AOL*	1.57 ± 0.30	>0.05
			AOW	1.29 ± 0.23	
			LOL	1.25 ± 0.18	
			LOW	1.0 3± 0.14	

TL—total length, *n*—sample size, *AOL*—asteriscus otolith length, *AOW*—asteriscus otolith width, *LOL*—lapillus otolith length, *LOW*—lapillus otolith width, *statistically different width.

independent term “a” has no sense in morphometrics (Lleonart et al., 2000). In addition, ANOVA was used to compare lapillus and asteriscus otoliths shape indices of *B. tauricus* between localities. Discriminant function analysis (DFA) were performed to detect differences in otolith shape variations between the sampling sites. Wilks’ lambda assessed the performance of DFA.

RESULTS

The min–max *TL* of individuals were 6.6–18.7, 5.7–21.1, 7.5–24.2, 10.8–26.1, and 8.7–18.1 cm sampled from Akçay, Engiz, Terme, Karadere and Değirmenağzı, respectively. There is no significantly differences in terms of otolith characteristics in Engiz, Karadere and Değirmenağzı samples for both asteriscus and lapillus otoliths between male and female but there were differences in terms of *OW* and *OL* from Akçay and Terme samples ($p < 0.05$). According to left and right otoliths comparisons, there were no differences in terms of *OW* and *OL* for asteriscus and *OW* for lapillus ($p > 0.05$). However, the left and right otoliths are different from each other in terms of *OL* for Engiz and Terme Streams (Table 1). Relationships between *TL* and *OW*, *OL* were determined using both power and linear regression equations and best fit was obtained among *TL* and *OW* for asteriscus ($r^2 > 0.945$), *OL* for lapillus ($r^2 > 0.941$) (Table 2). In addition, all the relationships between otolith characteristics and *TL* were found statistically important ($p < 0.001$)

The standardized model which removes the effects of fish size on otolith measurements was used for analysis of *B. tauricus* samples from 5 different localities. Shape indices were calculated for the utricular and lagenar otolith pairs of Crimean Barbel and *Luciobarbus* for right otoliths (Table 3). There was no statistical difference between the shape index values of right and left otoliths ($p > 0.05$), for this reason only right otoliths were used in the analysis. FF, C, E and AR were found statistically important for lapillus and asteriscus between localities (ANOVA, $p < 0.001$). Furthermore, REC is similar between all localities ($p > 0.05$) (Table 4). However, RO is statistically important for lapillus but not asteriscus according to localities.

Morphological variation should be analyzed considering multivariate analysis because the univariate approach used in population studies does not include the combined effects of variables (Murta, 2000). Differences between the six localities according to the

Table 2. Equations (formula, r^2) of relationships between *TL* and otolith characteristics of *B. tauricus*

Locality, Stream	Otolith	
	asteriscus	lapillus
Akçay	$OL = 0.2008TL^{0.846}$, 0.914 $OW = 0.2325TL^{0.724}$, 0.946	$OL = 0.2407TL^{0.684}$, 0.942 $OW = 0.1716TL^{0.704}$, 0.885
Engiz	$OL = 0.2352TL^{0.769}$, 0.883 $OW = 0.2333TL^{0.731}$, 0.906	$OL = 0.260TL^{0.647}$, 0.922 $OW = 0.2018TL^{0.610}$, 0.862
Terme	$OL = 0.2921TL^{0.69}$, 0.815 $OW = 0.3206TL^{0.592}$, 0.785	$OL = 0.4199TL^{0.475}$, 0.548 $OW = 0.4325TL^{0.334}$, 0.575
Karadere	$OL = 0.2281TL^{0.751}$, 0.602 $OW = 0.2674TL^{0.626}$, 0.604	$OL = 0.311TL^{0.568}$, 0.480 $OW = 0.236TL^{0.552}$, 0.491
Değirmenağzi	$OL = 0.2131TL^{0.802}$, 0.910 $OW = 0.1997TL^{0.752}$, 0.927	$OL = 0.2709TL^{0.616}$, 0.908 $OW = 0.2721TL^{0.537}$, 0.788

OL – otolith length, *OW* – otolith width.

Table 3. Summary of descriptive statistics of shape index values according to localities

Shape indices	Akçay Stream		Değirmenağzi Stream		Engiz Stream		Karadere Stream		Terme Stream		Sakarya River	
	RL	*RA*	*RL*	*RA*	*RL*	*RA*	*RL*	*RA*	*RL*	*RA*	*RL*	*RA*
FF	0.83 ± 0.04	0.78 ± 0.05	0.85 ± 0.10	0.82 ± 0.07	0.85 ± 0.03	0.77 ± 0.07	0.82 ± 0.03	0.76 ± 0.07	1.55 ± 2.46	0.70 ± 0.12	0.84 ± 0.03	0.81 ± 0.05
C	15.08 ± 0.50	16.11 ± 1.00	14.69 ± 0.34	15.43 ± 1.46	14.75 ± 0.45	16.37 ± 1.54	15.34 ± 0.62	16.78 ± 1.73	14.22 ± 4.30	18.42 ± 4.04	15.05 ± 0.52	15.56 ± 1.03
RO	0.73 ± 0.05	0.84 ± 0.07	0.79 ± 0.05	0.83 ± 0.04	0.70 ± 0.04	0.86 ± 0.05	0.70 ± 0.04	0.83 ± 0.05	0.75 ± 0.26	0.80 ± 0.06	0.72 ± 0.05	0.83 ± 0.08
REC	0.76 ± 0.03	0.77 ± 0.03	0.78 ± 0.03	0.78 ± 0.02	0.77 ± 0.02	0.72 ± 0.03	0.76 ± 0.29	0.77 ± 0.03	0.83 ± 0.29	0.81 ± 0.25	0.76 ± 0.06	0.77 ± 0.03
E	0.14 ± 0.03	0.08 ± 0.04	0.10 ± 0.03	0.10 ± 0.02	0.17 ± 0.03	0.07 ± 0.03	0.16 ± 0.03	0.09 ± 0.03	0.18 ± 0.03	0.11 ± 0.09	0.15 ± 0.06	0.08 ± 0.03
AR	1.34 ± 0.08	1.17 ± 0.08	1.19 ± 0.05	1.21 ± 0.04	1.14 ± 0.08	1.15 ± 0.06	1.38 ± 0.07	1.20 ± 0.03	1.44 ± 0.09	1.28 ± 0.33	1.36 ± 0.13	1.18 ± 0.08

Here and in Table 4: *FF*—form factor, C—circularity , *RO*—roundness, *REC*—rectangularity, *E*—ellipticity, *AR*—aspect ratio; *RL*—right lapillus, *RA*—right asteriscus, (±)—standard deviation.

shape of otoliths and basic morphometric characteristics were determined by a canonical DFA. The DFA performed for all otolith shape indices and measurements explains the intra-specific variability among localities. The first 5 discriminant functions were used in the analysis. This method is a classification approach that investigates differences between the localities, species vice versa by finding a linear combination of the descriptors that maximize the Wilk's lambda (λ) (Ramsay and Silveman, 2005). According to DFA results, the first discriminant function explains 82.6% of the variability and the second one explains 9.9% of total variance.

According to DFA results, 75.3% of the *B. tauricus* stocks were correctly classified with the outgroup *Luciobarbus* sp. (Table 5). The highest classification % result was found as 83.6 in Engiz Stream and according DFA, all *Luciobarbus* samples were classified together (Fig. 3).

DISCUSSION

The relationships between length and otolith dimensions generates a baseline for fish biology and fisheries researches. In addition, linear and nonlinear functions are preferred to describe relationships of

Table 4. ANOVA result for shape indices and otolith morphometrics (*p* values) between all localities

Shape Indices	Otoliths	
	asteriscus	lapillus
FF	<0.001*	<0.001*
RO	>0.050	<0.001*
C	<0.001*	<0.001*
REC	>0.050	>0.050
E	<0.001*	<0.001*
AR	<0.001*	<0.001*
OW	<0.001*	<0.001*
OL	<0.001*	<0.001*
OA	<0.001*	<0.001*
OP	<0.001*	<0.001*

OW—otolith width, *OL*—otolith length, *OA*—otolith area, *OP*—otolith perimeter, *statistically important.

otolith dimensions and fish size (Skeljo and Ferri, 2012; Jawad et al., 2017b). In this study, nonlinear equation was prefered for total length and otolith dimensions relationships because of higher r^2. Furthermore, both asteriscus and lapillus otolith pairs were used in analysis but there are a lot of studies only one otolith pair were used (only lagenar or utricular, sagittal otoliths are very small in Cyprinid fishes that is why not prefered). The studies between fish length and otolith dimensions relationships of *B. tauricus* are very limited. When the relationships between fish length and otolith measurements were evaluated, the best fit was obtained among *TL* and *OW* for asteriscus ($r^2 > 0.945$), *OL* for lapillus ($r^2 > 0.941$). Kontaş and Bostanci (2015) found a strong relationship between fork length and asteriscus otolith length ($r^2 = 0.80$). There are differences between present study and Kontaş and Bostanci (2015). In Kontaş and Bostanci (2015) fork length were used in analysis. It is thought that these differences may result from the number of samples used in the studies, different sampling areas and total length ranges. However, there are some studies about relationships between otolith morphometrics and fish length in other *Barbus* species (Schulz-Mirbach and Reichenbacher, 2006; Dusukcan and Calta, 2018).

This study provides biometric relationships between total length and otolith measurements for *B. tauricus* sampled from different localities in Black Sea Region and also represents the first quantitative analysis of total length and utricular and lagenar otolith size parameters for coastal streams of Black Sea. There is no study that evaluated both utricular and lagenar otoliths together. This research will contribute towards future stock assessment studies, and can be useful for sustainable utilization and management of fishery resources in this region.

Studies in recent years have shown that different methods like various external measurements elliptic fourier analysis, principal component analysis, DFA have been used to determine the shape of otoliths for detecting variations between localities or species (Boudinar et al., 2016; Zhao et al., 2017; He et al., 2018; Tuset et al., 2018). Otolith shape varies from a round in fish larvae to a specific shape in adults (Gauldie, 1988; Lagardère et al., 1995). Therefore, the data is standardized to eliminate the length and size factor in several studies (Leonart et al., 2000; Zischke et al., 2016; Zhao et al., 2017; Tuset et al., 2018) and in this study, too.

In this study, the variation of utricular and lagenar otoliths in *B. tauricus* was tested using six shape indices (form factor, roundness, circularity, rectangularity, ellipticity and aspect ratio indices) and 4 otolith morphometrics (width, length, area and perimeter) for differentiation of populations in Crimean barbel. In literature there is only one study that was examined shape index values of *B. tauricus*. Kontaş and Bostanci (2015) investigated six shape indices of *B. tauricus* (FF, C, RO, AR, E and REC) from Melet River (Ordu-Turkey). The results of both studies are similar.

The discriminant analysis creates a function to classify individuals within a group (Camacho, 1995). In this study, according to DFA, the first two components explained 92.5% of the total variance relevant to otolith shape indices and dimensions. Several studies

Table 5. Discriminant analysis classification matrix of predicted group membership, %

Locality, Stream	Predicted Group Membership					
	Akçay	Değirmenağzi	Engiz	Karadere	Terme	Sakarya
Akçay	62.7	3.9	*	9.8	23.5	*
Değirmenağzi	15.4	69.2	*	15.4	*	*
Engiz	5.5	*	83.6	5.5	5.5	*
Karadere	6.0	2.0	2.0	80.0	10.0	*
Terme	10.9	1.8	*	16.4	70.9	*
Sakarya	*	*	*	*	*	100.0

* Not available.

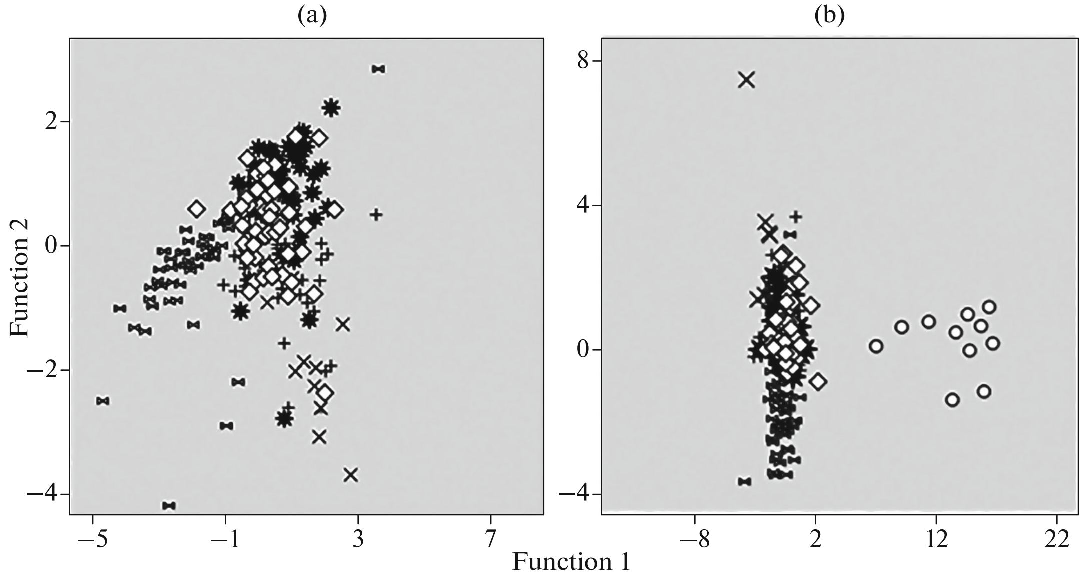

Fig. 3. Discriminant Function Analysis for the classification of *B. tauricus* according to otolith shape and morphometrics, (a) *B. tauricus* samples from (+) Akçay Stream, (×) Değirmanağzı Stream, (⋈) Engiz Stream, (⁎) Karadere Stream, (◇) Terme Stream and (b) *Luciobarbus* sp. from (○) Sakarya River.

have shown that otolith shape analysis used discrimination of fish stocks (Tuset et al., 2003, 2018; Vignon and Morat, 2010; Boudinar et al., 2016; Zhao et al., 2017; Bostanci and Yedier, 2018). In the present study, our results in the shape of otoliths from five different localities were determined and suggest that otolith shape indices differences in astriscus and lapillus are detectable for Crimean barbel.

According to shape indices results, FF, C, E and AR of both lapillus and asteriscus can be used for population discrimination of *B. tauricus* (ANOVA, $p < 0.001$). Furthermore, REC is similar between all localities ($p > 0.05$). However, DFA results support discrimination of *B. tauricus* populations as 75.3%. The Wilks' lamda allows assessment of the performance of the discriminant function analysis. This statistic is the ratio between the intragroup variance and the total variance and provides a means of calculating the chance-corrected percentage of agreement between true and predicted groups. The Wilks' lamda values range from 0 to 1, and the closer the λ is to 0, the better the discriminating power of the DFA (Bourehail et al., 2015). In this study the Wilks' lamda scores were in Table 6 ($p < 0.001$) and the first 5 functions used in analyses.

The relationship between fish size and otolith shape reflects both effects of ontogeny and the environment on otolith shape. Considering the findings of this study, it is evident that the asteriscus and lapillus shape are useful for the encouragement of further research on verifying the role of the otolith in identification, discriminating and taxonomy of fish. In the future, various approaches such as genetic, the microchemical of otoliths or fourier analyses are necessary for understanding the use of otoliths as an indicator of stock differentiations. This study indicated that multiple morphometric variables were important for separating the *Barbus taurícus* populations from the several coastal streams of Black Sea region. In conclusion, the data analysed in this study, indicates a variability in otolith shape and that is a good tool for species identification in *Barbus tauricus*. The results of this study will constitute a serious literature knowledge for the studies to be carried out thereafter.

Table 6. Results of Wilks' lamda test according to discriminant function analysis

Test of function(s)	Wilks' lambda	Chi-square	df	*p*
1–5	0.022	860.620	60	0
2–5	0.227	333.696	44	0
3–5	0.484	163.403	30	0
4–5	0.698	80.799	18	0
5	0.925	17.483	8	0.025

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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