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Otolith phenotypic analysis for the endemic Anatolian fish species, Caucasian bleak *Alburnus escherichii* Steindachner, 1897 (Teleostei, Leuciscidae), from Selevir Reservoir, Akarçay Basin, Turkey

by

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Abstract

Otolith phenotypic variability was analyzed in the Caucasian bleak (*Alburnus escherichii*) from the Selevir Reservoir in Turkey. Utricular (lapillus) and lagenar (asteriscus) otoliths were removed, while distinguishing between left and right otoliths. All otoliths were photographed on the distal (for asterisci) and dorsal surface (for lapilli) using a Leica DF295 digital camera. Otolith morphometrics were measured to the nearest 0.001 mm using Leica Imaging Software. Linear and nonlinear (power) models were applied to determine the relationships between otolith measurements and total length of fish individuals. Two length classes (Class I: 6.7–10.9 cm L_t; Class II: 11.0–15.0 cm L_t) were established to analyze the shape of otoliths. The Form Factor, Circularity, Roundness, Rectangularity, Aspect Ratio and Ellipticity were used to analyze the shape of otoliths. A standardized model was used to remove the effect of size on otolith measurements. Multivariate analysis was performed to detect differences in otolith shape variation. The results of discriminant function analysis showed that 79.9% of *A. escherichii* specimens were correctly classified by length classes. In this study, intraspecific variation of asteriscus and lapillus otoliths in *A. escherichii* is reported for the first time. The results of this study provide the first comprehensive data on otolith shape analysis and the relationship between otolith morphometrics and total length in the Caucasian bleak.

Key words: otolith biometrics, shape indices, mature and immature samples, *Alburnus escherichii*

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1. Introduction

Otoliths are calcareous structures found in the inner ear (labyrinth) of fish, responsible for hearing and balance. The labyrinth consists of three otolithic organs called the sacculus, the utriculus and the lagena, each containing chemically and morphologically different otolith pairs (La Mesa et al. 2020). In ostariophysians, lagenar and utricular otoliths are larger than saccular otoliths (Wright et al. 2002). Otolith phenotypic variation has long been used for different types of ichthyological studies, especially taxonomy (Więcaszek et al. 2020), genetics and phylogeny (Firidin et al. 2017), predator–prey relationships (Więcaszek et al. 2020), fossil studies (Gierl & Reichenbacher 2015), mass asymmetry (Yedier et al. 2018), paleoichthyology (Gierl et al. 2018), bioarchaeology (Van Neer et al. 2004), trophic ecology (Assis et al. 2020), age determination (Sun et al. 2020) and stock identification (La Mesa et al. 2020; Ozpicak 2020).

In terms of fisheries management and fish biology, it is important to determine the phenotypic variation induced by environmental factors. Being species specific, the shape and morphometry of otoliths have been used as a natural marker and a useful tool for fish identification. However, there are some limitations in research on otoliths. Since mineralization and growth of otoliths in fish are affected by environmental factors, aberrant crystallization of otoliths and fluctuating asymmetry in otolith dimensions should be investigated (Bostanci et al. 2018; Yedier & Bostanci 2019; Mejri et al 2020; Yedier & Bostanci 2020). Fluctuating asymmetry in otoliths can indicate specific environmental effects, and otolith asymmetry in fish has been used as a bioindicator to study the status of different populations (Grønkjaer & Sand 2003).

Otolith shape can vary within and between species with sex, population, growth rates and ontogenetic stages (Więcaszek et al. 2020). Although genetics and environmental conditions can strongly affect otoliths and their morphometric features, they are considered valuable structures for stock identification (Koeberle et al. 2020). Furthermore, relationships between fish size or weigh and otolith dimensions have several advantages in estimating the size of prey (Škeljo & Ferri 2012; Yılmaz et al. 2019; Saygın et al. 2020). Otoliths are often found in the stomach contents of different organisms (fish, mammals and birds) or as fossils in sediments. Therefore, species identification using otoliths is a very useful tool in food chain, ecological and paleontological studies (Buckland et al. 2017).

The genus *Alburnus*, known as bleaks, from the family of Leuciscidae has been the subject of various faunistic (Yılmaz et al. 2006), toxicological (Uysal et al. 2009), systematic (Gülle et al. 2017), morphometric (Ozulug & Freyhof 2007) and age determination (Bostanci & Polat 2011) studies. It is represented by 51 species worldwide (van der Laan 2020) and is considered an excellent example of high diversity and endemism of western Palearctic freshwater fish (Mangıt 2014). Seventeen endemic *Alburnus* species occur in Turkey (Froese & Pauly 2019). Of this genus, *Alburnus escherichii* was described by Steindachner (1897) from the Tabakane and Porsuk rivers, which debouch into the Sakarya River in the northern Anatolian Black Sea basin (Bayçelebi et al. 2020). The species is categorized as least concern (LC) by the International Union for Conservation of Nature IUCN (Freyhof 2014).

Research on i.a. otolith biometrics or biological properties of *A. escherichii* are rather limited. The objectives of this study were (i) to gain knowledge about otolith shapes, (ii) to understand the phenotypic variation of lagenar and utricular otolith pairs and (iii) to provide a basis for various studies on *A. escherichii* using otolith phenotypic analysis.

2. Materials and methods

2.1. Sample collection

Alburnus escherichii samples were collected from the Selevir Reservoir in the Akarçay Basin (Turkey) by local fishermen (Fig. 1). First, *Alburnus* samples from the Selevir Reservoir were identified as *Alburnus nasreddini*, an endemic fish species from the Akarçay Basin (Bektaş et al. 2020). However, recent molecular (Bektaş et al. 2020) and morphometric (Bayçelebi et al. 2020) studies revealed that *Alburnus nasreddini* is a synonym of *Alburnus escherichii*. A total of 154 Caucasian bleak specimens were examined for total length (L_t; ± 0.1 cm) and weight (W; ± 0.01 g). Their sex was determined by macroscopic examination of the gonads.

2.2. Preparation of otoliths and basic morphometric characteristics of asterisci and lapilli

Utricular (lapillus) and lagenar (asteriscus) otoliths were removed, distinguishing between left and right ones. Otoliths were removed through an incision in the cranium, then cleaned with ethanol and stored in a dry place. All otoliths were photographed on the distal (asterisci) and dorsal (lapilli) surface using a Leica DF295 digital camera. The asteriscus length (AL), asteriscus height (AH), asteriscus perimeter (AP), asteriscus area (AA), lapillus length (LL) and lapillus width (LW), lapillus perimeter (LP) and lapillus area

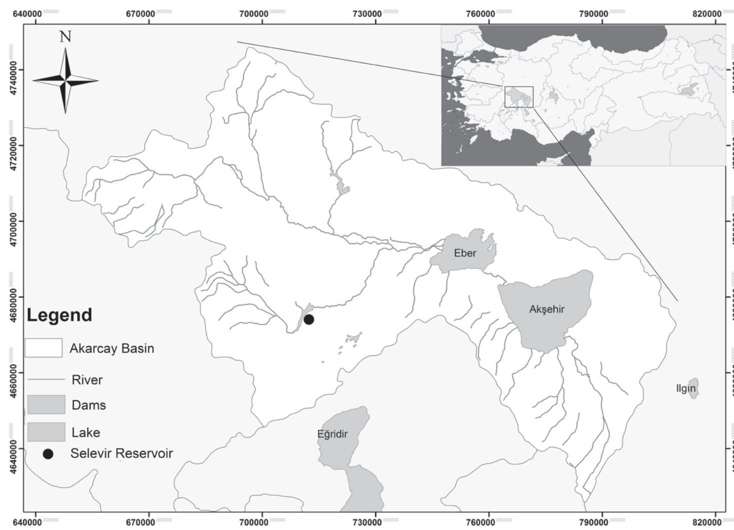

Figure 1

Sampling area of *A. escherichii*; (●) Selevir Reservoir

(LA) were measured to the nearest 0.001 mm using Leica Application Suit ver. 3.8 Imaging Software (Fig. 2) and weighted (AOW for asterisci and LOW for lapilli; 0.00001 g). The length of asteriscus and lapillus otoliths was defined as the greatest distance between their anterior and posterior margins. The height of asteriscus otoliths was defined as the greatest distance between their dorsal and ventral edges. The width of lapillus otoliths was determined as the greatest distance from their lateral to medial margins (Yilmaz et al. 2015). Lapilli and asterisci pairs were also photographed on their distal and proximal sides using a Hitachi SU 1510 Scanning Electron Microscope (SEM) for their phenotypic description (Fig. 3).

All variables were tested for normality and homogeneity of variance using Kolmogorov–Smirnov, Shapiro and Levene's tests, respectively. Different tests (paired t-test, Wilcoxon test, Independent Two Sample t test, Mann–Whitney U test) were used depending on whether the data were normally distributed or not. Differences between sexes were also examined by the independent t-test. A standardized model was used to remove the size effect on otolith measurements according to the following equation (Elliot et al. 1995; Lleonart et al. 2000):

$$Z_{ij} = Y_{ij}\left[\frac{X_0}{X_j}\right]^{bj}$$

where Y_{ij} is each original measurement of individual j, X_j is the total length of individual j, X_0 is the reference length, bj is the allometric parameter relating the dependent variable Y_i to the independent variable X, Z_{ij} is the value of the standardized measurement.

Linear and nonlinear (power) models ($y = a + bx$, $y = ax^b$, where y is the otolith measurement and x is the fish length) were applied to determine the relationships between the otolith morphometrics and L_t. Parameters a and b were estimated through linear regression analysis based on logarithms, log Y = log a + b log X (Zar 1999).

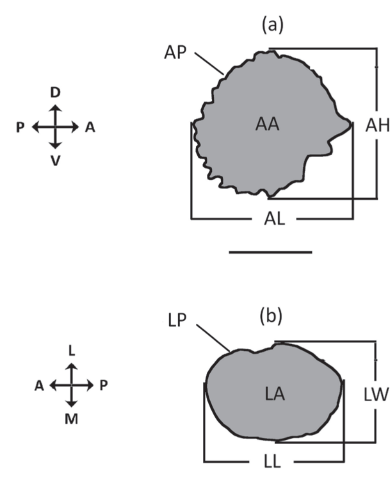

Figure 2

Lagenar (asteriscus) and utricular (lapillus) otoliths in *A. escherichii* sampled from Selevir Reservoir; the distal (a) view of the right asteriscus and the dorsal (b) view of the right lapillus (AL – asteriscus length; AH – asteriscus height; LL – lapillus length; LW – lapillus width; A – anterior; P – posterior; D – dorsal; V – ventral; L – lateral; M – medial), **Scale bar = 1mm**

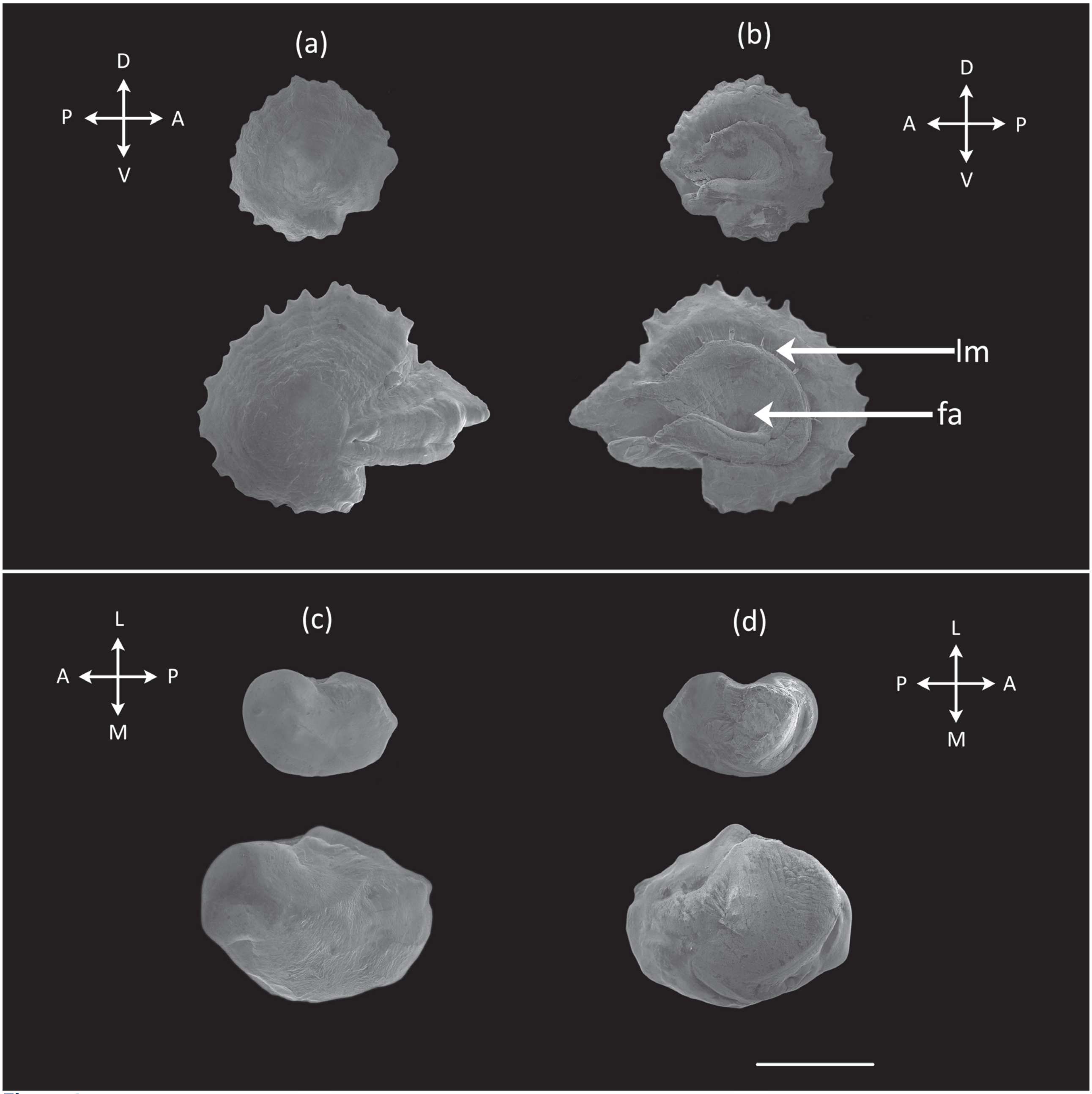

Figure 3

SEM pictures of utricular and lagenar otolith pairs in *A. escherrichii*. (a) Right asteriscus on the distal side; (b) right asteriscus on the proximal side (upper sample = 960 mm TL; lower sample = 1410 mm TL); (c) right lapillus on the dorsal side; (d) right lapillus on the ventral side (upper sample = 960 mm TL; lower sample = 1410 mm TL). A – anterior; P – posterior; D – dorsal; V – ventral; L – lateral; M – medial; lm – lobus major; fa – fossa acustica; **Scale bar = 1 mm**

2.3. Otolith shape analysis

The asteriscus and lapillus of *A. escherichii* were described morphologically according to the terminology used by Assis (2003) and Schulz-Mirbach & Reichenbacher (2006). Otolith shape indices such as the Form Factor (FF), Circularity (C), Roundness (RO), Rectangularity (RE), Aspect Ratio (AR) and Ellipticity (E) were used to analyze the shape of otoliths. They were calculated using the following formulas: Roundness = $(4 \times OA) / (\pi \times OL^2)$; Circularity = OP^2 / OA; Form Factor = $(4 \times \pi \times OA) / OP^2$; Ellipticity = $(OL - OB) / (OL + OB)$; Rectangularity = $OA / (OL \times OB)$ and Aspect Ratio = (OL / OB) (Tuset et al. 2003; Ponton 2006).

Two length classes (Class I: 6.7–10.9 cm L_t; Class II: 11.0–15.0 cm L_t) were generated for otolith shape analysis. Sexual maturity status, as determined by macroscopic examination of the gonads, was considered when establishing these classes. The population of *A. escherichii* from the Selevir Reservoir begins spawning in June when water temperature reaches 20°C and continues through mid-July (Gülle et al. 2017). The development of gonads in the Caucasian bleak was described using five stages following Brown-Peterson et al. (2011), which was also accepted by other researchers (Dopeikar et al. 2015; Keivany et al. 2017): stage I – testes and ovaries were very often clear and threadlike, blood vessels indistinct, and the sex could not be determined; stage II – ovaries and testes like an opaque reddish tube occupying almost half the body cavity; stage III – gonads filled the body cavity, ovaries were orange and testes were white; stage IV – ovaries were flaccid and blood vessels prominent, testes were small and flaccid and did not release milt under pressure; stage V – ovaries and testes were empty, loose and red.

In addition, multivariate analyses (PCA and DFA) were performed to detect morphometric differences in the shape of otoliths between mature and immature samples. Discriminant function analysis (DFA) was performed to detect differences in otolith shape variation between the sampling sites. Wilks' lambda (λ) was employed to assess the performance of DFA.

SPSS 20, Past 3.0, Minitab 15.0 and Excel were used for statistical analysis.

3. Results

3.1. Relationships between otolith morphometrics and total length of fish

The sex ratio was 1:2.02 based on the total sample size of females (n = 51; 33.12%) and males (n = 103; 66.88%). The maximum and minimum total length and weight of individuals ranged from 6.70 to 15.00 cm and from 2.58 to 33.48 g, respectively.

There are differences between females and males in terms of the total length and weight (Mann–Whitney U Test, $p < 0.05$). Therefore, a standardized model was used to remove the effect of size on otolith measurements. When asteriscus and lapillus otoliths from females and males were compared, there were no differences in AL, AH, AP, AA, AOW, LL, LW, LP, LA and LOW ($p > 0.05$). When comparing left and right otoliths, statistically significant differences were found in AH, AA, LL, LP and LA ($p < 0.001$). Descriptive statistics of otolith morphometrics are presented in Table 1.

The relationships between L_t and otolith dimensions were determined and the best fit for L_t and area for asterisci ($r^2 > 0.610$) and length for lapilli ($r^2 > 0.569$) was obtained (Table 2).

3.2. Differences in otolith shape

The medial face of asteriscus otoliths features a furrow. Although other authors used the term 'sulcus' to describe this furrow, the asteriscus does not look like a groove. For this reason, it is proposed that the name 'fossa acustica', used by Berinkey (1956) to name any furrow-like depression that exteriorly surrounds the fossa in cyprinids, is reintroduced. Asteriscus otoliths in the Caucasian bleak have serrated edges all around their periphery in both length classes. According to Assis (2003), the proximal surface has an acoustic pit (fossa acustica). The fossa acustica surrounded by a lobe (lobus major) can be easily detected in the asteriscus. However, the general shape of the lapillus is elongate. The antero- and posteromedial edges are small and rounded. In most lapilli, the antero- and posterolateral edges are well defined. In general, the posterolateral edge forms the pointed posterior end of the lapillus. The medial and posterior margins are slightly concave or rounded (Fig. 2). A bump (cranial umbo) is observed at its anterior end. The ventral region of the lapillus is convex and bumpy.

FF, C, RO, RE, AR and E were calculated for left and right asteriscus and lapillus otolith pairs separately. There were no differences in values of the shape indices for the left and right asteriscus and lapillus otolith pairs ($p > 0.05$) except Ellipticity for lapilli ($p < 0.001$). Otolith shape indices were calculated for asteriscus and lapillus otolith pairs (Table 3). The shape of otoliths was compared between the length classes. The discriminant function included otolith shape characteristics explaining the variation between mature and immature samples. According to the discriminant function analysis, 79.9% of *A. escherichii* samples were correctly classified, with Wilks' lambda (λ) score of 0.661 (Table 4).

4. Discussion

There are many studies describing *Alburnus* species based on morphological (Ozulug & Freyhof 2007; Elp et al. 2015; Gülle et al. 2017; Freyhof & Turan 2019; Bayçelebi et al. 2020), molecular (Mangıt & Yerli 2018; Bektaş et al. 2020) and morphological/molecular characters (Freyhof et al. 2018). Turkey is the fish speciation center. The territory of Turkey (especially the Anatolian part), located at the intersection of regions

Table 1

Descriptive statistics of otolith characteristics for Caucasian bleak by length classes

Character		Otolith length			Otolith height/width			Otolith weight			Otolith perimeter			Otolith area		
		Min.	Max	Mean ± SD	Min.	Max	Mean ± SD	Min.	Max	Mean ± SD	Min.	Max	Mean ± SD	Min.	Max	Mean ± DS
Class I (6.7–10.9 cm L_t) (N = 94)	RA	0.985	1.907	1.596 ± 0.15	1.082	1.991	1.596 ± 0.15	0.00036	0.00140	0.00095 ± 0.0002	3.500	6.507	5.591 ± 0.51	0.772	2.397	1.742 ± 0.22
	LA	0.998	1.915	1.591 ± 0.15	1.053	1.863	1.582 ± 0.12	0.00037	0.00143	0.00095 ± 0.0002	3.442	6.764	5.633 ± 0.52	0.767	2.362	1.756 ± 0.23
	RL	0.919	1.616	1.420 ± 0.11	0.733	1.460	1.058 ± 0.10	0.00024	0.00174	0.00115 ± 0.0002	2.644	4.610	3.965 ± 0.30	0.506	1.502	1.128 ± 0.16
	LL	0.919	1.678	1.452 ± 0.11	0.733	1.770	1.063 ± 0.12	0.00024	0.00183	0.00115 ± 0.0002	2.644	4.678	4.028 ± 0.30	0.506	1.553	1.160 ± 0.16
Class II (11.0–15.0 cm L_t) (N = 60)	RA	1.356	2.681	1.782 ± 0.20	1.304	2.197	1.720 ± 0.13	0.00031	0.00301	0.00122 ± 0.0003	4.707	8.865	6.347 ± 0.70	1.224	3.471	2.094 ± 0.34
	LA	1.257	2.542	1.776 ± 0.19	1.330	2.290	1.730 ± 0.19	0.00032	0.00307	0.00122 ± 0.0004	4.117	9.444	6.267 ± 0.92	1.254	3.519	2.108 ± 0.34
	RL	1.176	2.074	1.555 ± 0.15	0.807	1.693	1.154 ± 0.13	0.00050	0.00468	0.00155 ± 0.0006	3.205	5.959	4.344 ± 0.42	0.724	2.489	1.350 ± 0.27
	LL	1.151	2.170	1.591 ± 0.15	0.815	1.770	1.161 ± 0.141	0.00050	0.00482	0.00155 ± 0.0006	3.206	6.099	4.409 ± 0.43	0.709	2.615	1.187 ± 0.28
Total (N = 154)	RA	0.985	2.681	1.67 ± 0.19	1.082	2.197	1.630 ± 0.14	0.00031	0.00301	0.00105 ± 0.0003	3.500	8.865	5.886 ± 0.70	0.772	3.471	1.879 ± 0.32
	LA	0.998	2.542	1.66 ± 0.19	1.053	2.290	1.639 ± 0.15	0.00032	0.00307	0.00106 ± 0.0003	2.117	9.444	5.880 ± 0.77	0.767	3.519	1.893 ± 0.33
	RL	0.919	2.074	1.47 ± 0.14	0.733	1.693	1.096 ± 0.12	0.00024	0.00482	0.00131 ± 0.0005	2.644	5.959	4.113 ± 0.40	0.506	2.489	1.214 ± 0.24
	LL	0.919	2.170	1.51 ± 0.15	0.733	1.770	1.101 ± 0.14	0.00024	0.00468	0.00131 ± 0.0005	2.644	6.099	4.177 ± 0.41	0.506	2.615	1.248 ± 0.24

*(RA – right asteriscus, LA – left asteriscus, RL – right lapillus, LL – left lapillus)

with different ecological and geographical conditions, is characterized by high diversity and endemism of freshwater fish (Perea et al. 2010). In the present study, morphometrics and shape of otoliths in the endemic fish species, *A. escherichii*, were investigated.

4.1. General otolith morphometrics

Morphological characteristics of fish otoliths vary with species and fish size (Popper et al. 2005; Romero et al. 2020). The relationships between fish length and otolith dimensions are used as a basis for research in fish biology and fisheries. Linear and nonlinear functions are preferred to describe the relationships between otolith dimensions and fish size. In general, the nonlinear function is used to study the relationships between otolith morphometrics and total length of fish (Jawad et al. 2017; Yılmaz et al. 2019; Saygın et al. 2020). According to Lleonart et al. (2000), using a linear model to explain the relationship between otolith morphometrics and fish length is not useful because the independent coefficient "a" is not relevant in morphometry. There are no studies for *A. escherichii* on the relationship between the fish length and otolith dimensions. However, there are many studies involving other *Alburnus* species that investigated the relationships between otolith dimensions and fish length. Bostanci & Polat (2011) studied the relationships between otolith dimensions and fork length in *A. tarichi* from Lake Van and found strong correlations. Basusta et al. (2013) investigated the relationships between otolith morphometrics and total length of fish in the population of *A. heckeli*. Saygın et al. (2017) investigated the relationships between fork length and otolith dimensions in *A. tarichi* from different lakes across Van Basin. In present study, the best fit was obtained between L_t and area for asterisci ($r^2 > 0.610$), and length for lapilli ($r^2 > 0.569$).

Relationships between fish size and otolith morphometrics are a baseline for studies of prey–predator interactions. Analysis of otoliths retrieved from the stomachs or feces of piscivorous predators can provide information on the type, size, mass and energy content of their fish prey (Więcaszek et al. 2020). Results of research on the relationships between fish length and otolith biometrics can be used to determine the size distribution of fish consumed by predators and stock discrimination (Jawad et al. 2017; Park et al. 2018). In the Selevir Reservoir, *A. escherichii* (in previous studies described as *A. nasreddini*) feeds on zooplankton, amphipods, dipteran larvae and pupae, and rarely on plant material (Gülle et al. 2017). In this habitat, *Sander lucioperca* is the top predator species.

Table 2

Relationships between L_t and otolith dimensions

Variable		Linear a	Linear b	r^2	Power a	Power b	r^2
Length	Asteriscus	0.365	0.120	0.533	0.264	0.773	0.539
	Right lapillus	0.475	0.093	0.556	0.290	0.683	0.563
	Left lapillus	0.469	0.096	0.565	0.288	0.696	0.569
Height	Right asteriscus	0.628	0.093	0557	0.373	0.620	0.563
	Left asteriscus	0.623	0.094	0.546	0.369	0.627	0.551
Width	Lapillus	0.369	0.067	0.392	0.246	0.627	0.389
Weight	Asteriscus	−0.0009	0.0002	0.500	2E−05	1.713	0.474
	Lapillus	−0.0016	0.0003	0.488	8E−06	2.111	0.546
Perimeter	Asteriscus	0.886	0.463	0.581	0.806	0.835	0.585
	Right lapillus	1.346	0.256	0.550	0.856	0.659	0.548
	Left lapillus	1.338	0.263	0.554	0.850	0.669	0.550
Area	Right asteriscus	−0.466	0.217	0.594	0.093	1.255	0.610
	Left asteriscus	−0.482	0.220	0.594	0.092	1.266	0.606
	Right lapillus	−0.407	0.150	0.524	0.058	1.275	0.533
	Left lapillus	−0.422	0.155	0.527	0.058	1.289	0.539

Table 3

Descriptive statistics of shape indices for Caucasian bleak (right otolith) except Ellipticity; both right (R) and left (L) otolith pairs

	Class I		Class II		Total	
Shape Indices/Otoliths (Mean ± SD)	Asteriscus	Lapillus	Asteriscus	Lapillus	Asteriscus	Lapillus
Aspect Ratio	1.015 ± 0.06	1.347 ± 0.09	1.036 ± 0.07	1.352 ± 0.07	1.023 ± 0.06	1.349 ± 0.08
Form Factor	0.702 ± 0.06	0.897 ± 0.02	0.656 ± 0.06	0.891 ± 0.02	0.684 ± 0.07	0.894 ± 0.02
Roundness	0.873 ± 0.07	0.710 ± 0.04	0.844 ± 0.09	0.705 ± 0.04	0.862 ± 0.08	0.708 ± 0.04
Circularity	18.036 ± 1.69	14.011 ± 0.25	19.340 ± 1.96	14.105 ± 0.29	18.544 ± 1.90	14.048 ± 0.27
Rectangularity	1.717 ± 0.22	0.841 ± 0.14	2.022 ± 0.29	1.004 ± 0.24	1.837 ± 0.20	0.905 ± 0.20
Ellipticity	0.007 ± 0.03	0.147 ± 0.03 (R)	0.016 ± 0.03	0.149 ± 0.03 (R)	0.011 ± 0.03	1.147 ± 0.03 (R)
		1.826 ± 0.16 (L)		1.967 ± 0.17 (L)		1.880 ± 1.17 (L)

The presented study will also provide a reference for dietary and ecological studies on *S. lucioperca*.

This study provides data on biometric relationships between total fish length and otolith measurements for *A. escherichii* and presents the first quantitative analysis of total fish length and utricular and lagenar otolith size parameters. It may be useful for sustainable use and management of fisheries resources in this region.

4.2. Otolith shape

Asteriscus otoliths in the Caucasian bleak have serrated edges around their periphery. The proximal surface features an acoustic pit. Mesial and lateral surfaces are convex. Otherwise, the general shape of the lapillus (Fig. 3) is consistent with the description provided by Schulz-Mirbach & Reichenbacher (2006). Bostanci et al. (2015) described the shape of asteriscus otoliths in four *Alburnus* species. According to these authors, the shape of otoliths in *A. escherichii* is also oval, the mesial and lateral surfaces are convex, and their dorsal margins are rounded. The shape of otoliths is an important feature in the identification of fish species. The characteristics of otoliths are a useful tool for identifying intra- and interspecific relationships. Otolith shape analysis has been used in many studies (Mapp et al. 2017; Ozpicak 2020; Bano & Serujiddin 2021).

The otolith shape varies from round in fish larvae to a specific shape in adults (Lagardère et al. 1995).

Table 4

Classification matrices for predicted group membership of *A. escherichii* according to otolith shape

Length Classes		Predicted Group Membership*	
		Class I	Class II
%	Class I	81.9	18.1
	Class II	23.3	76.7

*79.9% of the original grouped cases were correctly classified

Therefore, in many studies, including this one, data are standardized to eliminate the length and size factor (Zhao et al. 2017; Tuset et al. 2018; Saygın et al. 2020). In the present study, two length classes were established according to the stages of gonadal development and the classification score was calculated as 79.9%. In recent years, many studies have been conducted using different length classes according to morphology, ontogenetic effects and sexual variation (Capoccioni et al. 2011; Cerna et al. 2019; Carvalho et al. 2020; Teimori et al. 2020; Więcaszek et al. 2020). Waessle et al. (2003) identified a strong modification in the morphology of *Micropogonias furnieri* otoliths, which was associated with sexual maturation. Capoccioni et al. (2011) indicated ontogenetic and environmental effects on the *Anguilla anguilla* otolith shape variation. Souza et al. (2020) investigated ontogenetic and interpopulation differences in the otolith shape for *Perca fluviatilis* and found that otoliths tend to be elongated when fish are young and old, while more rounded in middle age. Bostanci et al. (2015) used the shape of otoliths as a tool to distinguish between different *Alburnus* species. They established four length classes and the overall canonical discriminant analysis (CDA) classification score was 93.8% for different *Alburnus* species. However, there are still ambiguities in the taxonomical status of *Alburnus* species. Phenotypic variation is a dynamic and flexible concept that affects population structure within a short period of time. As a phenotypic approach, research on otoliths can support the taxonomy of this species because they are species specific.

Unfortunately, changes in climatic conditions, which affect the water regime, and human activities are the main threats to the basin and lakes. There are no doubts that all these negative phenomena will have a negative impact on the distribution of the species and the current status of its population. For all these reasons, it is necessary to develop a conservation strategy for *A. escherichii*.

5. Conclusion

The data presented here indicate that the stages of gonadal development affect the shape of otoliths. To this end, further research will be needed on environmental factors, genetic patterns, and the relationship at the individual level between microchemical composition, which carriers information on fish life history, and shapes of otoliths.

Conflicts of interest

The authors declare that there is no conflict of interest.

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