The effect of ontogenetic diet shifts on sagittal otolith shape of European perch, Perca fluviatilis (Actinopterygii: Percidae) from Lake Ladik, Turkey

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The effect of ontogenetic diet shifts on sagittal otolith shape of European perch, *Perca fluviatilis* (Actinopterygii: Percidae) from Lake Ladik, Turkey

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Abstract: European perch undergoes several ontogenetic dietary shifts during its lifetime. The present study focused on the variability in otolith shape caused by ontogenetic dietary changes in this species. A total of 172 specimens (7.5 to 27.5 cm TL) were caught by local fishermen in Lake Ladik from April to November 2019. Shape indices (SIs) and elliptic Fourier coefficients (EFCs) were calculated for each otolith. To detect ontogenetic changes in otolith shape, SIs and EFCs were compared among the three defined size classes (I, II, and III). Size-related groups were distinguished by both SIs and EFCs. Otolith shape analysis was more effective than morphometric analysis in distinguishing ontogenetic groups. Simultaneous use of both methods improved the classification success of each method alone. This study demonstrated that otolith shape of European perch is influenced by ontogenetic changes in diet. However, sexual maturity of the fish is also responsible for these variations in otolith shape.

Key words: *Perca fluviatilis,* otolith shape, morphometry, Fourier analysis, ontogenetic diet shift

1. Introduction

Otoliths are paired calcified structures in the skull of bony fishes that support hearing and balance. There are three pairs of otoliths called sagittae, lapilli, and asterisci (Quist et al., 2012). The shape and size of otoliths are primarily determined by genetic factors. However, there is also considerable variability caused by environmental conditions. Therefore, otoliths exhibit considerable intra and interspecific variations (Wright et al., 2002; Vignon and Morat, 2010). Due to the interspecific differences in otolith shape, these calcified structures have been used in fish taxonomy (Davoodi and Rahimian, 2016; Lin and Al-Abdulkader, 2019), in distinguishing populations or stocks (Vasconcelos et al., 2018; Wiff et al., 2020), in trophic ecology studies to determine the diet of piscivorous animals (Carvalho et al., 2019; Byrd et al., 2020), ecomorphological studies (Jaramillo et al., 2014; Assis et al., 2020), paleontological and paleo-ecological studies (Reichenbacher et al., 2007; Reichenbacher and Kowalke, 2009), age and growth analyses (Khan et al., 2019; Heral and Bayhan, 2020).

Although otoliths have a species-specific morphological structure, they may also exhibit intraspecific changes in shape and size due to the effects of internal (physiological) and external (environmental) factors (Mille et al., 2015). Otolith morphology can vary between populations (Morat

et al., 2012; Ozpicak et al., 2018) or stocks (Paul et al., 2013; Zhao et al., 2018) of the same species, and within a species depending on sex (Yılmaz et al., 2014; Başusta and Khan, 2021), diet (Gagliano and McCormick, 2004; Mille et al., 2016), and ontogeny (Campana, 2004). In addition, morphological differences (asymmetry) can be observed between the right and left otoliths of the same fish (Motamedi et al., 2021; Teimori et al., 2021). On the other hand, environmental factors such as water temperature (Hüssy, 2008; Mahé et al., 2019), depth (Tuset et al., 2003; Assis et al., 2020), substrate type (Volpedo and Fuchs, 2010; Jaramillo et al., 2014) and salinity (Capoccioni et al., 2011; Avigliano et al., 2014), as well as extreme habitat conditions (Schulz-Mirbach et al., 2011; Deng et al., 2011) can cause morphological variation in otoliths.

Ontogenetic allometry is a critical component in defining otolith shape (Monteiro et al., 2005; Xiong et al., 2015). Therefore, it is important to understand and describe the variability of otolith shape during ontogeny (Vignon, 2012). The literature reports that otolith morphology varies to some degree depending on the ontogenetic stage, which is determined by age (Ye et al., 2015; Villegas-Hernández et al., 2018), size (Gonzales Naya et al., 2012; Motamedi et al., 2021), year class (Bolles and Begg, 2000; Gonzales-Salas and Lenfant, 2007), sexual maturity status (Montanini et al., 2017; Cerna et al., 2019)

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or early life stages (larva to juvenile) (Yan et al., 2017; Coelho et al., 2019). In addition, Vignon (2012) found that otolith shape changed in a coral reef fish (*Lutjanus kasmira*) as it evolved from juveniles in the estuary to adults occupying either the channel or the outer reef off the coast of French Polynesia. Therefore, the researcher emphasized that otolith morphology is synergistically influenced by both ontogeny and environmental conditions. Similarly, Curcio et al. (2014) attributed differences in otolith shape between juvenile and adult *Lepidonotothen larseni* to different habitat preferences (pelagic and epibenthic) and ontogenetic shifts in feeding habits. In addition, Morat et al. (2012) demonstrated that ontogenetic shifts in feeding habits of red mullet populations in the northwestern Mediterranean affect otolith morphology.

The European perch (*Perca fluviatilis* L., 1758) is a carnivorous perch that is widely distributed throughout Eurasia but has also been successfully introduced into South Africa, Australia and New Zealand (Craig, 2000). Its size varies greatly depending on the water body in which it grows (Ceccuzzi et al., 2011). The diet of the European perch consists of zooplankton, benthic invertebrates and fish, and this species undergoes ontogenetic dietary changes (Persson et al., 1991, Yazıcıoğlu et al., 2016). Juveniles feed on pelagic zooplankton, then transition to benthic resources with age, and finally prey on fish when they are large enough (Hjelm et al., 2000). Although it is known that otolith shape and morphometry can change with fish ontogeny (Campana, 2004), little information is available on changes in otolith morphology during ontogeny for *P. fluviatilis* (Souza et al., 2020). Moreover, there is no study on the detection of the otolith morphological and morphometric changes based on ontogenetic diet differences in this species. Therefore, the main objective of our work is to assess the effects of the size-related ontogenetic shifts in feeding habits on the sagittal otolith shape of the European perch from Lake Ladik, Turkey. This research is a valuable contribution to studies on the biology of the species and the trophic ecology of its potential predators.

2. Materials and methods

2.1. Study area and sample collection
Lake Ladik (35° 40′–36° 05′ E and 40° 50′–41° 00′ N) is located on the borders of Ladik district, south of Samsun province, northern Anatolia (Figure 1). It is 10 km away from Ladik district. This lake has a tectonic character in terms of formation, an area of 1000 ha and a maximum depth of 6 m (Yılmaz et al., 2015). It was classified as a eutrophic and shallow lake (Apaydın Yağcı et al., 2015).
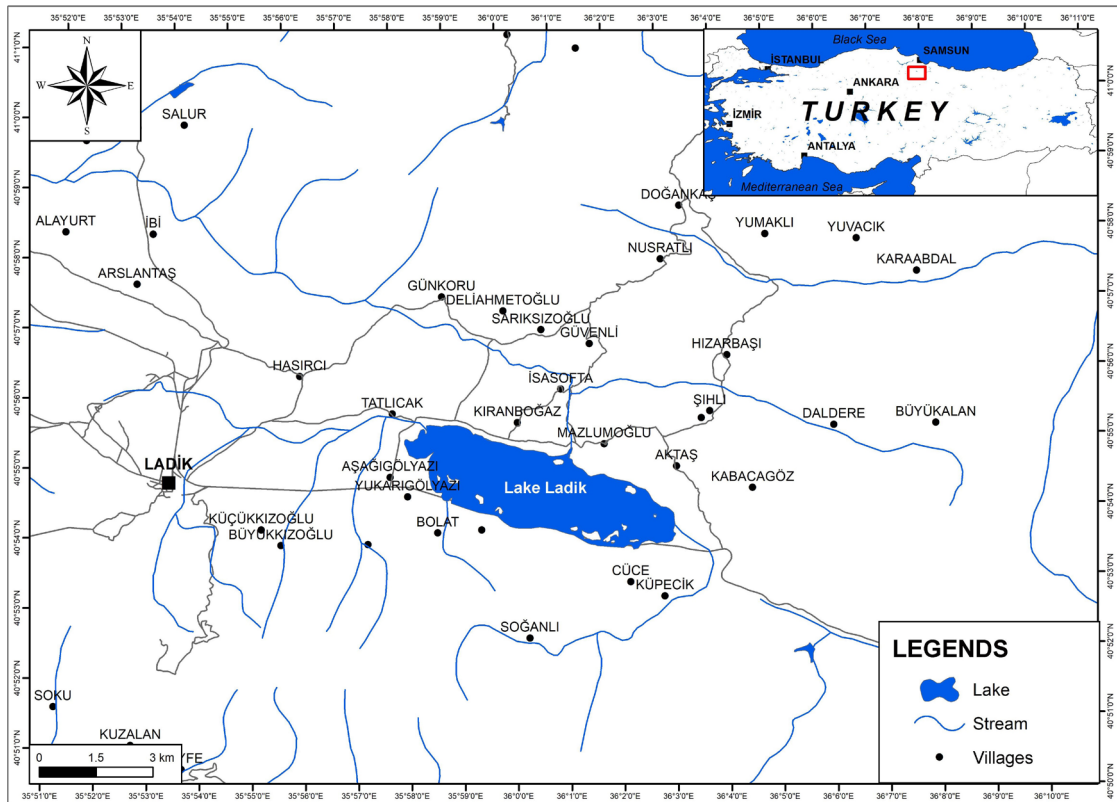

Figure 1. Map of the study area.

Sampling was conducted during the period April-November 2019. European perch samples were collected from commercial fishermen in Lake Ladik. A total of 172 specimens (90 females and 82 males) were collected, stored in a cooler, and brought to the laboratory.

2.2. Laboratory analysis

The total length (TL in cm) and sex of each fish were recorded. The studied specimens were divided into three size classes (Table 1): small (< 14 cm TL, N = 60), medium (14–20.9 cm TL, N = 60), and large fish (> 21 cm TL, N = 52). The boundaries of these size classes were established as a consequence of a diet analysis study that revealed the main shifts in feeding habits of the European perch individuals in Lake Ladik (Yazıcıoğlu et al., 2016). The sagittal otolith (sagitta) pairs were removed, washed, dried and stored in labeled plastic vials. All analyses were performed using only the right otoliths, as no significant morphometric difference between the left and right otoliths of the European perch was found in previous works (Yılmaz et al., 2014; Şimşek et al., 2019). Otoliths were viewed under a binocular microscope (Leica S8APO) at 10× magnification. Each otolith was systematically positioned with the *sulcus acusticus* facing upward and the rostrum facing left. Two-dimensional digital images of the otoliths were taken with a digital camera (Leica DFC295). Reflected light was used to obtain high-contrast digital images. Otoliths were photographed as white silhouettes on a black background.

The following morphometric variables were measured using image analysis software (Leica Application Suit ver. 3.8): otolith length (OL, mm), otolith height (OH, mm), otolith perimeter (OP, mm) and otolith area (OA, mm^2) (Figure 2). Then, six shape indices (SIs) such as shape factor, aspect ratio, circularity, roundness, rectangularity, and ellipticity were calculated based on these measurements. The formulae and biological meanings of these indices are given in Table 2 (Tuset et al., 2021).

The shape of each otolith was evaluated with the elliptic Fourier analysis. This method represents the outline using several components known as harmonics. Each harmonic has four coefficients (a, b, c, d), which are the result of projecting each point of the contour onto the (x) and (y) axes. The precision of the outline description improves as the number of harmonics increases (Kuhl and Giardina, 1982). Shape 1.3 software (Iwata and Ukai, 2002) was used to calculate the elliptic Fourier coefficients (EFCs). A total of 80 coefficients were obtained for the maximum of 20 harmonics. The EFCs were normalized according to the first harmonic in the shape program and were thus made invariant against the differences in the otolith size, its orientation and the starting point of the outline. In addition, the number of harmonics required to adequately describe the otolith outline was determined using the

Table 1. Number of specimens (N) and fish size composition for each ontogenetic group of European perch from Lake Ladik.

Ontogenetic groups	Sex	N	Total length (cm)	
			Mean ± SD	Range
I	F	30	11.03 ± 1.90	7.5–13.9
	M	30	10.32 ± 1.63	8.0–13.9
II	F	30	17.38 ± 1.57	14.2–20.8
	M	30	17.15 ± 1.76	14.3–20.8
III	F	30	23.54 ± 1.76	21.3–27.5
	M	22	23.38 ± 1.63	21.1–26.5

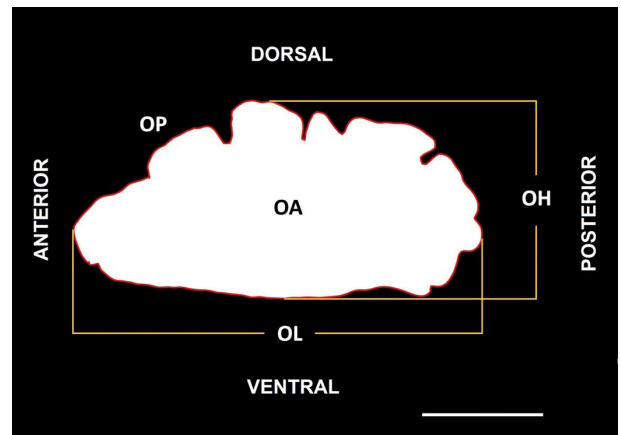F, female; M, male; SD, standard deviation.

Figure 2. Proximal photograph and measurements of the right sagittal otolith of European perch from Lake Ladik. OL: otolith length, OH: otolith height, OP: otolith perimeter (red line), OA: otolith area, scale bar = 2 mm.

Fourier power spectrum (Crampton, 1995). For the nth harmonic, the Fourier power (FP_n) is given by expression:

$$FP_n = \frac{a_n^2 + b_n^2 + c_n^2 + d_n^2}{2},$$

where a_n, b_n, c_n and d_n are the Fourier coefficients of the nth harmonic. Then, the percentage Fourier power ($FP_n\%$) and the cumulative percentage of the Fourier power ($CFP_n\%$) were calculated by the following formulas:

$$FP_n\% = \left(\frac{FP_n}{\sum_1^n FP_n} \right) \times 100$$

$$CFP_n\% = \sum_1^n FP_n\%.$$

Table 2. Otolith shape indices calculated from morphometric measurements.

Shape indices	Equation	Explanation
Form factor	$(4\pi \times OA) / OP^2$	It estimates the irregularity of the otolith surface area, and takes a value of 1.0 for a perfect circle.
Aspect ratio	OL / OH	It refers to the degree of otolith elongation.
Circularity	OP^2 / OA	It compares the otolith shape to a perfect circle.
Roundness	$(4 \times OA) / (\pi \times OL^2)$	It compares the otolith shape to a perfect circle.
Rectangularity	$OA / (OL \times OH)$	It describes the variations in length and height with respect to the otolith area, and 1.0 corresponds to the perfect square.
Ellipticity	$(OL - OH) / (OL + OH)$	It gives information about whether the change in axes is proportional or not.

OL, otolith length; OH, otolith height; OP, otolith perimeter; OA, otolith area.

As 99.99% of the cumulative power was described by the first nine harmonics, the otolith shape of the European perch was summarized by 36 Fourier coefficients. However, the first three coefficients (a1, b1, c1) derived from the 1st harmonic were not included because they degenerated during the normalization process. Therefore, the total number of EFCs for each otolith was determined as 33 (4×9−3). Each of the EFCs was treated as an independent variable.

2.3. Data analysis
Before each analysis, the data were subjected to a normality and homogeneity tests, i.e. the Kolmogorov–Smirnov test (or Shapiro–Wilk test) and Levene's test, respectively. The independent two-sample *t*-test or Mann–Whitney *U* test was used to compare the fish size of females and males in each ontogenetic group. The Kruskal–Wallis test was used to examine the distribution of fish size between ontogenetic groups.

The sex effect (female-male) on the otolith variables (SIs and EFCs) in each of the ontogenetic groups was controlled with the analysis of covariance (ANCOVA), using sex as the main factor and fish size as a covariate (Song et al., 2019). If the interaction "sex×TL" was found to be significant in the ANCOVA, the corresponding variable was not included in subsequent analyzes because it could not be accurately standardized.

The effects of fish size on otolith parameters (SIs and EFCs) need to be examined to allow valid comparisons between ontogenetic groups. To this end, the ANCOVA test was performed by using group as the main factor and fish size as a covariate (Song et al., 2019). If the interaction "group×TL" was significant in the ANCOVA, the parameter in question was excluded from further analyses because it could not be precisely adjusted. If it was not, this variable was standardized according to the allometric growth model (Lleonart et al., 2000):

$$M_S = M_O \left(\frac{\overline{x}}{x}\right)^b,$$

where M_s is the standardized variable; M_o is the original variable; $\overline{x}$ is the mean fish size (16.85 cm) for all specimens from three ontogenetic groups; x is the fish size; b is a parameter calculated for each variable as the slope of the regression between log M_o and log x for each variable. However, the EFCs were not subjected to the fitting process because they were already generated in a standardized way in the Shape software (Agüera and Brophy, 2011). The standardized values of the different otolith variables (SIs and EFCs) were compared between ontogenetic groups using one-way ANOVA or the Kruskal–Wallis test by choosing an appropriate pairwise comparison test.

Since multicollinearity problem was detected among the otolith variables, a principal component analysis (PCA) based on the variance-covariance matrix was performed to reduce the dimensionality of the data. Thus, a new set of orthogonal variables, principal component scores (PCs), for the subsequent canonical discrimination analysis (CDA) was obtained to separate ontogenetic groups from each other (Song et al., 2019). The PCA was performed three times with SIs, EFCs and a combination of both variables.

The CDA was performed with the adopted PCs to compare otolith shape variations among the ontogenetic groups. Three CDA were carried out: one using only the selected PCs from SIs, another using only the selected PCs from EFCs and the last one combining both. The quadratic discriminant function analysis was employed because the assumption of the homogeneity of group covariance matrices was not met (Box's M test, p < 0.01). The performance of the discriminant analysis was evaluated with the Wilks' lambda (λ) values. The classification accuracy was estimated by using leave-one-out cross-

validation by means of the jackknife method. Comparisons among the ontogenetic groups were conducted using a permutational multivariate analysis of variance (one-way PERMANOVA; Anderson, 2001). The one-way PERMANOVA was based on the Euclidean distance and 9999 permutations.

All statistical analyzes were performed using SPSS 21.0, Minitab 17.0, PAST 3.0 (Hammer et al., 2001) and the Microsoft Excel package.

3. Results

3.1. Fish morphometry
There was no significant difference in fish size between the sexes in each group (*t*-test, p > 0.05 for group I and II; Mann–Whitney *U* test, p > 0.05 for group III). Fish size differed considerably among ontogenetic stages (Kruskal–Wallis test, p < 0.001).

3.2. Otolith morphometric analysis
The effect of sex difference on the SIs was not significant (ANCOVA, p > 0.05 in all cases). For this reason, the SI values from both sexes were combined for the next analysis. The form factor and circularity indices were omitted from further analyses because of the interaction between group and fish size observed for these parameters (ANCOVA, p < 0.05). The remaining four SIs (aspect ratio, roundness, rectangularity and ellipticity) were allometrically standardized with the fish size. All shape indices, except roundness, were significantly different among ontogenetic groups (one-way ANOVA, Table 3). The highest values of the SIs were observed in group II. In the PCA using only SIs, the first two PCs accounted for 100% of the total variance (99.43% for PC1, 0.57% for PC2). PC1 differentiated the groups based on the aspect ratio (correlation coefficient, R = 0.78) and roundness (R = 0.58). Roundness (R = −0.76), aspect ratio (R = 0.48) and rectangularity (R = 0.44) were responsible for the variation in the PC2. The results of the CDA performed using the SIs are presented in Table 4 and shown in Figure 3. The first two canonical discriminant functions were used in the CDA (λ = 0.887 for function 1–2, p = 0.000; λ = 1.000 for function

2, p = 0.859). The first function (F1) explained 99.9% of the total variance (eigenvalue, E = 0.127) and was closely correlated with PC1 (R = 0.96). The F1 distinguished group II from the other groups well. The second function (F2) explained 0.1% of the total variance (E = 0.00) and was closely correlated with PC2 (R = 1.00). The F2 was unable to separate between ontogenetic groups. The percentages of well-classified individuals obtained with the CDA were very low for group I (38.3%) and group III (30.8%). A moderate classification percentage was obtained for group II (68.3%) using the SIs. The CDA generated a 46.5% overall classification success rate. The nonparametric multivariate analysis verified the CDA results. The one-way PERMANOVA did not show significant differences among ontogenetic groups (F = 1.82, p = 0.139).

3.3. Fourier shape analysis
In the analysis of the sex effect on the EFCs, the ANCOVA test showed that seven EFCs (a2, b3, b5, b7, b8, a9, b9) differed between females and males (p < 0.05). Therefore, these coefficients were removed from the subsequent analysis. Regarding the effects of the fish size on the EFCs, it was determined that four EFCs (c2, d3, c4, a7) differed among ontogenetic groups (ANCOVA, p < 0.05). Thus, they were not included in the further analysis. According to statistical tests, four of the remaining 22 EFCs were significantly different among groups (One-way ANOVA, c3, F = 3.36, p = 0.03; d8, F = 3.54, p = 0.03; Kruskal–Wallis test, d1, H = 105.17, p = 0.00; a3, H = 9.42, p = 0.01). In the PCA using only EFCs, the first two PCs described 100% of the total variance (98.999% for PC1, 1.001% for PC2). PC1 differentiated the groups based on the coefficient d1 (R = 0.99). The coefficients b2 (R = 0.58) and c3 (R = −0.54) were responsible for the variation in the PC2. The first two canonical discriminant functions were used in the CDA (λ = 0.409 for function 1-2, p = 0.000; λ = 0.955 for function 2, p = 0.005). The F1 explained 96.6% of the total variance (E = 1.333) and was closely correlated with PC1 (R = 0.99). The F1 distinguished group I from the other two groups well. The F2 explained 3.4% of the total variance (E = 0.047) and was closely correlated with PC2 (R = 1.00).

Table 3. Standardized values (mean ± SD) of the shape indices for the three size classes of European perch, and their statistical comparisons among the ontogenetic groups.

Shape indices	Group I	Group II	Group III	F	p
Aspect ratio	1.95 ± 0.07[b]	1.98 ± 0.08[a]	1.94 ± 0.12[ab]	3.68	0.03
Roundness	2.67 ± 0.15	2.70 ± 0.12	2.66 ± 0.14	0.91	0.40
Rectangularity	0.69 ± 0.02[b]	0.70 ± 0.02[a]	0.69 ± 0.02[b]	3.58	0.03
Ellipticity	0.32 ± 0.02[b]	0.33 ± 0.02[a]	0.32 ± 0.02[b]	4.28	0.02

Means with different lowercase letters are significantly different. F, ANOVA test statistic; p, significance.

Table 4. Leave-one-out cross-validation matrix of the quadratic discriminant function analysis for the three ontogenetic groups of European perch, based on different variables of the otolith.

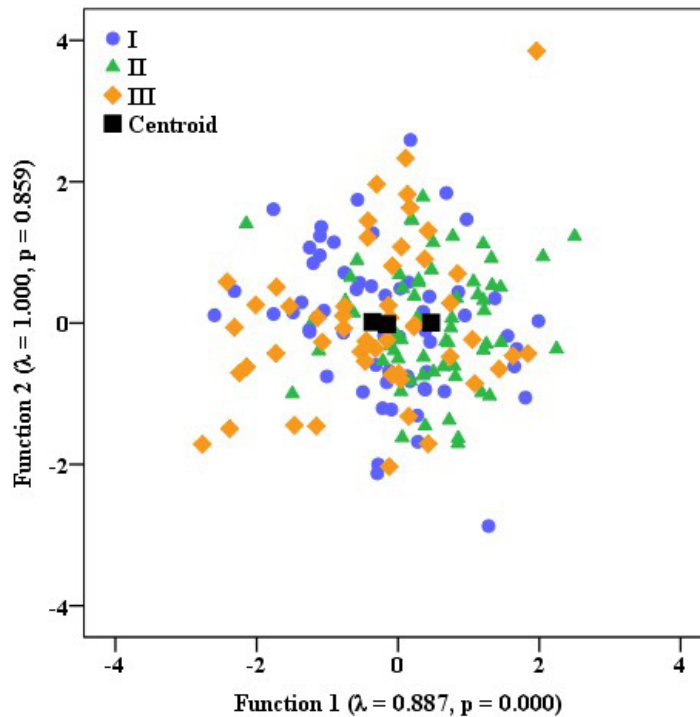| Variable	Group	Predicted group membership			Total	Overall
		I	II	III		
SIs	I	**38.3**	40.0	21.7	100.0	
	II	20.0	**68.3**	11.7	100.0	46.5
	III	32.7	36.5	**30.8**	100.0	
EFCs	I	**88.3**	10.0	1.7	100.0	
	II	18.3	**56.7**	25.0	100.0	69.9
	III	3.8	32.7	**63.5**	100.0	
SIs + EFCs	I	**88.3**	8.3	3.4	100.0	
	II	16.7	**66.6**	16.7	100.0	73.3
	III	5.8	30.7	**63.5**	100.0	

Correct classifications are in bold.

Figure 3. Quadratic discriminant function analysis plot indicating separation of the three ontogenetic groups (I, II, III) of European perch using the SIs.

The F2 separated group II from group III (Figure 4). The CDA generated an overall classification success rate of 69.9% (Table 4). The highest rate was obtained for group I (88.3%), followed by group III (63.5%) and group II (56.7%). The nonparametric multivariate analysis verified the CDA results. The one-way PERMANOVA yielded significant differences among the three ontogenetic groups (F = 37.79, p = 0.0001). Pairwise comparison detected considerable differences between groups I and II (p < 0.0002), groups I and III (p < 0.0002), and groups II and III (p < 0.0002). The reconstruction of the otolith shape outline from the average EFCs for each ontogenetic group

is shown in Figure 5. The main shape differences appeared on the dorsal margin, and also the posterior-dorsal and posterior-ventral regions of the otolith.

3.4. Combination of the two methods

In PCA with a combination of SIs and EFCs, the first two PCs accounted for 100% of the total variance (72.05% for PC1, 27.95% for PC2). The coefficient d1 (R = 0.98) was responsible for the major variation in the PC1. The second component (PC2) was defined by aspect ratio (R = 0.77) and roundness (R = 0.56). The first two canonical discriminant functions were used in the CDA (λ = 0.385 for function 1-2, p = 0.000; λ = 0.892 for function 2, p = 0.000). The F1 (E = 1.317) was strongly related to PC1 (R = 0.96) and explained 91.6% of the total variance, distinguishing group I well from the other two groups. The F2 (E = 0.121) was strongly correlated with PC2 (R = 0.99) and explained 8.4% of the total variance, separating groups II and III (Figure 6). The CDA achieved an overall classification success rate of 73.3% (Table 4). The highest rate was obtained for group I (88.3%), followed by group II (66.6%) and group III (63.5%). These results were also confirmed by the one-way PERMANOVA which showed a significant difference among groups (F = 5.67, p < 0.001).

4. Discussion

The results of this study indicated the presence of ontogenetic variations in the sagittal otolith shape of

European perch captured from Lake Ladik. In many studies carried out with different fish species, it has been reported that otolith morphology varies according to ontogenetic stages represented by size groups (Xiong et al., 2015; Montanini et al., 2017; Cerna et al., 2019; Motamedi et al., 2021; Teimori et al., 2021). Traditional morphometry analysis based on different shape indices showed that the sagittal otolith of the species is elongated and oval-shaped. The same observation was also reported by some researchers who have studied the otolith morphometry of European perch (Yilmaz et al, 2014; Sapota and Dabrowska, 2019). However, Sapota and Dabrowska (2019) reported that there was no change in European perch otolith shape during fish growth. Contrary to Sapota and Dabrowska (2019), we detected some differences in aspect ratio, rectangularity, and ellipticity indices according to fish size classes. While the highest values of shape indices were obtained for the fish in the medium-sized group, the lowest values were calculated for the individuals in the large-sized group. This finding indicates that the otoliths of medium-sized specimens are more elongated and have a more oval shape. Similar to our results, Biolé et al. (2019) reported that medium-sized individuals of *Odontenthes argentinensis* have a higher aspect ratio and rectangularity values than small- and large-sized ones. Callicó Fortunato et al. (2017) found that the smaller individuals of *Mugil liza* have a lower aspect ratio and therefore a more rectangular
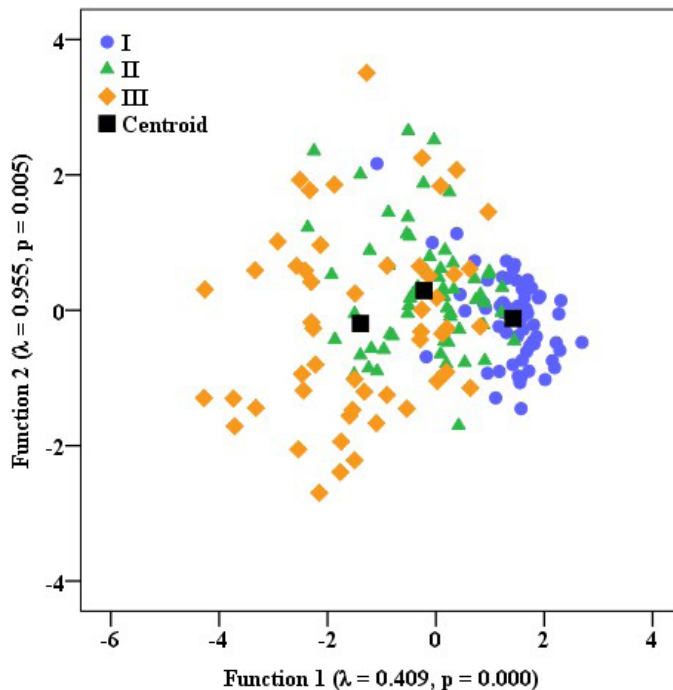

Figure 4. Quadratic discriminant function analysis plot indicating separation of the three ontogenetic groups (I, II, III) of European perch using the EFCs.
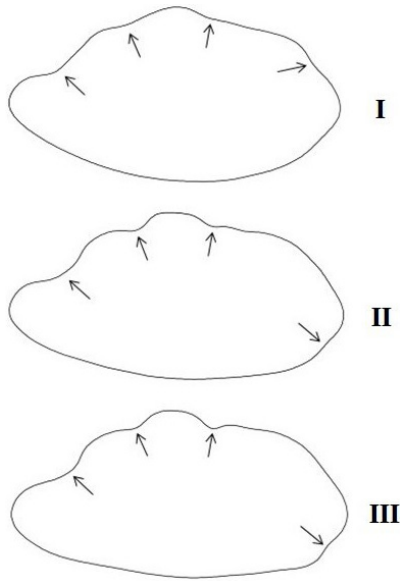
Figure 5. Average shapes of the otoliths of European perch in the three ontogenetic groups, based on the mean EFCs. Arrows show the main shape differences.

otolith shape, while larger specimens tend to have a more circular otolith. Bostanci et al. (2015) determined that the ellipticity index increased with total length in *Alburnus mossulensis*, while it decreased in *Alburnus tarichi*. Tuset et al. (2003) observed that the otoliths turned into a more oval shape with the increased length in *Serranus scriba*.

Our work showed that the aspect ratio, roundness, and rectangularity indices are more effective in discriminating the ontogenetic stages. However, we were able to achieve a low overall classification success of 46.5% for the ontogenetic group discrimination of European perch, with otolith shape indices mentioned above. The failure of shape indices in the differentiation of ontogenetic groups may be associated with the lack of sufficient and independent morphometric variables. Recently, the insufficiency of classical indices in identifying fish species has been documented, and their routine usage is no longer recommended (Tuset et al., 2021). Although the biological interpretation of elliptic Fourier analysis is more complex than linear morphometry (Stransky and MacLellan, 2005), it is considered the most objective and powerful shape analysis technique to capture all shape variations and small-scale individual differences in otolith outlines (Campana and Casselman, 1993). In the present study, three ontogenetic groups of European perch were classified with an accuracy of 69.9% using elliptical Fourier coefficients. Similarly, Biolé et al. (2019) achieved a high overall classification success of 98.4% in separating the three ontogenetic developmental stages of *Odontesthes argentinensis* using elliptical Fourier descriptors. Our study

also disclosed that using elliptical Fourier coefficients alone or combining them with morphometric variables in describing ontogenetic changes in sagittal otoliths of European perch provides higher classification success than using shape indices alone. Similar findings have been obtained in other species, such as *Sebastes spp.* (Zhuang et al., 2015), *Astyanax spp.* (Avigliano et al., 2018), and *Nibea albiflora* (Song et al., 2019).

Otolith morphology is regulated by a complex combination of physiological (sexual maturity, growth, etc.) and environmental (temperature, salinity, depth, diet, etc.) factors (Vignon and Morat, 2010; Mille et al., 2015). The effects of these factors on the otolith shape may be more or less depending on the fish species and otolith type (Bounket et al., 2019). Hüssy (2008) suggested that the general shape of the otolith is an ontogenetic process and that finer details can be changed by environmental conditions, particularly feeding level (Gagliano and McCormick, 2004) and nutrient availability (Cardinale et al., 2004). On the other hand, it has been reported that diet composition may be a source of otolith shape variation through direct and/or indirect (otolith growth) processes (Mille et al., 2016). Moreover, ontogenetic changes in diet composition may contribute to the differentiation of otolith morphology (Morat et al., 2012; Biolé et al., 2019). The diet of European perch inhabiting Lake Ladik displays marked changes according to size classes. The small-sized fish (<14 cm TL) fed only on macroinvertebrates, while large-sized individuals (>21 cm TL) only consume prey fish. The diet of medium-sized specimens (14-20.9 cm TL) is composed of more prey fish and fewer macroinvertebrates (Yazıcıoğlu et al., 2016). These ontogenetic shifts in diet were reflected in the otolith shape, and the three size groups were correctly classified with a ratio of 73.3%. However, the most marked differences were observed in otoliths of the small-sized fish, with a classification accuracy of 88.3%. The classification accuracies were 66.6% and 63.5%, respectively, for medium- and large-sized fish. This case indicates that the otolith shape does not change much after the European perch reaches 14 cm in length. According to some authors, the most visible change in otolith shape occurs in a size corresponding to the onset of sexual maturity (Tuset et al., 2003; Gonzalez Naya et al., 2012; Xiong et al., 2015). This is the size in which metabolism is markedly altered, sexual maturation affects fish growth and thus has an impact on otolith shape (Morat et al., 2012). Therefore, in addition to the morphological differences between juvenile and adult fish, it is also possible to identify variations in otolith growth rates before and after first reproduction (Carvalho et al., 2015). The size of the first sexual maturity of the European perch specimens in Lake Ladik is unknown. However, Kottelat and Freyhof (2007) reported that sexual maturity of this species was reached in the size range
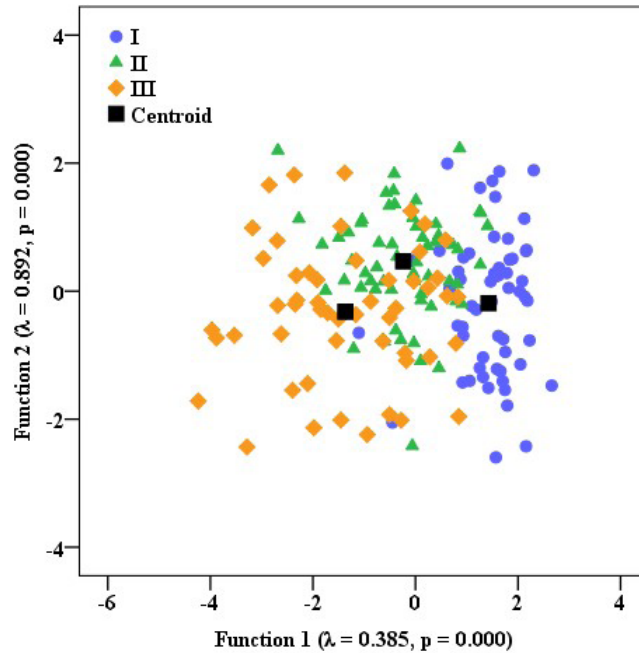
Figure 6. Quadratic discriminant function analysis plot indicating separation of the three ontogenetic groups (I, II, III) of European perch using the SIs plus EFCs.

corresponding to 1–2 years of age in males and 2–4 years of age in females. When these age ranges are accepted for the European perch specimens in the study area, the first maturity corresponds to 11.9 cm TL (8.7–14 cm) in males and 15.7 cm TL (11–21.5 cm) in females (Saygin et al., 2016). In this case, group I, which contains 80% of the immature fish, represents the juvenile stage of the species, while group II and III, which contains 97% of the mature individuals, corresponds to the adult stage. Discrimination analyses revealed the morphological differences in the otoliths of fish belonging to the two stages.

In conclusion, this study showed that there is clear ontogenetic variation in the otolith shape, which could be valuable to characterize different life stages of European perch. In distinguishing the ontogenetic stages represented by size groups, otolith shape analysis was more effective than morphometric analysis. Simultaneous use of both methods strengthened the analysis. The observed changes in sagittal otolith shape throughout the ontogeny of European perch living in Lake Ladik were associated with both dietary shifts and sexual maturity. The outcomes of the present work can be used in future studies on the trophic ecology of European perch predators and in population studies based on the contour of otoliths of this species.

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

Ethical statement
Ethical approval was not required for the animal study because fish samples were obtained from the local fishermen. The present work did not conduct any type of experiments on living animals.

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