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Stock Discrimination of Northern Pike *Esox lucius* L., 1758 Inhabiting Lakes Simenlik and Ladik (Samsun-Turkey) Using Otolith Biometry and Shape Analysis

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A B S T R A C T

This study was carried out to investigate the relationships between total length and otolith dimensions and to detect the otolith shape index values of Northern pike (*Esox lucius*) which has high economic value, sampled from Ladik and Simenlik Lakes. A total of 140 specimens (Lake Ladik: 82 individuals, Lake Simenlik: 58 individuals) were sampled. Otolith height, length, perimeter, and area were determined by Imaging Software. Nonlinear and linear models were 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. It was found that the relationship between the total length-otolith length was found to have the highest r^2 value (Lake Ladik r^2=0.949, Lake Simenlik r^2=0.914) among the total length-otolith morphometrics at both localities. Otolith shape indices were calculated by using otolith measurements. As a result of comparing shape indices, it was found that there was a significant difference in two localities using roundness, ellipticity, and aspect ratio (P <0.05). The results indicated otolith shape indices could be used as a suitable tool to discriminate Northern pike populations.

Keywords: *Esox lucius*, otolith shape, biometry, Ladik Lake, Simenlik Lake

A R T I C L E I N F O

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Ladik ve Simenlik Gölleri'nde Yaşayan Turna Balığının Otolit Biyometrisi ve Şekil Analizleri Kullanılarak Stok Ayrımları

Öz: Bu çalışma, Ladik ve Simenlik Gölleri'nden örneklenmiş ve ekonomik değeri oldukça yüksek olan turna balığının (*Esox lucius*) total boyu ve otolit özellikleri arasındaki ilişkileri incelemek ve otolit şekil indeks değerlerini saptamak için gerçekleştirilmiştir. Toplamda 140 örnek (Ladik Gölü: 82 birey, Simenlik Gölü: 58 birey) örneklenmiştir. Otolit eni, boyu, çevresi ve alanı görüntü analiz programı ile belirlenmiştir. Otolit ölçümleri ve balık total boyu arasındaki ilişkinin belirlenmesinde doğrusal ve doğrusal olmayan ilişkilerden faydalanılmıştır. Otolit şekil analizleri için Şekil Faktörü, Yuvarlaklık, Dairesellik, Dikdörtgensellik, En-Boy Oranı ve Eliptiklik parametreleri kullanılmıştır. Her iki lokalite için de otolit ölçümleri ve balık boyu arasındaki ilişkilerde otolit boyunun en yüksek r^2 değerine sahip olduğu bulunmuştur (Ladik Gölü r^2=0,949, Simenlik Gölü r^2=0,914). Otolit şekil indeksleri otolit ölçümleri kullanılarak hesaplanmıştır. Şekil indekslerinin lokaliteler arasında karşılaştırma sonuçlarına göre, Yuvarlaklık, Eliptiklik ve En-Boy Oranı parametrelerinin önemli derecede farklı olduğu bulunmuştur. Sonuçlar, turna popülasyonlarının ayrım için otolit şekil indekslerinin kullanışlı bir araç olduğunu belirtmektedir.

Anahtar kelimeler: ***:*** *Esox lucius*, otolit şekli, biyometri, Ladik Gölü, Simenlik Gölü

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Introduction

Northern pike (*Esox lucius*) is a species of significant importance to both commercial and recreational fisheries with a wide range of distribution from arctic to subtropical waters. *E. lucius* can tolerate a wide variety of environmental conditions, but in terms of development stages; it is a mesothermal piscivore species that prefers shallow, moderately productive, mesotrophic-eutrophic freshwater environments (Casselman 1996).

Northern pike is considered as a top predator in the food web in most freshwater ecosystems (Soupir et al. 2000). And also, cannibalism occurs in both the early developmental stages of pike and adult fish, most instances of cannibalistic behavior refer to the consumption of larvae or juvenile individuals (Pereira et al. 2017). Pike can regulate the populations of prey species in its ecosystem, as well as its own population due to high cannibalistic behavior (Sharma and Borgstrøm 2008; Harvey 2009). In addition, stocking of pike is used in bio-manipulation experiments as an indirect tool to reduce the eutrophication process (Prejs et al. 1997). Because of all these features, the pike has been the subject of many different studies such as migration (Karás and Lehtonen 1993), feeding biology (Yazicioglu et al. 2018), otolith chemical studies (Stańczak et al. 2017; Möller et al. 2019), ecology (Craig 2008) and genetic (Nordahl et al. 2019; Sunde et al. 2020).

Otoliths, a true biological and environmental archive of fishes, are one of the most preferred methods in stock separation studies, especially being species specific and can reflect phenotypic plasticity. The otoliths facilitate reconstruction of environmental parameters (temperature, salinity) and life history traits of fish (age, growth, reproduction, and migration) (Radhakrishan et al. 2009). The otoliths record the life history features of the individuals (age, chemical elements, reproduction, etc.) and have been described as a "flight recorder" of fish (Lecomte-Finiger 1992). They are considered as valuable markers for distinguishing different fish populations (Tuset et al. 2003; Petursdottir et al. 2006; Zengin et al. 2015; Renán et al. 2016; Avigliano et al. 2019; Mahè et al. 2019; Vu and Kartavtsev 2020; Labidi et al. 2020; Ghanbarifardi and Zarei 2021; Chen et al. 2021). It is critical to determine the phenotypic variations produced by environmental influences in fisheries management and biology. At this point, because of being species-specific, otolith shape and morphometry have been used as a natural marker and a useful tool for the identification of fish stocks (Tracey et al. 2006). However, the analysis of otoliths retrieved from the stomachs or feces of piscivorous predators can be used to provide information on the type, size, mass, and energetic content of their fish prey (Morley and Belchier 2002). Fish size-otolith biometry 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 (Battaglia et al. 2010; Yilmaz et al. 2015; Mehanna et al. 2016; Bostanci et al. 2017; Saygin et al. 2017; Yazicioğlu et al. 2017; Ozpicak 2020; Fey and Greszkiewicz 2021; Osman et al. 2021).

The aim of this study is (i) to examine morphological variations and shape analyses in sagittal otoliths, (ii) to reveal relationships between total length and otolith measurements, and determine the intraspecies variation in populations of *E. lucius*, sampled from Lakes Simenlik and Ladik, Turkey.

Materials and Methods

Study Area and Sampling

Esox lucius samples were obtained from fishermen between March 2017-February 2018 in Lake Ladik, and between February 2017-November 2017 from Lake Simenlik (Figure 1). Specimens were measured to the nearest 0.1 cm for total length (*TL*) and weighted to the nearest 0.01g. The sex was determined by macroscopic examination of the gonads.

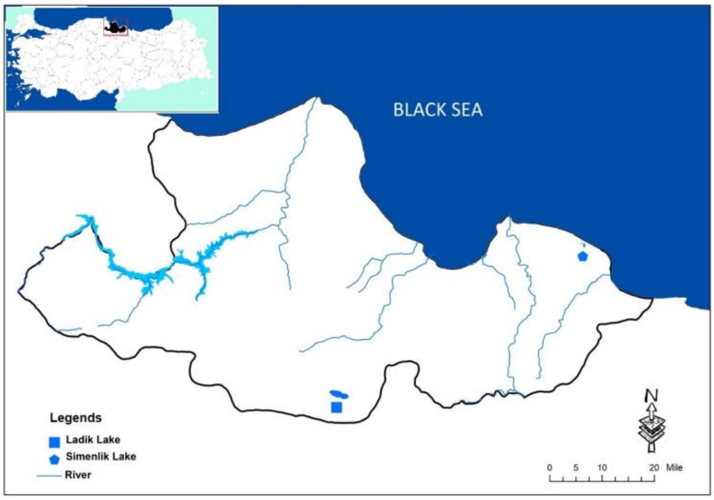

Figure 1. The map of Lakes Simenlik and Ladik

Otolith Preparation and Statistical Analysis

Sagittal otoliths were removed by making left and right distinctions. Otoliths were removed through a cut in the cranium, then cleaned with ethanol and stored dry. All otolith pairs were weighted with Precisa scales (*OW*) (±0.0001 g). All otoliths were photographed on the distal side with a Leica DFC295 digital camera. Otolith height (*OH*), 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 $(E) = (OL - OH)/ (OL + OH)$; rectangularity $(REC) = (OA/(OL \times OH)$ and aspect ratio $(AR) = (OL/OH)$ (Tuset et al. 2003; Ponton 2006) (Figure 2).

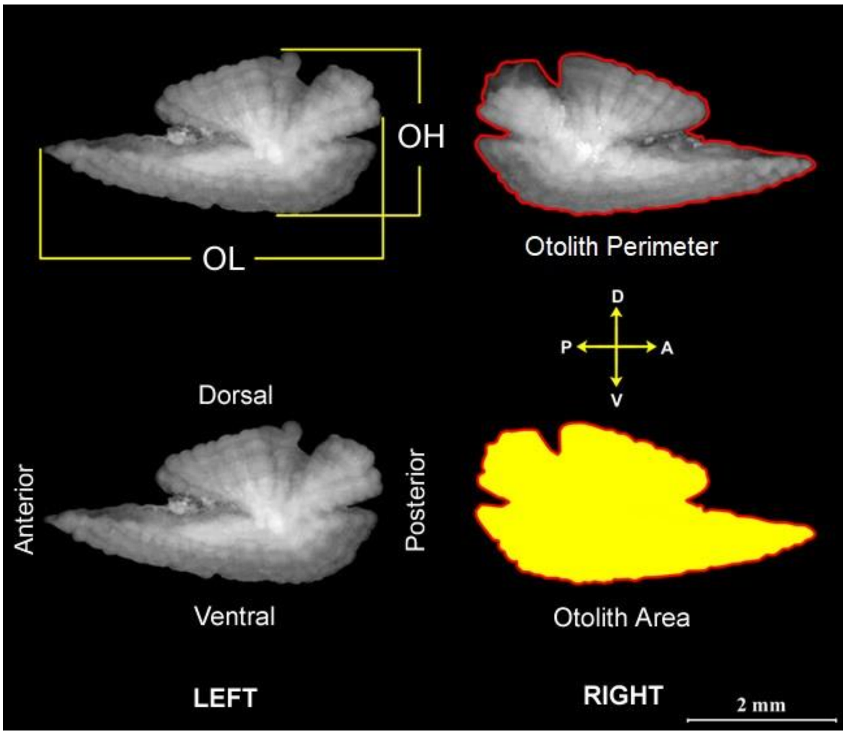

Figure 2. Sagittal otolith pairs of *Esox lucius* (*OH*: Otolith Height, *OL*: Otolith Length)

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

Result

A total of 140 individuals were sampled from Lake Ladik (*N*, 82; min-max, 33.0-74.0; Mean±SD, 42.83±8.87 cm *TL*) and Lake Simenlik (*N*=58; min-max, 28.5-58.1; Mean±SD, 41.60±7.72 cm *TL*). Descriptive statistics of sagittal otoliths were offered in Table 1 for both localities. According to left and right otoliths comparisons, there were no differences in terms of otolith characteristics for Ladik Lake samples but there were differences in *OL* for Simenlik samples ($P<0.05$). However, there were statistically differences in terms of *OL*, *OH*, and *OA* in Lake Ladik and also *OP* in Lake Simenlik between males and females ($P<0.05$).

As a result of the comparison analyzes performed, right otoliths were preferred in the measurements where there was no significant difference between the right and left otolith measurements of both samples. In addition, shape indices were calculated according to sex (Table 2). According to analysis there were no statistically significant differences between right and left otolith pairs in terms of shape indices ($P>0.05$).

The otolith shape indies of all individuals belonging to the Ladik Lake and Simenlik Lake samples were determined whether there was a significant difference in the otolith shape of the individuals sampled from these two localities (Figure 3).

Table 1. Descriptive statistics of sagittal otoliths according to localities (*OL*: Otolith Length, *OH*: Otolith Height, OW: Otolith Weight, *OP*: Otolith Perimeter, *OA*: Otolith Area, *R*: Right, *L*: Left)

SEX	Variable/Locality		Ladik Lake			Simenlik Lake		
♀			Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum
	OL	*R*	6.524±1.34	5.216	10.024	6.551±1.079	4.740	8.758
		L	6.503±1.30	5.197	9.698	6.489±1.175	4.294	9.098
	OH	*R*	3.178±0.56	2.533	4.630	3.087±0.487	2.189	4.004
		L	3.190±0.59	2.552	4.754	3.071±0.502	2.223	4.086
	OW	*R*	0.0162±0.01	0.008	0.045	0.0184±0.01	0.006	0.039
		L	0.0163±0.01	0.008	0.048	0.0183±0.01	0.005	0.040
	OP	*R*	19.513±4.00	14.919	30.351	19.245±3.086	14.036	25.660
		L	19.370±4.02	15.161	30.603	18.872±3.257	12.457	25.479
	OA	*R*	13.390±5.50	8.531	28.499	12.911±4.304	6.127	21.767
		L	13.321±5.58	8.378	29.610	12.814±4.463	6.024	22.873
♂	*OL*	*R*	5.942±0.841	5.066	8.527	6.056±0.855	4.443	7.937
		L	5.920±0.852	4.976	8.575	6.019±0.895	4.443	8.058
	OH	*R*	2.921±0.395	2.514	4.014	2.938±0.453	2.164	4.034
		L	2.925±0.401	2.512	4.116	2.943±0.441	2.141	3.991
	OW	*R*	0.0130±0.006	0.00810	0.033	0.0165±0.009	0.006	0.044
		L	0.0130±0.006	0.00800	0.033	0.0166±0.009	0.006	0.049
	OP	*R*	17.447±2.49	14.062	24.475	17.390±2.610	12.415	23.265
		L	17.551±2.53	14.251	24.182	17.503±2.716	12.341	23.144
	OA	*R*	11.156±3.38	8.285	21.224	11.617±3.437	5.848	20.269
		L	11.163±3.45	7.872	21.570	11.517±3.618	5.810	21.081
♀+♂	*OL*	*R*	6.219±1.135	5.066	10.024	6.303±0.997	4.443	8.758
		L	6.198±1.121	4.976	9.698	6.254±1.062	4.294	9.098
	OH	*R*	3.043±0.497	2.514	4.630	3.012±0.472	2.164	4.034
		L	3.051±0.511	2.512	4.754	3.007±0.473	2.141	4.086
	OW	*R*	0.0146±0.008	0.008	0.045	0.0174±0.009	0.006	0.044
		L	0.0145±0.009	0.008	0.048	0.0175±0.01	0.005	0.049
	OP	*R*	18.430±3.43	14.062	30.351	18.318±2.983	12.415	25.660
		L	18.416±3.43	14.251	30.603	18.187±3.051	12.341	25.479
	OA	*R*	12.219±4.63	8.285	28.499	12.264±3.915	5.848	21.767
		L	12.189±4.69	7.872	29.610	12.165±4.079	5.810	22.873

Table 2. Descriptive statistics of otolith shape indices of Ladik Lake and Simenlik Lake according to sex (*FF*: Form Factor, *RO*: Roundness, *C*: Circularity, *REC*: Rectangularity, *E*: Ellipticity, *AR*: Aspect Ratio)

Sex		Ladik Lake				Simenlik Lake			
	Shape Indices	Mean	SE	Min	Max	Mean	SE	Min	Max
♀	*FF*	0.432	0.006	0.367	0.489	0.429	0.008	0.342	0.519
	RO	0.390	0.004	0.338	0.434	0.374	0.003	0.347	0.410
	C	29.319	0.396	25.705	34.264	29.594	0.544	24.232	36.796
	REC	6.500	0.399	4.143	13.795	6.081	0.371	2.830	10.077
	E	0.343	0.003	0.310	0.395	0.359	0.003	0.319	0.396
	AR	2.047	0.015	1.897	2.308	2.122	0.016	1.935	2.310
♂	*FF*	0.454	0.005	0.380	0.538	0.475	0.009	0.389	0.630
	RO	0.395	0.002	0.361	0.424	0.395	0.003	0.355	0.436
	C	27.841	0.323	23.379	33.039	26.671	0.449	19.931	32.335
	REC	5.483	0.249	4.119	10.305	5.649	0.329	2.848	10.302
	E	0.340	0.002	0.302	0.368	0.347	0.003	0.316	0.389
	AR	2.034	0.010	1.866	2.167	2.066	0.014	1.922	2.272
♀+♂	*FF*	0.443	0.004	0.367	0.538	0.452	0.006	0.342	0.630
	RO	0.393	0.002	0.338	0.434	0.384	0.003	0.347	0.436
	C	28.544	0.265	23.379	34.264	28.132	0.399	19.931	36.796
	REC	5.967	0.236	4.119	13.795	5.865	0.247	2.830	10.302
	E	0.342	0.002	0.302	0.395	0.353	0.002	0.316	0.396
	AR	2.040	0.009	1.866	2.308	2.094	0.011	1.922	2.310

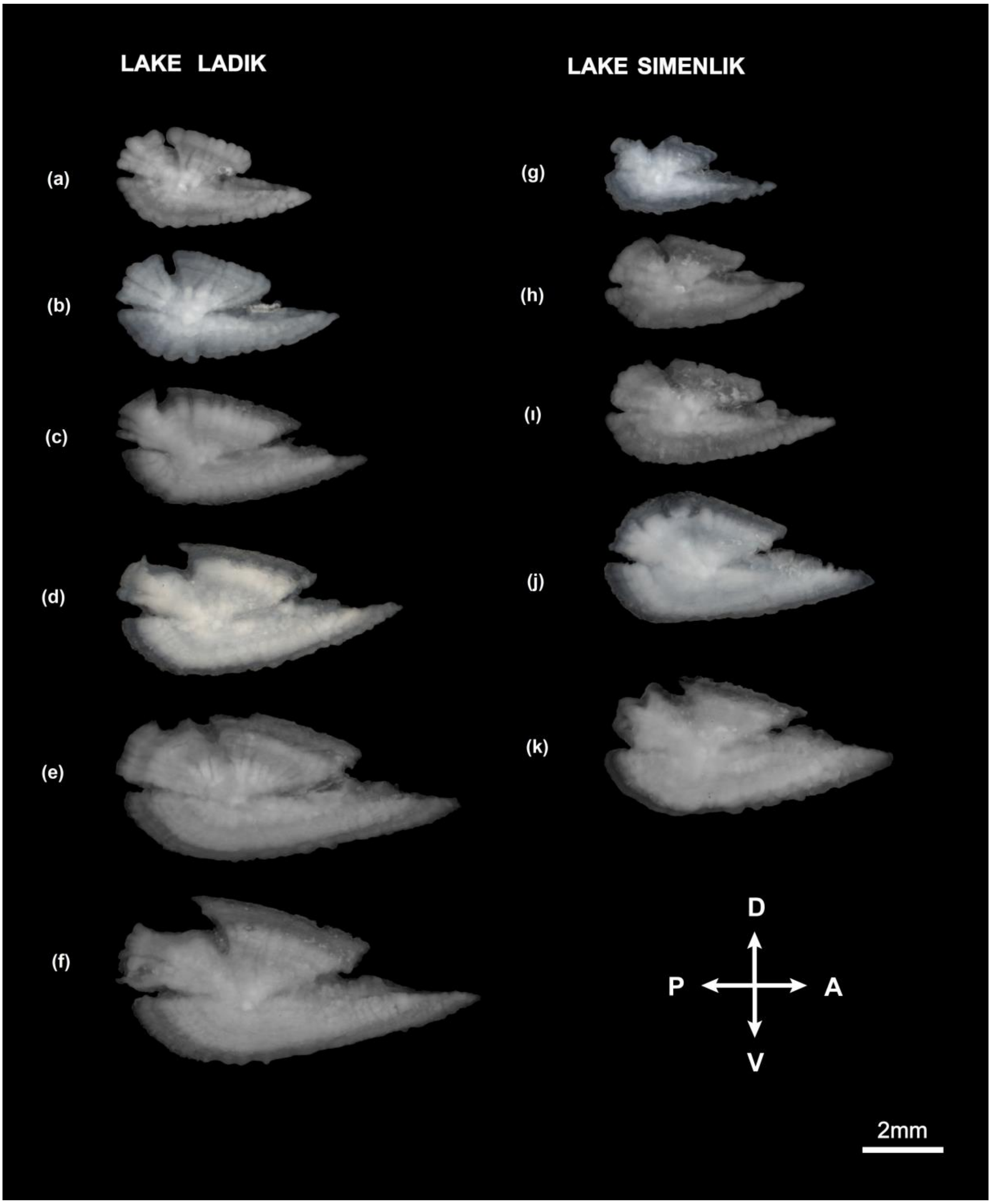

Figure 3. Otolith morphological development according to *TL*: (a) 34.6 cm, (b) 41.1 cm, (c) 48.4 cm, (d) 57.7 cm, (e) 68.2 cm, (f) 74.0 cm, (g) 28.5 cm, (h) 34.6 cm, (ı) 40.9 cm, (j) 48.5 cm, (k) 57.8 cm

As a result of the shape index comparisons, it was determined that there was a significant difference between the two localities in terms of roundness, ellipticity, and aspect ratio (P<0.05). There is no significant difference in terms of form factor, circularity, and rectangularity (P>0.05).

However, in the otolith measurements with differences, relationships were determined for both otolith pairs. Relationships between *TL* and otolith characteristics were determined using both power and linear regression equations and the best fit was obtained among *TL* and *OL* for Ladik (r^2> 0.949) and Simenlik Lakes (r^2 > 0.914) (Table 3). In addition, all the relationships between otolith characteristics and TL were found statistically important (P<0.001).

Table 3. Relationships between otolith parameters and total length according to localities

Relationship	Variable/Locality		Ladik Lake			Simenlik Lake		
Linear			a	b	r^2	a	b	r^2
	OL	*R*	0.879	0.125	0.949	1.174	0.123	0.911
		L				0.787	0.131	0.912
	OH		0.754	0.053	0.910	0.631	0.057	0.875
	OW		-0.023	0.001	0.886	-0.028	0.001	0.844
	OP		2.514	0.372	0.921	3.531	0.355	0.845
	OA		-9.339	0.503	0.931	-7.755	0.481	0.899
Power	*OL*	*R*	0.244	0.863	0.936	0.310	0.808	0.913
		L				0.252	0.862	0.914
	OH		0.169	0.770	0.899	0.161	0.786	0.876
	OW		3.293E-006	2.215	0.882	1.074E-006	2.579	0.885
	OP		0.691	0.874	0.905	0.908	0.806	0.837
	OA		0.028	1.609	0.924	0.029	1.613	0.906

Discussion

Otoliths are considered as an invaluable source of information for reconstructing a fish's life cycle (Campana and Thorrold 2001). In addition, otolith morphology and shape analysis are often used for stock discrimination (Begg and Brown 2000; Galley et al. 2006; Leguá et al. 2013; Bostancı and Yedier 2018; Ozpicak 2020; Bano and Serujiddin 2021; Yedier 2021). However, the relationships between length and otolith dimensions generate a baseline for fish biology and fisheries research (Ozpicak 2020). Because being otolith shape is species-specific, they are widely used in several different studies, such as species differentiation with otolith shape (Cardinale et al. 2004), identifying fossil samples (Gierl and Reichenbacher 2015) or dietary items in a stomach content (Škeljo and Ferri 2012).

In this study otolith biometric measurements, relationships between total length and otolith dimensions, and shape index of *E. lucius* sampled from Ladik and Simenlik lakes were determined. In addition to, *E. lucius* populations were compared by calculating the otolith shape indices for both localities.

Otoliths morphometrics and total length relationships

Fish size-otolith size relationships will be useful for researchers examining the food habits of piscivores and the size of fish in archaeological samples (Harvey et al. 2000). The relationships between *TL* and otolith measurements of fish species could provide info for the back-calculation of the fish total length from otolith measurements (Zan et al. 2015).

The relationships between otolith measurements of individuals sampled from two different lakes and total fish length were determined. Both linear and nonlinear regression models were used to determine the relationships of the samples in both localities. Linear and nonlinear functions are preferred to describe relationships of otolith dimensions and fish size. Generally, the nonlinear function was used in otolith morphometrics and total length relationships (Waessle et al. 2003; Saygin et al. 2017; Jawad et al. 2017; Kanjuh et al. 2018; Zengin Özpiçak et al. 2018; Yilmaz et al. 2019; Saygin et al. 2020; Bulatović et al. 2021). However, in Ladik Lake, the linear regression model was found more stronger, and it was determined that the nonlinear regression is more useful in explaining the relationships between otolith measurements and total length. Yazicioğlu et al. (2017) investigated otolith biometry-total length relationships in the population of *E. lucius* from Ladik Lake. They found a strong relationship between the otolith length and total length (r^2>0.89). In this study, otolith morphometrics and total length relationships were determined, and the best fit was obtained among *OL* and *TL* for both localities (Ladik Lake, r^2>0.949; Simelik Lake, r^2>0.914).

Relationships between fish size and otolith morphometrics are a baseline for prey-predator studies. Analyzing otoliths retrieved from the stomachs or faeces of piscivorous predators can provide information on the type, size, mass, and energy content of their fish prey (Więcaszek et al. 2020). *E. lucius* is an important recreational, predator, and top-level piscivore. For this reason, it is thought that the results of this study will also contribute to the prey-predator relations. Also, this is the first study about otolith morphometrics and the total length relationships of *E. lucius* for Simenlik Lake.

Otolith shape indices and stock discrimination

In this study, six different shape indices (form factor, roundness, circularity, rectangularity, ellipticity, aspect ratio) were calculated using the sagittal otoliths of *E. lucius* belonging to the Ladik and Simenlik Lake populations. This is the first study about the otolith shape of *E. lucius* from different lakes. Otolith properties are useful tools to identify intra and interspecific relationships (Leguá et al. 2013; Mapp et al. 2017; Saygin et al. 2020; Ozpicak 2020; Bano and Serujiddin 2021) and stock discrimination (Begg and Brown 2000; Galley et al. 2006; Agüera and Brophy 2011; Vieira et al. 2014; Jemaa et al. 2015; Bacha et al. 2016; Afanasyev et al. 2017; Duncan et al. 2018; Zengin Özpiçak et al. 2018). In the literature, no other study on this subject related to *E. lucius* could be found. When the otolith shape indices of the Ladik and Simenlik Lake samples were compared, the difference between the shape index values of the otoliths in the two populations of roundness (P<0.05), ellipticity (P<0.001), and aspect ratio (P<0.001) was found to be statistically significant. This showed that the two populations could be distinguished according to their roundness, ellipticity, and aspect ratio values.

In conclusion, considering the findings of this study, it is evident that the sagittal otolith shape is useful for the encouragement of further research on verifying the role of the otolith in the identification, discrimination, 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 differentiation and prey-predator relationships.

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