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Research Article

Otolith shape analyses of *Squalius cephalus* (Linnaeus, 1758) (Actinopterygii: Cyprinidae) inhabiting four inland water bodies of the middle Black Sea region, Turkey

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Abstract: This study was carried out to determine the intra- and inter- population variations of chub, *Squalius cephalus*, utricular and lagenar otolith shapes sampled from four localities in the Black Sea region. Otolith shape indices, including Form Factor (FF), Circularity (C), Roundness (RO), Rectangularity (RE), Aspect Ratio (AR) and Ellipticity (E) were used for otolith shape analyses and multivariate analyzes (Canonical Discriminant Analysis) were used to assess interspecies variations. The FF and C were found statistically significant for lapillus, but not for asteriscus otoliths in the four habitats. Furthermore, RO was similar for all localities for both asteriscus and lapillus. CDA results showed that 66.5% of the chub individuals were correctly classified. The results indicated otolith shape can be used as a suitable tool to discriminate chub populations.

Keywords: Otolith shape analysis, Lapillus, Asteriscus, Chub, Population.

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Introduction

Morphological based inter-population variations are especially important for understanding the evolutionary interpretation of ancient life. For this reason, new methods are always required to support this information (Teimori & Eslami 2017). One of the this methods is otolith analysis which can be used as an important indicator in the studies of fish populations (Begg & Brown 2000; Tuset et al. 2003) and systematics (Tuset et al. 2008). The otoliths accumulate the environment perception elements in fish, 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). Morever, they are considered as valuable markers for distinguishing different fish populations (Begg et al. 2001; Devries et al. 2002; Watkınson & Gillis 2005; Galley et al. 2006; Petursdottir et al. 2006). Otolith shape is species specific both in extant and extinct fish species (Campana & Casselman 1993; Reichenbacher & Cappetta 1999; Wakefield et al. 2014; Bostancı et al. 2015; Gierl & Reichenbacher 2015; Mapp et al. 2017), and thus partially are subjected to genetics (Vignon & Morat 2010). Otolith morphology varies also within the populations of the same species (Tuset et al. 2003, Bourehail et al. 2015; Zengin et al. 2015; Renán et al. 2016; Avigliano et al. 2017; Ibáñez et al. 2017; Teimori & Eslami 2017). Also, otolith shape analysis for stock differentiation is a useful, cheap, practice and time-efficient method. In recent years, with the developing technology, the realization of otolith shape analysis using various programs together reduces the errors in the studies (Ponton 2006; Tuset et al. 2006; Boudinar et al. 2016; Hussy et al. 2016; Avigliano et al. 2017).

Squalius cephalus (Linnaeus, 1758) is found in Europe and Asia Minor inhabiting fresh and brackish waters (Kottelat 1997). The chub is an opportunistic and mobile species and common in almost all

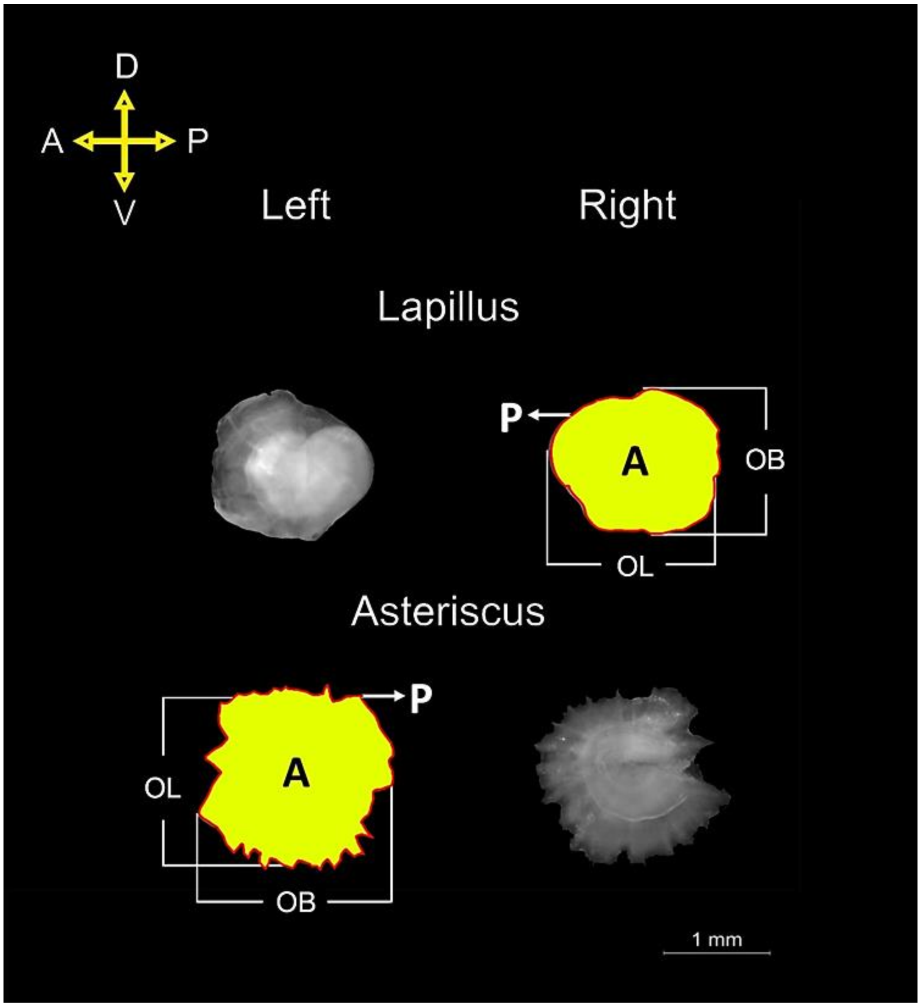

Fig.1. Otolith characteristics of asteriscus and lapillus (OL: Otolith Length, OB: Otolith Breadth/width, A: Otolith Area (Yellow colour), P: Otolith Perimeter (Red colour around the yellow).

running waters in Turkey (Geldiay & Balık 2007). According to investigated literature, there are no studies about otolith shape indices of chub between different regions. However, there are some studies about otolith biometry of the chub (Tarkan et al. 2007; Bostancı et al. 2009; Kurucu & Bostancı 2018). The aim of this study is to determine the shape index values of *S. cephalus*, sampled from Akçay Stream, Abdal Stream, Terme Stream and Yedikır Dam Lake in the Middle Black Sea region and detect the regional differences between the localities in terms of otolith features.

Materials and Methods

Study Area and Sampling. *Squalius cephalus* individuals were sampled from different inland waters of middle Black Sea region including Abdal Stream (N=44), Akçay Stream (N=58), Terme Stream (N=55) and Yedikır Dam Lake (N=62) by using SAMUS 725 MP electrofishing device during October 2015-April 2017. Specimens were measured to the nearest 0.1cm for total length (TL) and weighted to the nearest 0.01g. The sex was determined by macroscopic examination of the gonads.

Otolith Preparation and Statistica Analysis. Utricular (lapillus) and lagenar (asteriscus) otoliths were removed by making head dissection. Otoliths were weighted using precision scales (OW) (± 0.0001g). All lapillus and asteriscus otolith pairs were photographed on the distal side with a Leica DFC295 digital camera. Otolith breadth/width (OB), otolith

Table 1. Descriptive of asteriscus otolith pairs (Min: Minimum, Max: Maximum, OB: Otolith breadth, OL: Otolith Length, P: Perimeter, A: Area, FF: Form factor, R: Roundness, C: Circularity, REC: Rectangularity, E: Ellipticity, AR: Aspect Ratio).

Locality/ Variable	Abdal Stream		Akçay Stream		Terme Stream		Yedikır Dam Lake	
	Right Ast.	Left Ast.	Right Ast.	Left Ast.	Right Ast.	Left Ast.	Right Ast.	Left Ast.
	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max
OB	0.816-2.909	0.828-2.866	0.745-2.272	0.787-2.272	0.884-2.141	0.869-2.096	0.891-2.578	0.876-2.278
OL	0.828-3.431	0.840-3.431	0.872-2.667	0.804-2.739	0.928-2.321	0.966-2.321	0.943-2.673	0.951-2.685
OW	0.0004-0.0108	0.0003-0.0108	0.0002-0.0048	0.0003-0.0048	0.0004-0.004	0.0004-0.004	0.0003-0.0054	0.0003-0.0055
P	2.862-15.228	2.292-16.226	2.578-10.103	2.609-10.956	1.081-9.288	0.756-8.727	3.130-9.464	3.227-10.089
A	0.545-6.051	0.545-6.193	0.440-3.579	0.417-3.583	0.610-3.301	0.616-3.124	0.576-3.991	0.603-3.887

length (OL), area (A) and perimeter (P) (± 0.001mm) were determined by Leica Application Suit Ver. 3.8 Imaging Software (Fig. 1).

Form Factor (FF), Circularity (C), Roundness (RO), Rectangularity (RE), Aspect Ratio (AR) and Ellipticity (E) were used for otolith shape analyses. Otolith shape can be described in some ways, and the simplest and useful method is distance measurement. Such measurements can be used in a series of mathematical equations that calculate shape indices (Russ 1990). These shape indices were calculated for the right and left utricular and lagenar otolith pairs of *S. cephalus*. FF is used to estimate the surface area irregularity, taking values of 1.0 when it is a perfect circle and <1.0 when it is irregular. RO and C give information on the similarity of various features to a perfect circle. RE describes the variations of length and width with respect to the area (Tuset et al. 2003), while E indicates if the changes in the axes are proportional (Russ 1990). All the variables were tested for normality and homogeneity of variance using the Shapiro and Levene's test respectively. Different tests were implemented in statistical analysis if the variables were normally distributed or not (Paired t-test, Wilcoxon test, Independent Two Sample t-test, Mann-Whitney U test and ANOVA-Tukey test). Left and right otolith measurements (OL, OB, OW, A and P) were tested by normality test. If the data are normally distributed, the comparisons of right-left otoliths measurements were tested by paired t-test. If the any of the comparative data are not normally distributed, the comparisons of right-left otoliths measurements were tested by Wilcoxon test. Differences between sexes were also examined by independent t-test. ANOVA was used to compare lapillus and asteriscus otoliths shape indices of chub among localities of the Black Sea. Canonical Discriminant Analysis (CDA) were performed to detect differences in otolith shape variations among the sampling sites. The Wilks' Lambda assessed the performance of the discriminant analyses. The classification success of the discriminant analysis was checked using jackknifed cross validation. SPSS 21, Minitab 17.0 and the Excel software were utilized in the evaluation of data.

Results

A total of 218 chub individuals were sampled from Abdal Stream (N=44; 29.4±0.972cm TL; 328.10±9.70g) Akçay Stream (N=57; 18.00±0.518 cm TL; 67.57±2.57g), Terme Stream (N=55; 15.60±0.289cm TL; 46.99±1.16g) and Yedikır Dam Lake (N=62; 17.70±0.327cm TL; 71.30±2.13g). Descriptive statistics of asteriscus and lapillus otoliths are given in Tables 1 and 2. There was no statistically differences between sex in terms of OB, OL and OW in different localities ($P>0.05$) and right and left asteriscus otoliths ($P>0.05$). But when right and left lapillus otoliths were compared, there were significant differences in terms of OL of Abdal, Akçay and Terme Streams and OB and OW of Yedikır Dam Lake Samples($P<0.05$).

Shape indices of both right and left otolith pairs of utricular and lagenar otoliths are given in Table 3. In CDA, both asteriscus and lapillus otoliths were used. FF and C were found statistically important for

Table 2. Descriptives of lapillus otolith pairs (Lap: Lapillus, Min: Minimum, Max: Maximum, OB: Otolith breadth, OL: Otolith Length, P: Perimeter, A: Area, FF: Form factor, R: Roundness, C: Circularity, REC: Rectangularity, E: Ellipticity, AR: Aspect Ratio).

Locality/ Variable	Abdal Stream		Akçay Stream		Terme Stream		Yedikır Dam Lake	
	Right Lap. Min-Max	Left Lap. Min-Max	Right Lap. Min-Max	Left Lap. Min-Max	Right Lap. Min-Max	Left Lap. Min-Max	Right Lap. Min-Max	Left Lap. Min-Max
OB	0.666-2.244	0.677-2.258	0.587-2.842	0.576-1.946	0.711-1.707	0.696-1.677	0.674-1.966	0.659-1.930
OL	0.834-3.083	0.840-3.098	0.779-2.141	0.777-2.165	0.884-2.022	0.898-2.000	0.876-2.362	0.824-2.421
OW	0.0005-0.0162	0.0006-0.0162	0.0005-0.007	0.0004-0.007	0.0007-0.0058	0.0006-0.0059	0.0006-0.0064	0.0006-0.0065
P	2.484-8.930	2.484-8.966	2.054-6.428	2.054-6.202	2.591-5.935	2.546-5.899	2.461-7.014	2.501-7.034
A	0.423-4.835	0.423-4.761	0.292-2.725	0.292-2.618	0.452-2.431	0.464-2.397	0.395-3.118	0.412-3.108

Table 3. Shape Indices for right and left otolith pairs of utricular and lagenar otoliths.

Shape Indices	Abdal Stream				Akçay Stream			
	RL	LL	RA	LA	RL	LL	RA	LA
Form Factor	0.81±0.008	0.82±0.006	0.61±0.02	0.67±0.07	0.85±0.004	0.84±0.005	0.64±0.018	0.65±0.017
Circularity	15.52±0.19	15.27±0.11	21.90±0.86	21.44±0.99	14.91±0.09	14.91±0.09	20.67±0.62	20.32±0.64
Roundness	0.73±0.008	0.75±0.007	0.87±0.02	0.87±0.02	0.75±0.01	0.77±0.006	0.83±0.013	0.83±0.017
Rectangularity	1.18±0.12	1.20±0.12	1.70±0.17	1.64±0.14	0.71±0.009	0.71±0.004	0.68±0.01	0.69±0.01
Ellipticity	0.10±0.004	0.10±0.004	0.03±0.004	0.04±0.01	0.90±0.007	0.83±0.004	0.025±0.04	0.027±0.04
Aspect Ratio	1.23±0.01	1.21±0.01	1.06±0.009	1.11±0.05	1.21±0.02	1.19±0.01	1.05±0.009	1.06±0.008
	Terme Stream				Yedikır Dam Lake			
	RL	LL	RA	LA	RL	LL	RA	LA
Form Factor	0.86±0.003	0.85±0.004	0.79±0.16	0.96±0.33	0.85±0.004	0.83±0.005	0.63±0.01	0.63±0.01
Circularity	14.61±0.06	14.72±0.07	20.11±0.62	20.26±0.82	14.77±0.07	15.07±0.08	20.28±0.34	20.11±0.33
Roundness	0.78±0.05	0.78±0.05	0.85±0.08	0.84±0.01	0.79±0.04	0.79±0.04	0.82±0.03	0.85±0.04
Rectangularity	0.96±0.05	0.96±0.05	1.35±0.07	1.32±0.07	0.75±0.03	0.76±0.03	0.71±0.02	0.72±0.03
Ellipticity	0.08±0.003	0.08±0.003	0.03±0.003	0.03±0.004	0.10±0.007	0.11±0.007	0.05±0.004	0.04±0.006
Aspect Ratio	1.17±0.008	1.17±0.008	1.06±0.007	1.07±0.007	1.23±0.02	1.25±0.02	1.10±0.008	1.09±0.01

Table 4. ANOVA result for shape indices based on the studied localities.

Shape Indices/Otoliths	Lapillus P values	Asteriscus P values
Form Factor	<0.001*	>0.05
Roundness	>0.05	>0.05
Circularity	<0.001*	>0.05
Rectangularity	<0.001*	<0.001*
Ellipticity	<0.001*	<0.001*
Aspect Ratio	<0.001*	<0.001*

Table 5. Eigenvalues of canonical discriminant function analysis.

Function	Eigenvalue	Variance %	Cumulative %	Canonical Correlation
1	2.085[a]	85.2	85.2	0.822
2	0.252[a]	10.3	95.6	0.449
3	0.109[a]	4.4	100.0	0.313

[a]. First 3 canonical discriminant functions were used in the analysis.

lapillus, but not asteriscus otoliths. Furthermore, RO was similar in all the localities (ANOVA, $P>0.05$). When all data evaluated together RE, E and AR were found statistically different in different localities (ANOVA, $P<0.001$) (Table 4).

The Canonical Discriminant Function Analysis performed for all otolith shape indices (FF, C, RO, RE, AR and E), explains the intra-specific variability among localities. The first 3 canonical discriminant functions were used in the analysis ($P<0.001$, Wilks' Lamda scores 1-3: 0.233, 2-3: 0.720, 3: 0.902). The aim of CDA is to investigate the integrity of

Table 6. Results of the Canonical Discriminant Function of classifying chub individuals.

	Locality	Predicted Group Membership				Total
		Terme Stream	Akçay Stream	Yedikır Dam Lake	Abdal Stream	
	Terme Stream	46	0	2	7	55
	Akçay Stream	1	37	19	0	57
	Yedikır Dam Lake	2	23	36	1	62
	Abdal Stream	15	1	2	26	44
%	Terme Stream	**83.6**	0	3.6	12.7	100
	Akçay Stream	1.8	**64.9**	33.3	0	100
	Yedikır Dam Lake	3.2	37.1	**58.1**	1.6	100
	Abdal Stream	34.1	2.3	4.5	**59.1**	100

66.5% of original grouped cases correctly classified.

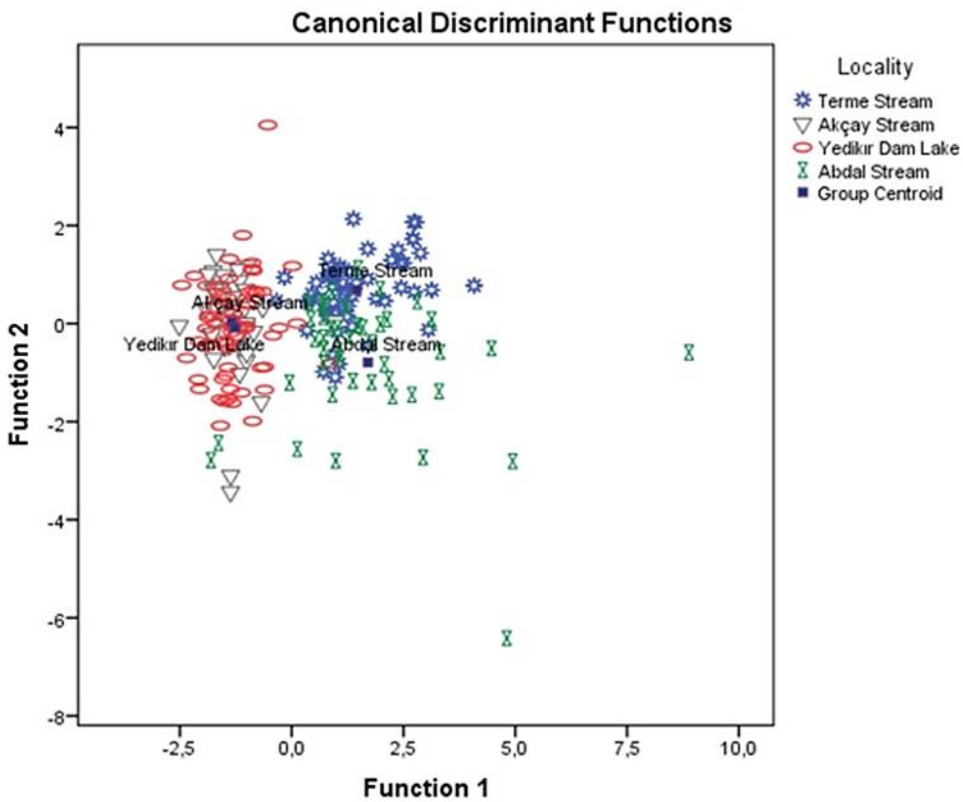

Fig.2. CDA of shape indices in the chub individuals (both otolith pairs asteriscus and lapillus were used in analysis together-rigth otolith).

predefined groups, i.e., individuals belonging to combined a group such as species, morphometric characteristics, through finding linear combinations of descriptors that maximise the Wilks Lambda (λ) (Ramsay & Silveman 2005).

Functions 1, 2 and 3 shows 85.2%, 10.3% and 4.4% of variance (Table 6). According to CDA results 66.5% of the chub samples were correctly classified (Table 6, Fig. 2).

Discussion

Morphological and morphometric differences of particular structures like otoliths are important for identifying organisms and separating of the populations. In recent years, otoliths are preferred in many studies, because otolith shape is species specific. They are widely used several different studies, such as species differentiation with otolith shape (Aguirre & Lombarte 1999; Cardinale et al. 2004), identifying fossil samples (Reichenbacher et al. 2007; Gierl & Reichenbacher 2015) or dietary items in a stomach content (Škeljo & Ferri 2012).

Several studies have shown that otolith shape analysis allowed discrimination of fish stocks (Cardinale et al. 2004; Vignon & Morat, 2010; Bostancı et al. 2015; Pavlov 2016; Montanini et al. 2017; Zhao et al. 2017). In the present study, the shape analysis of otoliths from four different localities revealed that differences in astriscus and lapillus of studied chub, *S. cephalus*. FF, C, REC, E and AR for lapillus and REC, E and AR for asteriscus were found to be important elements do differentiate four studied populations. The usefulness of otolith shape analysis for stock identification and differentiation have already been supported for several fish species (Campana 1999; Tuset et al. 2006; Ferguson et al. 2011; Zengin et al. 2015; Renan et al. 2016; Avigliano et al. 2017; Teimori & Eslami 2017). Some researchers believe that the otolith shape is regulated by the genetics of the fish samples. Also environmental changes are really important for otolith shape of fish. The species specific otoliths is genetically regulated, while environmental effects on otolith shape are mainly expressed at an intraspecific level (Vignon & Morat 2010). Although there are several studies regarding otolith dimensions, genetics, age, growth, feeding, length-weight relationships and reproduction features of the chub inhabiting European and Turkish waters (Stefanova et al. 2008; Bostancı 2009; Ozuluğ & Freyhof 2011; Cejko & Krejszeff 2016; Ozcan et al. 2017; Kurucu & Bostancı 2018; Ozpicak et al. 2018) but no study has focused on the otolith shape differences of *S. cephalus* in different localities.

According to shape indices results, R, AR and E of both asteriscus and lapillus otoliths can be used for discrimination of chub populations. Also, FF of lapillus otoliths and C of asteriscus otoliths were statistically important for populations, too.

Based on the comparison of otolith shape indices in chub using ANOVA and CDA, the intraspecific differences are supported. The shape indices are comparatively independent on otolith size (OL, OB, A, P). 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. The discriminant analysis creates a function to classify individuals within a group (Camacho 1995). According to CDA, the first two components explained 95.5% of the total variance relevant to otolith shape indices.

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 CDA (Bourehail et al. 2015). In this study the Wilks' Lamda scores are closer to 0 for Function 1-3, Function 2-3 and Function 3.

In the present study, the discriminant analysis indicated a clear differentiation in otolith shape between the *S. cephalus* from different localities with more than 65% of individuals correctly classified. There are different results in the literature. Begg & Brown (2000) were reported a greater classification success of 56-81% in year classes in the identification of *Melanogrammus aeglefinus* stocks. Devries et al. (2002) obtained 81.6% accuracy in stocks of king mackerel *Scomberomorus cavalla* between the eastern Gulf of Mexico and Atlantic Ocean. Tuset et al. (2003) demonstrated that regional differences in the comber *Serranus cabrilla* between the Canary Islands and Alicante with an accuracy of 68.8%. According to Bourehail et al. (2015) 80% of individuals correctly classified in otolith shape between the two species of *S. sphyraena* and *S. viridensis*.

In conclusion, the results of the present study confirm that otolith shape analysis can be used to distinguish populations of *S. cephalus* from the Abdal, Akçay, Terme Streams and the Yedikır Dam Lake.

Conflict of Interest Statement

The authors have no conflict of interest.

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مقاله پژوهشی

آنالیز ریختی سنگ ریزه‌های شنوایی ماهی سر مخروطی معمولی *Squalius cephalus* (Linnaeus, 1758)(شعاع بالگان: کپورماهیان) ساکن چهار منبع آب‌های داخلی ناحیه میانی دریای سیاه، ترکیه

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چکیده: این مطالعه به‌منظور مشخص نمودن تغییرات درون و بین جمعیتی اتولیت‌های اوتریکول و لاژنای ماهی سر مخروطی معمولی نمونه‌برداری شده از چهار منبع آب‌های داخلی ناحیه میانی دریای سیاه ترکیه انجام گرفت. نمایه‌های ریختی اتولیت شامل فاکتور فرم (FF)، نمایه مدور بودن (C)، نمایه گردی (R)، نمایه چهارگوشی (RE)، نمایه نسبی ابعاد (AR) و نمایه بیضوی برای آنالیز ریختی اتولیت و تحلیل چند متغیره (CDA) برای بررسی تغییرات درون گونه‌ای مورد استفاده قرار گرفت. دو نمایه FF و C اتولیت لاپیلوس از نظر آماری در چهار جمعیت اختلاف معنی‌داری نشان دادند در حالی‌که این نمایه‌ها برای اتولیت آستریسکوس اختلافی را نشان ندادند. به‌علاوه RO هر دو نوع اتولیت در ۴ جمعیت مقادیر مشابهی را نشان داد. بر اساس تحلیل چند متغیره (CDA) ۶۵/۵ درصد ماهیان سرمخروطی معمولی به‌طور صحیحی دسته‌بندی شده بودند. نتایج نشان داد که از شکل اتولیت می‌توان به‌عنوان ابزاری مناسب در متمایز نمودن جمعیت‌های ماهی سرمخروطی معمولی استفاده نمود.

کلمات‌کلیدی: آنالیز ریختی اتولیت، لاپیلوس، آستریسکوس، ماهی سرمخروطی، جمعیت.