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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Assessing of fluctuating asymmetry in otolith of the *Alburnus* spp. from Anatolian lotic and lentic systems

Anadolu lotik ve lentik sistemlerindeki *Alburnus* spp. otolitlerinde dalgalı asimetrinin değerlendirilmesi

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Abstract: Fluctuating asymmetry can cause developmental disorders in fish, and particularly high levels of asymmetry adversely affect fish life. In this study, the fluctuating asymmetry levels of the otoliths of four *Alburnus* species (*A. chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897, and *A. mossulensis* Heckel, 1843) found in Turkish inland waters were investigated. A total of 160 fish were collected in the Turkish inland waters. The fluctuating asymmetry level was calculated for the width and length characters of the asteriscus. *Alburnus* species, which were examined in the present study, were divided into four total length classes and fluctuating asymmetry of otolith was evaluated according to both the total length classes and species. There was significant difference between the right and left asteriscus otolith measurements for *A. escherichii* (P<0.05). Besides, there were no significant differences between females and males' otoliths measurements of the species (P>0.05). The highest and lowest asymmetry levels in the otolith length and otolith width were calculated in the *A. escherichii* among the *Alburnus* species. However, the highest and lowest asymmetry levels in otolith length and otolith width were calculated in the *A. mossulensis* among the total length groups of the *Alburnus* species. In this study, it was determined that the fluctuating asymmetry level varies according to different fish species and habitats.

Keywords: *Alburnus*, asteriscus, asymmetry, freshwater, otolith

Öz: Dalgalı asimetri, balıklarda gelişimsel bozukluklara neden olabilir ve özellikle yüksek düzeyde asimetri balık yaşamını olumsuz etkiler. Bu çalışmada Türkiye iç sularında bulunan dört *Alburnus* türünün (*A. chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897 ve *A. mossulensis* Heckel, 1843) otolitlerinin dalgalı asimetri düzeyleri araştırılmıştır. Türkiye iç sularından toplam 160 adet balık örneklenmiştir. Asteriskus otolitlerinin genişlik ve boy karakterleri için dalgalı asimetri düzeyi hesaplanmıştır. Bu çalışmada incelenen *Alburnus* türleri dört boy sınıfına ayrılmış ve otolitin dalgalı asimetrisi hem total boy sınıflarına hem de türlere göre değerlendirilmiştir. Sağ ve sol asteriskus otolit ölçümleri arasında *A. escherichii* için önemli farklılıklar bulunmuştur (P<0.05). Ayrıca, türlerin dişi ve erkek bireylerinin otolit ölçümleri arasında önemli bir farklılık yoktur (P>0.05). *Alburnus* türlerinden otolit boyu ve otolit genişliğindeki en yüksek ve en düşük asimetri düzeyleri *A. escherichii* türünde hesaplanmıştır. Ancak *Alburnus* türlerinin total boy grupları arasında otolit uzunluğu ve otolit genişliğindeki en yüksek ve en düşük asimetri düzeyleri *A. mossulensis* türünde hesaplanmıştır. Bu çalışmada dalgalı asimetri düzeyinin farklı balık türlerine ve habitatlara göre değişiklik gösterdiği tespit edilmiştir.

Anahtar kelimeler: *Alburnus*, asteriskus, asimetri, tatlı su, otolit

INTRODUCTION

In fish species, otoliths are constituted by three pairs of calcium carbonate structures such as asteriscus, lapillus, and sagitta which are commonly used in many studies because of their species-specific features, morphological diversity, and chemical compositions (Campana, 1999; Tuset et al., 2008; Bostancı et al., 2015; Pavlov, 2019). The otoliths are bony structures that play an active role in the vital functions of fish, such as balance and sound. Therefore, asymmetric otoliths can negatively affect the balance and sensory sensitivity of the fish (Lychakov and Rebane, 2005; Gagliano et al., 2008). In many studies, otolith asymmetry has been used as an indicator to test the similarities and differences between fish populations and even has been used to test different environmental effects such as temperature (Miller, 2011), salinity (Elsdon and Gillanders, 2002), and pollution (Hardersen, 2000), in the relevant populations. When the literature is examined, it is determined that fish otoliths are used extensively in age determination studies, but few studies have observed the fluctuating asymmetry in otoliths (Jawad et al., 2012; Yedier et al., 2018; Abdulsamad et al., 2020).

The inconsistency between the left and right features of an organism due to the different development of the bilateral characteristics is called asymmetry (Leary and Allendrof, 1989; Yedier et al., 2018). Fluctuating asymmetry (FA) is expressed as random deviations from the perfect bilateral symmetry observed in many animal groups and reflects variable growth during the development of organisms (Fey and Hare, 2008). Besides, FA is thought to reflect the environmental pressures and genetic that the organism experiences throughout its development. For instance, environmental factors that cause developmental disorders, have been reported to cause deviations from the symmetrical character of bilateral organisms (Fey and Hare, 2008). It was stated that asymmetry observed in the otoliths is a valuable index for body and health conditions during the early development and growth of fish (Gagliano and McCormick, 2004; Allenbach, 2011).

The genus *Alburnus* (Bleaks) belongs to the Leuciscidae family, one of the largest families of teleost and is represented by 48 species from Europe to the southern regions of Southwest Asia (Freyhof and Kottelat, 2007; Çiçek et al., 2015). The genus is an excellent example for endemism and high diversity in the western Palaearctic freshwater fishes in Turkey with 26 species, of which 17 are endemic (Gülle et al., 2017; Frose and Pauly, 2020). A comprehensive evaluation of FA in otolith length and width characteristics of four *Alburnus* species in Turkish waters was not investigated. Therefore, the main objective of the present study was to provide some valuable information about the otolith asymmetry of *A.chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897, and *A. mossulensis* Heckel, 1843 in Turkish inland waters.

MATERIALS AND METHODS

All fish samples were obtained from Turkish inland waters (*A.chalcoides* from Cevizdere Stream - 41°05'10.1"N, 37°19'28.6"E, *A. escherichii* from Seydisuyu Stream - 39°24'51.9"N, 31°07'29.7"E, *A. mossulensis* from Munzur River - 39°05'23.2"N, 39°32'31.6"E and *A. tarichi* from Lake Van - 38°32'02.9"N, 43°17'16.7"E) using different sized (7-10 cm) fishing nets and also from local fishermen. *A. tarichi* is an endemic species for Lake Van Basin, *A. escherichii* from Anatolia, and *A. mossulensis* from Tigris–Euphrates Basin. Total length of the samples was measured to the nearest ± 1 mm. The gender of fish samples was determined and their left and right asteriscus otoliths were removed. The cleaned and undamaged otoliths were examined. The otolith width (OW, mm) and length (OL, mm) of the left and right otoliths were taken by the same research scientist to exclude any unwanted error and were repeated three times. The right and left otoliths were photographed using a microscope with a computer-connected camera system (Leica S8APO) (Figure 1). Otolith width and length were measured by ImageJ software (Ver. 1.50b).

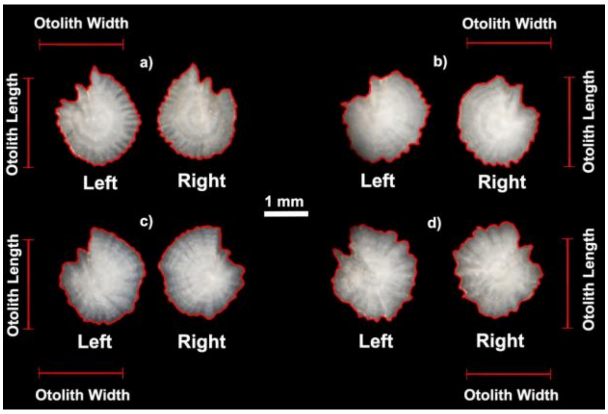

Figure 1. The otolith length and width of asteriscus pairs for a) *A. chalcoides*, b) *A. escherichii*, c) *A. mossulensis*, and d) *A. tarichi* from the Anatolian freshwaters

Kolmogorov-Smirnov and Levene's tests were used for testing whether the variables have normal distribution and homogeneity of variances, respectively. While t-test was applied to determine differences between females and males' otolith measurements, paired t-test was used to determine differences between right and left otoliths measurements (Bostancı et al., 2015). In the present study, otolith length and width were used to calculate the Fluctuating Asymmetry (FA) in the *Alburnus* species from Turkish waters. The statistical analysis included calculating the squared coefficient of asymmetry variation (CV^2_a) for otolith length and width, according to Valentine et al. (1973): $CV^2_a = (S_{r-l}*100/X_{r+l})^2$, where X_{r+l} is the mean of the character, which is calculated by adding the absolute scores for both sides and dividing by the sample size and S_{r-l} is the standard deviation of signed differences. ANOVA test was used to compare CV^2_a values in the otoliths between the different total length classes. The relationships between total length classes and fluctuating asymmetry values in otolith length and width were calculated with linear regression model (y=ax+b). Minitab 17.0 statistical program was used for all statistical analyses.

RESULTS

A total of 160 specimens (n=40 for each species) were collected from Turkish inland waters in 2021. The samples were divided into four total length classes, with the differences between the total length classes being equal and each class containing at least one individual. Total length classes are 100-120 mm, 121-140 mm, 141-160 mm, and 161-180 mm for *A. chalcoides* in the Cevizdere Stream (Turkey), 80-100 mm, 101-120 mm, 121-140 mm, and 141-160 mm for *A. escherichii* in the Seydisuyu Stream (Turkey), 110-130 mm, 131-150 mm, 151-170 mm and 171-190 mm for *A. mossulensis* in the Munzur River (Turkey) and 170-190 mm, 191-210 mm, 211-230 mm and 231-250 mm for *A. tarichi* in the Lake Van (Turkey) (Table 1).

All variables were passed the Kolmogorov-Smirnov test of normality (P>0.05) and Levene's test of homogeneity (P>0.05). The otolith measurements of both sexes were pooled in the study because there were no significant differences between female and male otoliths measurements of the species (t-test, *P*>0.05). There were no statistical differences between left and right otolith measurements of *A. chalcoides*, *A. mossulensis,* and *A. tarichi* (paired t-test, *P*>0.05); however, statistically differences were found between left and right otoliths of *A. escherichii* (paired t-test, *P*<0.05).

Table 1. Squared coefficient of asymmetry and otolith characters mean, min-max values by total length class of *A. chalcoides*, *A. escherichii*, *A. mossulensis*, *A. tarichi*

	Character	Total length class (mm)	N	CV_a^2	Character mean	Character Min-Max	% of individuals with asymmetry
A. chalcoides Cevizdere Stream	Otolith Length	100-120	7	10.922	1.352	1.246-1.450	85.71
		121-140	9	15.320	1.752	1.573-1.981	88.89
		141-160	17	16.192	2.272	1.955-2.582	88.24
		161-180	7	21.642	2.588	2.254-2.980	100.00
	Otolith Width	100-120	7	7.755	1.335	1.252-1.527	85.71
		121-140	9	9.829	1.678	1.522-1.895	77.78
		141-160	17	13.981	2.055	1.852-2.326	82.35
		161-180	7	26.011	2.310	2.054-2.597	100.00
A. escherichii Seydisuyu Stream	Otolith Length	80-100	11	13.118	1.558	1.438-1.724	90.91
		101-120	11	20.800	1.827	1.587-1.997	90.91
		121-140	14	23.418	1.919	1.720-2.193	78.57
		141-160	4	35.024	2.105	1.838-2.258	100.00
	Otolith Width	80-100	11	10.974	1.447	1.229-1.644	90.91
		101-120	11	29.682	1.632	1.381-1.929	81.82
		121-140	14	30.835	1.797	1.472-2.109	85.71
		141-160	4	39.614	1.952	1.758-2.223	100.00
A. mossulensis Munzur River	Otolith Length	110-130	5	5.783	1.944	1.775-2.096	80.00
		131-150	15	14.487	2.131	1.935-2.392	93.33
		151-170	14	18.447	2.353	2.096-2.613	92.86
		171-190	6	59.103	2.535	2.281-2.898	100.00
	Otolith Width	110-130	5	5.841	1.735	1.578-1.846	80.00
		131-150	15	14.622	1.907	1.719-2.149	86.67
		151-170	14	21.949	2.115	1.826-2.396	85.71
		171-190	6	77.367	2.403	2.104-2.711	100.00
A. tarichi Lake Van	Otolith Length	170-190	11	17.130	2.498	2.226-2.697	81.82
		191-210	15	21.551	2.693	2.511-2.983	86.67
		211-230	10	25.344	2.752	2.515-3.106	80.00
		231-250	4	33.438	2.904	2.582-3.253	100.00
	Otolith Width	170-190	11	18.344	2.330	2.112-2.511	90.91
		191-210	15	20.330	2.450	2.183-2.825	93.33
		211-230	10	22.459	2.570	2.240-2.960	90.00
		231-250	4	26.492	2.829	2.711-3.079	100.00

In the current study, results exhibited that the level of FA for the two otolith traits at its lowest values in the fish species ranging in total length between 100–120, 80–100, 110–130 and 170–190 for *A. chalcoides, A. escherichii, A. mossulensis,* and *A. tarichi,* respectively. The highest FA levels for the two otolith traits were determined in total length groups such as 161-180 for *A. chalcoides*, 141-160 for *A. escherichii*, 171-190 for *A. mossulensis,* and 231-250 for *A. tarichi*, respectively. In all of the four *Alburnus* species examined in our study, the percentage of individuals showing asymmetric characteristics in both otolith length and otolith width is in the largest total length class (Table 1). Besides, the FA values of total length classes for each species were significantly different (ANOVA, *P*<0.05).

In the current study, it was determined that fluctuating asymmetry values in both otolith length and otolith width tend to increase with the total length of the four *Alburnus* species and there was a linear relationship between them for all bleaks. The highest and lowest relationships between total length classes and fluctuating asymmetry values in otolith length and width were determined in *A. tarichi* (r^2= 0.968 and 0.967) and *A. mossulensis* (r^2= 0.798 and 0.787), respectively.

Similarly, the highest and lowest correlations in total length classes and fluctuating asymmetry values in otolith width were determined in *A. tarichi* and *A. mossulensis*, respectively.

Fluctuating asymmetry results of the otolith width and length of the four *Alburnus* species from Turkish inland waters are presented in Table 2. FA level of the otolith width was greater than otolith length for *A. escherichii*, *A. mossulensis,* and *A. tarichi,* but it was not the case with *A. chalcoides* (Table 2). The percentage of individuals showing asymmetry in otolith length characteristics was highest for *A. mossulensis*, while the percentage of otolith width was calculated as the highest in *A. tarichi*. In addition, the percentage of individuals showing asymmetry in these two otolith characters in *A. escherichii* was equal to each other (Table 2).

Table 2. Squared coefficient of fluctuating asymmetry values and otolith characters means with minimum and maximum values for four bleaks from Turkish inland waters

	Character	Total Length (mm)	n	CV_a^2	Mean	Min-Max	% of individuals with asymmetry
Alburnus chalcoides	Otolith Length	100-180	40	17.540	2.049	1.246-2.980	90.00
Cevizdere Stream	Otolith Width	100-180	40	15.914	1.889	1.252-2.597	85.00
Alburnus escherichii	Otolith Length	80-160	40	23.358	1.813	1.438-2.258	87.50
Seydisuyu Stream	Otolith Width	80-160	40	27.857	1.671	1.229-2.223	87.50
Alburnus mossulensis	Otolith Length	110-190	40	22.455	2.246	1.775-2.898	92.50
Munzur River	Otolith Width	110-190	40	26.962	2.033	1.578-2.711	87.50
Alburnus tarichi	Otolith Length	170-250	40	21.744	2.675	2.226-3.253	85.00
Lake Van	Otolith Width	170-250	40	22.946	2.485	2.112-3.079	92.50

DISCUSSION

In the literature, it was observed that most otolith asymmetry studies are conducted extensively on marine species (Jawad at al., 2012; Kontaş at al., 2018; Yedier et al., 2018; Abdulsamad et al., 2020), while few studies were carried out in inland fishes (Green and Lochmann, 2005; Estes et al., 2006; Green and Lochmann, 2006). The lack of data regarding the natural asymmetry of the fish is present in the Turkish inland waters as in many parts of the world. Therefore, it is difficult to decide whether the asymmetry values obtained in the present study are higher or lower than normal. However, in the previous study, it was reported that asymmetry has many negative effects on fish species such as hearing problems (Lychakov and Rebane, 2005), abnormal swimming activity (Helling et al., 2003), and adaptations problems in the habitat (Jorgensen and Fiksen, 2010).

This is the first study to determine the fluctuating asymmetry in otolith of *Alburnus* spp. in Turkish inland waters. The FA values of the otolith length in the compared *Alburnus* species were ranged from 10.922 to 21.642 for the *A. chalcoides*, 13.118 to 35.024 for the *A. escherichii,* 5.783 to 59.103 for the *A. mossulensis* and 17.130 to 33.438 for the *A. tarichi.* On the other hand, the lowest and highest fluctuating asymmetry values in the otolith width were varied such as 7.755 and 26.011 in *A. chalcoides* from the Cevizdere Stream, 10.974 and 39.614 in *A. escherichii* from the Seydisuyu Stream, 5.841 and 77.367 in *A. mossulensis* from the Munzur River and 18.344 and 26.492 in *A. tarichi* from the Lake Van. The highest asymmetry value differs among otolith characters in fish species. For example, some researchers were determined the highest asymmetry value in the otolith length (Al- Mamry et al., 2011; El-Regal et al., 2016), while others were indicated in the otolith width (Sadighzadeh et al., 2011; Jawad, 2012; Jawad et al., 2012; Abdulsamad et al., 2020). Similarly, some differences also were identified with the highest asymmetry value of otolith characters for four *Alburnus* species from the inland waters of Turkey. Although the highest asymmetry value was found in the otolith length in *A. chalcoides* species, it was determined in otolith width in the *A. escherichii, A. mossulensis* and *A. tarichi*. The highest and lowest asymmetry values in the otolith length and otolith width were found in the *A. escherichii* among the *Alburnus* species. However, the highest and lowest asymmetry in otolith length and otolith width were calculated in the *A. mossulensis* species among the total length groups of *Alburnus* species.

When these four *Alburnus* species are compared, it is concluded that the fish from the Seydisuyu Stream and Munzur River are more stressed than from Cevizdere Stream and Lake Van.

In order to evaluate the fluctuation asymmetry values in both otolith length and otolith width of the four *Alburnus* species, there is no study conducted with the same species in different regions or different species in the same regions. Therefore, fluctuating asymmetry values of the otolith characters of *Alburnus* spp. examined in the study were compared among themselves. In many studies, a relationship between otolith asymmetry and total length was examined and it was stated that asymmetry increases with total length (Jawad, 2001; Al-Mamry et al., 2011). In the current study, it was determined that the value of otolith asymmetry was positively correlated with the fish length for four *Alburnus* species from Turkish inland waters. Similar results are available in several studies in different habitats (Jawad et al., 2012; Mabrouk et al., 2014; Abdulsamad et al., 2020). The increase in asymmetry levels depending on the total length may be the result of longer exposure to fish under environmental conditions depending on the age and fish size (Thiam, 2004). In many studies, it has been reported which fluctuating asymmetry in the morphometric character of the organism, is negatively correlated with the animal fitness (Martin and Lopez, 2001; Jawad et al., 2012). Pollutants such as heavy metals, pesticides, DDT, and detergents in water can accumulate in the fish body and can then be transferred to humans through the food chain (Ongley, 1996; Afshan et al., 2014). Determining the environmental stress of heavy metals and other pollutants in aquatic ecosystems is very important for both humans and fish. For this, it should be well known to collect detailed information about habitat conditions and animal welfare of the fish species. In such cases, habitats should be protected for populations by measuring the effects of these stresses in fish species before they are irreversibly affected (Lens et al., 2002). Therefore, fluctuating asymmetry is one of the methods applied in determining the quality and health of individuals and populations, since it is both easy to apply and not destructive (Møller and Thornhill, 1998; Lens et al., 2002). In addition, it was stated in previous studies that developmental instability was determined using fluctuating asymmetry values and this asymmetry could be an indicator of environmental stress (Parsons, 1990; Alados et al., 1993). Furthermore, based on previous research in this field, it is likely that there is a relationship between asymmetry in fish species and environmental factors (Yedier et al., 2018). The environmental problems in aquatic habitats are also present in the sampling stations of the current study, as in many inland waters of Turkey. Cevizdere Stream (*A. chalcoides*), which is surrounded by hazelnut lands, is located in the Ordu province (Turkey) and it is one of the main drinking water resources of the Ordu district of Ünye (Anonymous, 2018). In the literature, there seems to be no study to determine water pollution in this stream, although there are some quarries and cement plants as the obvious pollutants around the stream. Seydisuyu Stream Basin (*A. escherichii*), which has important agricultural lands and borate deposits on its basin, is located in Eskişehir province (Turkey) (Atıcı et al., 2018). The pollution of Seydisuyu Stream has been expressed in many studies and the main reasons for the pollution of the stream system are the discharge of agricultural pesticides, urban sewage water from settlements, and mineral mining activities (Çiçek et al., 2013; Köse et al., 2014; Tokatlı et al., 2014). It is reported that these pollutants can be an important limiting factor in aquatic life in the region unless a precaution is taken for the pollution in Seydisuyu Stream (Köse et al., 2014). Munzur River (*A. mossulensis*), which is an important water source, is located in Tunceli province (Turkey). Unfortunately, it is stated that domestic wastewater and agricultural activities cause pollution by causing changes in some parameters of the water quality of Munzur River (Yıldırım et al., 2011a; Ural et al., 2012). It was reported that this pollution in the Munzur River causes oxidative stress in fish and this stress adversely affects the life of the fish (Yıldırım et al., 2011b; Lushchak, 2016). Lake Van (*A. tarichi*), which is the largest soda lake, is located in Turkey's eastern Anatolian region and the primary water sources feeding the lake are rains and streams (Poyraz and Mutlu, 2017). It has been reported that Lake Van is under the influence of many pollutants such as quarries, mining activities, non-mining mineralization areas, geothermal discharges in the basin, natural radioactive contaminations, and domestic wastes (Öğün et al., 2005; Çiftçi et al., 2008).

Since fish are very sensitive to changes in their habitats (Bassem, 2020), they are one of the model organisms used in the assessment of environmental and ecological factors in aquatic ecosystems (Ali et al., 2008). While the asymmetry caused by environmental stress has direct or indirect effects on fish species, such as developmental disorders, it may cause problems in the short or long term. For this reason, fish populations should be checked at regular intervals and the increase or decrease in asymmetry should be monitored.

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