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Research article

Fluctuating asymmetry in the sagittal otoliths of *Atherina boyeri* from the Hirfanlı Dam Lake (Kırşehir, Türkiye)

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Abstract: Fish may face stress due to many different pollutants in their habitats. They may express this negative situation they experience with fluctuating asymmetry (FA) as a developmental disorder. In particular, high levels of asymmetry may affect the activities of fish and even cause serious problems in their lives. In the present study, fluctuating asymmetry levels of the otoliths of the *Atherina boyeri* distributed in Hirfanlı Dam Lake were investigated. A total of 100 fish samples were evaluated within the scope of the study, and these fish individuals were divided into six total length groups, taking into account the sample size. The left and right sagittal otoliths of fish individuals in each length group were removed. Otolith characters such as area, length, perimeter, and width were measured. The FA values of these four otolith characters of the *A. boyeri* were calculated separately for both population-based and total-length groups. Additionally, the relationship between FA values and total length groups was investigated. It was determined that there was no statistical difference between the left and right sagittal otolith measurements of *A. boyeri* individuals (P>0.05). In these four otolith characteristics, the highest FA level was calculated in the otolith width, and the lowest FA level was calculated in the otolith perimeter. It was determined that the FA values in the otolith characters differed between the total length groups. The highest FA value was calculated in the otolith area in total length group I, while the lowest FA value was calculated in the otolith perimeter in total length group IV. For *A. boyeri* individuals, the percentage of asymmetrical individuals was calculated to be highest in otolith area and otolith perimeter (100%), and lowest in otolith width (83%). Additionally, there are individuals with asymmetrical features in all total length groups. It was determined that there was no significant relationship between the FA values of otolith in the *A. boyeri* and the total length groups. The results of this study show that the population of *A. boyeri* in Hirfanlı Dam Lake could be under environmental stress.

Keywords: Fluctuating asymmetry, *Atherina boyeri*, Invasive species, Environmental stress.

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Introduction

Bilateral symmetry is an important adaptation mechanism that allows fish to adapt to survive in aquatic environments. This symmetry plays critical roles in many aspects of fish, such as their interactions with their environment, their hunting and predator avoidance strategies, their ability to catch their prey, and their adaptation to their environment (Holló, 2017; Corballis, 2020; Toxvaerd, 2021; Stundl et al., 2021). For instance, bilateral symmetry enables fish to move quickly and efficiently in the aquatic environment, saving energy. Moreover, this symmetry may help fish better blend in with their environment and camouflage. In addition, fish individuals with bilateral symmetry may be found more attractive to the opposite sex in terms of mate selection, thus increasing reproductive success (Thornhill & Gangestad, 1999; Wade, 2010; Shang et al., 2020). The symmetrical body structure in fish ensures that the otoliths, which are the balance and hearing organs in the inner ear, work properly, allowing fish to perceive sounds in the water and maintain their balance ability (Mille et al., 2015; Mahé et al., 2021; Yedier et al., 2022).

Otoliths are one of the most frequently used bony structures in ichthyology studies due to their unique shape and structure. Otoliths are very important organs for the adaptation of fish to habitats and the sustainability of their stocks, as they play an active role in the vital functions of fish such as sound and balance (Popper & Lu, 2000; Taylor et al., 2020; Chen, 2022). Otoliths are among the organs that are also important for fisheries and fisheries biology. For instance, otoliths can be used for many different purposes, such as age determination, monitoring growth rate, understanding population dynamics, determining food sources and ecological role, responding to environmental changes, and monitoring fishing pressure (Newman et al., 2000; Campana & Thorrold, 2001; Zengin et al., 2006; Sponaugle, 2010; Mille et al., 2016; Zhang & Ye, 2021). Otoliths are a critically important research tool to ensure the sustainability of the fishing industry and the conservation of fish stocks. Fisheries biology relies on this data to ensure the protection of ecosystems and the sustainability of fish resources for future generations (Botsford et al., 2009; Green et al., 2009; Gebremedhin et al., 2021). The discrepancy between the right and left characteristics of an organism due to different development of bilateral characteristics is called asymmetry (Leary & Allendrof, 1989; Yedier et al., 2018). Fluctuating asymmetry (FA) can also be defined as random deviations from perfect bilateral symmetry observed in many animal groups (Fey & Hare, 2008) or as the differential development of bilateral characters in an organism (Van Vallen, 1962; Leary & Allendroff, 1989). In aquatic systems, organisms' developmental instability may be reflected by fluctuating asymmetry, which is a random deviation from the perfect binary system. In other words, the organism cannot compensate for disorders during development, and it is reported that this inability can be associated with stress, mostly caused by environmental or genetic conditions in many studies (Zakharov, 1992; Jawad & Adams, 2021; Yedier et al., 2022; Jawad et al., 2023).

Fluctuating asymmetry in otoliths refers to the distortion of otoliths to develop symmetrically and asymmetric structural changes that occur when exposed to certain environmental stress factors (Jawad et al., 2020; Gao et al., 2023). Fluctuating asymmetry is a complex result of genetic and environmental influences during the development process of the organism and is considered an indicator used to evaluate the stress level of the organism (Gagliano & McCormick, 2004). Therefore, asymmetric otoliths may negatively affect the fish's balance and sensory sensitivity (Lychakov & Rebane, 2005; Gagliano et al., 2008). Consequently, high-fluctuation asymmetry may be indicative of the worst condition of fish exposed to unfavorable environments. Therefore, otolith asymmetry can be used as an environmental indicator when comparing similar species living in different regions (Gagliano & McCormick, 2004; Allenbach, 2011). For instance, asymmetry in fish otoliths has been used to compare different environmental impacts such as pollution (Hardersen, 2000), salinity (Elsdon & Gillanders, 2002), and temperature (Miller, 2011) in different habitats.

Atherina boyeri (Risso, 1810) is a euryhaline teleost fish that lives in estuarine and coastal waters, as well as in salt marshes, lagoons, shallow brackish, and inland waters (Froese & Pauly, 2023). *A. boyeri* is distributed on the eastern coasts of the Mediterranean, the Black Sea, and the Atlantic Ocean (Froese & Pauly, 2023), as well as attracting attention as an invasive fish species with abundant and successful populations in inland waters in Anatolia. Since *A. boyeri* is an invasive fish species in the Turkish inland waters, the fluctuating asymmetry values in the otoliths of this species have not been investigated, although there are many studies on *A. boyeri* (Gençoğlu & Ekmekçi, 2016; Bostancı & Yedier, 2018; Apaydın Yağcı et al., 2018; Yedier et al., 2019; Gençoğlu et al., 2020; Kale et al., 2023). Therefore, the main objective of the present study was to determine fluctuating asymmetry for four sagittal otolith characters such as area, length, perimeter, and width of *A. boyeri* from Hirfanlı Dam Lake (Kırşehir, Türkiye).

Materials and Methods

Atherina boyeri samples were obtained by commercial fishermen in Hirfanlı Dam Lake (Kırşehir, Türkiye). The total length of the fish samples was measured to the nearest ± 1 mm. The right and left sagittal otoliths were removed from each fish sample. Images of the right and left otoliths were obtained using a light microscope and

Scanning electron microscope (Figure 1). The otolith area (OA, mm^2), length (OL, mm), perimeter (OP, mm), and width (OW, mm) of the right and left otoliths were preferred. OA, OL, OP, and OW were measured by ImageJ software (Ver. 1.50b). In order to eliminate possible errors and problems related to otolith measurements in the study, otoliths were analyzed three times by the same researcher, and the average values of the relevant measurements were used in the study. Squared coefficient of asymmetry variation (CV_a^2) values were calculated to determine the Fluctuating Asymmetry (FA) values of OA, OL, OP, and OW data of *A. boyeri*. The square coefficient of asymmetry change was calculated using the formula; $CV_a^2 = (S_{r-l} * 100 / X_{r+l})^2$, where X_{r+l} is the mean value of the character, and S_{r-l} is the standard deviation of signed differences of the character (Valentine et al., 1973). Kolmogorov-Smirnov and Levene's tests were used to determine whether parametric or nonparametric statistical tests would be used in the study. According to Kolmogorov-Smirnov and Levene's test results, relevant statistical tests were used. The relationships between total length groups and FA values in otolith area, length, perimeter, and width were calculated with a linear regression model (y=ax+b). All statistical analyses were performed using the Minitab 19.0 statistical program.

Results

In the study, a total of 100 *A. boyeri* samples from Hirfanlı Dam Lake (Kırşehir, Türkiye) were examined. Fish samples were divided into six total length groups of equal length sizes, taking into account the differences between total length groups. Total length groups are 40-49 mm, 50-59 mm, 60-69 mm, 70-79 mm, 80-89 mm, and 90-99 mm for *A. boyeri* in the Hirfanlı Dam Lake (Table 1). Otolith measurement data passed Levene's test of homogeneity (P>0.05) and the Kolmogorov-Smirnov test of normality (P>0.05). Therefore, parametric statistical tests were used in the study. Paired t test was used to compare left and right otolith data. There were no statistical differences (paired t-test, P>0.05) between left and right otolith measurements of *A. boyeri* samples.

The highest and lowest FA levels for the otolith area of *A. boyeri* were determined in total length groups I and V, respectively. The lowest and highest FA levels for the otolith length of *A. boyeri* were determined in total length groups III and II, respectively. The highest and lowest FA levels for the otolith perimeter of *A. boyeri* were determined in total length groups III and IV, respectively. The lowest and highest FA levels for otolith width of *A. boyeri* were determined in total length groups IV and II, respectively.

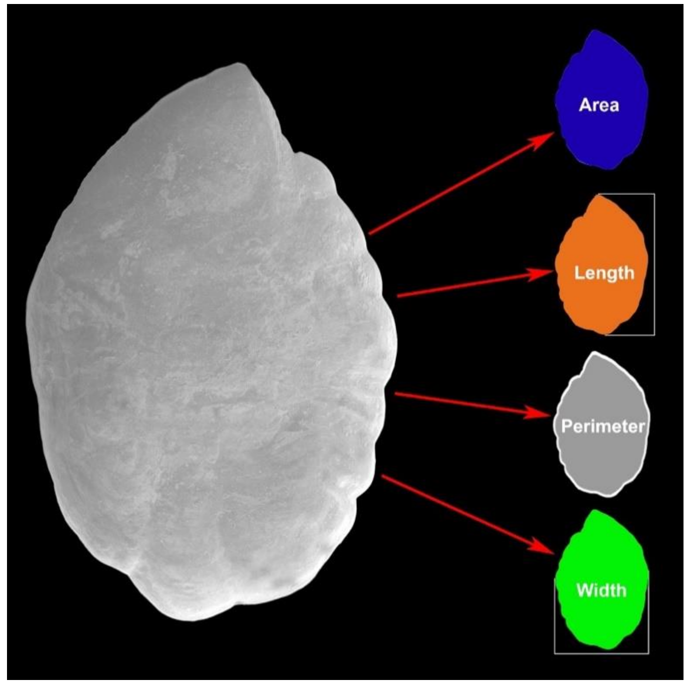

Figure 1. The area, length, perimeter, and width of sagittal otolith for *Atherina boyeri* from Hirfanlı Dam Lake (Kırşehir, Türkiye)

The otoliths of *A. boyeri* examined in our study show fluctuating asymmetrical features in all four otolith characters. Additionally, it was determined that there were individuals with asymmetric otoliths in all size groups (Table 1). In all total length groups of *A. boyeri*, the percentage of asymmetric individual data with fluctuating asymmetry in terms of otolith area and otolith perimeter was determined as 100% (Table 1). The highest percentage of asymmetric individuals showing fluctuating asymmetry in terms of otolith length was calculated as 100% in total length groups IV, V, and VI, and the lowest was 82.50% in total length group III (Table 1). The highest percentage of asymmetric individuals showing fluctuating asymmetry in terms of otolith width was calculated as 100% in total length groups V and VI, and the lowest was 75% in total length group IV (Table 1).

Among these four otolith characteristics of *A. boyeri*, the highest FA level was calculated in the otolith width, and the lowest FA level was calculated in the otolith perimeter (Table 2). Among the four otolith characters of *A. boyeri* examined in our study, otolith perimeter and otolith area showed the highest percentage of asymmetric individuals (100%), while otolith width showed the lowest percentage of asymmetric individuals (83%) (Table 2).

Table 1. Squared coefficient of asymmetry values, otolith characters mean, and min-max values in six total length groups of *Atherina boyeri from Hirfanlı Dam Lake* (Kırşehir, Türkiye)

Otolith Character	Total Length Groups (mm)		n	CV_a^2	Character Mean	Character Min-Max	Percentage of Asymmetric individual
Area	**I**	40-49	15	10.231	1.396	1.056-1.744	100.00
	II	50-59	37	5.488	1.901	1.502-2.356	100.00
	III	60-69	40	5.102	2.539	1.811-3.084	100.00
	IV	70-79	4	6.196	3.097	2.880-3.493	100.00
	V	80-89	2	4.965	4.824	4.541-5.131	100.00
	VI	90-99	2	8.186	5.487	5.384-5.603	100.00
Length	**I**	40-49	15	3.402	1.717	1.487-1.926	86.67
	II	50-59	37	7.233	1.992	1.720-2.254	89.19
	III	60-69	40	2.165	2.305	1.955-2.682	82.50
	IV	70-79	4	3.013	2.527	2.424-2.711	100.00
	V	80-89	2	4.428	3.202	3.128-3.324	100.00
	VI	90-99	2	2.750	3.369	3.339-3.396	100.00
Perimeter	**I**	40-49	15	1.411	4.518	3.875-5.064	100.00
	II	50-59	37	1.305	5.275	4.727-6.017	100.00
	III	60-69	40	2.906	6.105	5.132-6.969	100.00
	IV	70-79	4	0.176	6.770	6.397-7.166	100.00
	V	80-89	2	0.331	8.486	8.317-8.658	100.00
	VI	90-99	2	1.646	9.150	9.073-9.206	100.00
Width	**I**	40-49	15	6.531	1.135	0.942-1.284	80.00
	II	50-59	37	7.638	1.330	1.184-1.484	78.38
	III	60-69	40	7.185	1.528	1.284-1.698	95.00
	IV	70-79	4	1.353	1.700	1.642-1.798	75.00
	V	80-89	2	2.242	2.031	2.000-2.069	100.00
	VI	90-99	2	2.720	2.277	2.254-2.311	100.00

Table 2. Squared coefficient of fluctuating asymmetry values, otolith characters mean, and min-max values in *Atherina boyeri* from Hirfanlı Dam Lake (Kırşehir, Türkiye)

Otolith Character	Total Length Range (mm)	n	CVa2	Character Mean	Character Min-Max	Percentage of Asymmetric individual
Area	40-99	100	6.314	2.258	1.056-5.603	100.00
Length	40-99	100	4.001	2.149	1.487-3.396	87.00
Perimeter	40-99	100	2.003	5.695	3.875-9.206	100.00
Width	40-99	100	6.670	1.428	0.942-2.311	83.00

The relationships between total length groups and fluctuating asymmetry values in otolith area, length, perimeter, and width are determined as $y=-0.3057x + 7.7647$ ($R^2=0.0745$), $y=-0.3093x + 4.9145$ ($R^2=0.1001$), $y=-0.1279x + 1.7435$ ($R^2=0.0583$), and $y=-1.1736x + 8.719$ ($R^2=0.6136$), respectively (Figure 2). In the relationships between the total length groups of the fish and the fluctuating asymmetry values, the highest and lowest determination coefficients were calculated in the otolith width and otolith area characters, respectively (Figure 2).

Discussion

This is the first study to determine the fluctuating asymmetry in sagittal otoliths of *Atherina boyeri* in Hirfanlı Dam Lake. It was observed in the literature that fluctuating otolith asymmetry studies are concentrated on marine fish species (Abdulsamad et al., 2020; Jawad & Adams, 2021; Gao et al., 2023; Reis et al., 2023; Zhang et al., 2023), while there are few studies on freshwater fish species (Jawad & Mahé, 2022; Yedier et al., 2022; Jawad et al., 2023). There is a lack of data on fluctuating otolith asymmetry in many fish species in both freshwater and marine aquatic habitats. In addition, the threshold limits of the fluctuating otolith asymmetry value in fish species have not been reported in the literature. Therefore, it is very difficult to decide whether the asymmetry values obtained in our study are high or low. However, it was reported in previous studies on this subject that asymmetry has many negative effects on fish species, such as adaptation problems in habitats (Jorgensen & Fiksen, 2010), abnormal swimming activity (Helling et al., 2003), and hearing problems (Lychakov & Rebane, 2005).

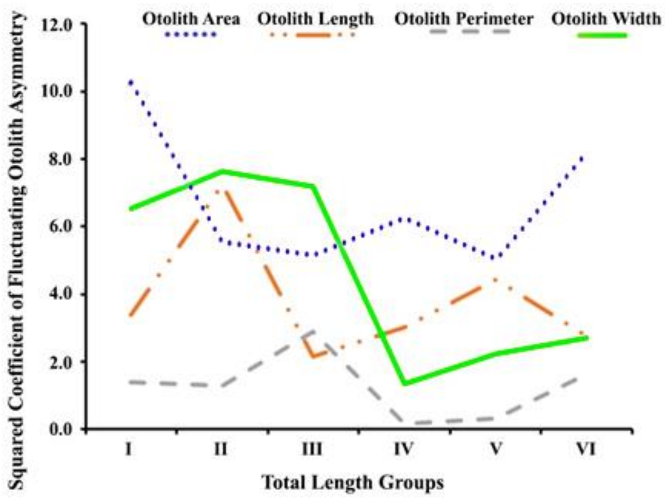

Figure 2. The relationship between total length groups and squared coefficient of fluctuating otolith asymmetry of *Atherina boyeri* from Hirfanlı Dam Lake (Kırşehir, Türkiye)

In studies to determine the fluctuating asymmetry in otoliths, the high asymmetry value in otolith characters varies among fish species. For instance, it was reported that the highest asymmetry values in otolith length of *Rastrelliger kanagurta* from the Sea of Oman (Al-Mamry et al., 2011), *Hipposcarus harid* and *Chlorurus sordidus* from the Red Sea coast of Egypt (El-Regal et al., 2016), *Trachurus mediterraneus* (Yedier et al., 2018) from Middle Black Sea, and *Alburnus chalcoides* from Anatolian lotic and lentic waters (Yedier et al., 2022). In addition, the otolith area showed the highest asymmetry in *Merlangius merlangus* (Kontaş et al., 2018) from the Middle Black Sea and *Trachurus mediterraneus* from the North Aegean Sea (Bostancı et al., 2018). Contrary to these studies, in our study, the highest asymmetry value was determined in the otolith width in *Atherina boyeri* individuals in Hirfanlı Dam Lake. There are also studies in the literature that are similar to the results of our study. For instance, it was also reported that the highest asymmetry values in otolith width of *Liza klunzingeri* (Sadighzadeh et al., 2011), *Sillago sihama* and *Sardinella sindensis* from Persian Gulf (Jawad et al., 2012), *Lutjanus bengalensis* from the Sea of Oman (Jawad, 2012), *Alburnus escherichii*, *Alburnus sellal, Alburnus tarichi* from Anatolian lotic and lentic waters (Yedier et al., 2022). There is no study in the literature conducted with the same species in different regions or with different species in the same region to compare the fluctuating asymmetry values in the otolith characters of *Atherina boyeri*. For this reason, the fluctuating asymmetry values of the otolith characters of *A. boyeri* were compared with other fish species in the literature according to their otolith characters. Many studies examined the relationship between otolith asymmetry and the total length of fish samples, and different results were reported regarding this relationship. There are studies stating that asymmetry increases with total length in the literature (Al-Mamry et al., 2011; Yedier et al., 2022). It was reported that increased levels of asymmetry depending on total length may be a result of longer exposure to adverse environmental conditions, depending on the size and age of the fish (Thiam, 2004). However, in the current study, it was concluded that the relationship between the total length of individuals belonging to the *A. boyeri* in Hirfanlı Dam Lake and the asymmetry value of otolith characters is quite complex. The coefficient of identification between fluctuating asymmetry in the otolith perimeter and total length was determined as a quite low value as $R^2=0.0583$, while the coefficient of identification of the relationship between fluctuating asymmetry in otolith width and total length of the same species was calculated as $R^2=0.6136$. As a result, it was concluded that there is not clearly positive or negative relationship between the fluctuating asymmetry values in otolith characters and the total length of the *A. boyeri*. The results obtained in our study regarding the relationship between the asymmetry in otoliths and fish length are compatible with many studies in the literature (Lychakov & Rebane, 2005; Albusaidi et al., 2010; Kontaş et al., 2018; Jawad & Mahé, 2022).

Aquatic systems, whether freshwater or marine systems, are exposed to different types of pollutants such as pesticides, herbicides, heavy metals, detergents, DDT, industrial and textile wastewater, etc (Wasi et al., 2013; Madhav et al., 2020; Karri et al., 2021). Fish, which are permanent users of these habitats, can accumulate such pollutants in their bodies. As a result of these accumulations, some cellular and functional deteriorations may occur in fish. These pollutants can even reach humans through the food chain (Afshan et al., 2014; Reyes-Calderón et al., 2022). Therefore, determining the environmental stress caused by pollutants in aquatic ecosystems is very important not only for fish, the permanent users of aquatic systems but also for humans and other creatures that consume them. For this reason, collecting detailed information about the habitat conditions and welfare of fish, which have an important place in food chains, may be of vital importance for many living groups.

In many studies conducted with different living groups, it has been reported that this asymmetry may be an indicator of the environmental stress that living beings are exposed to and that the developmental instability observed in these living groups can be determined by using fluctuating asymmetry values (Parsons, 1990; Alados et al., 1993). Such negative situations in aquatic habitats can be determined by calculating fluctuating asymmetry values on fish species. As a result of taking these values regularly at certain intervals, the relevant habitats can be monitored and, if necessary, protection practices can be created for the relevant habitats (Lens et al., 2002). It was reported in the literature that both the Hirfanlı Dam Lake and the Kızılırmak River which provides water to the lake are exposed to pollution from different pollutants (Yılmaz, 2006; Çakıroğulları et al., 2011; Gül et al., 2011; Kavurmacı et al., 2013). The results of the current study confirm the fluctuating asymmetry values in *A. boyeri* individuals distributed in the Hirfanlı Dam Lake as an indication that the fish may have been exposed to environmental stress. In addition, fluctuating asymmetry is among the preferred applications in determining the quality and health of individuals and populations because it is easy to apply and does not require much equipment (Moller & Thornhill, 1998; Lens et al., 2002).

In addition, the subject of fluctuating asymmetry in otoliths is based on different studies examined in many disciplines and conducted on the otoliths of various organisms. Fluctuating asymmetry can serve many different purposes. For example, we can summarize this into groups below. In research on fish populations, FA attracts a lot of attention, especially in studies on fish populations. For this reason, the asymmetry of otoliths has the potential to provide important information about the living conditions, stress level, and health of fishes, and can be achieved by monitoring the asymmetry changes in otoliths, especially in response to environmental changes. In environmental pollution studies, different pollutants such as chemicals, heavy metals, or toxins can affect FA in otoliths. Therefore, researchers can also use the asymmetry in otoliths to evaluate the level of environmental pollution. This issue may become even more important, especially in studies on marine organisms. In research on natural resource management, FA of otoliths is an important research topic for natural resource management and fisheries management. Researchers can use asymmetry in otoliths to assess the impact of stressors such as overfishing or environmental changes on fish populations. In climate change research, factors such as temperature, sea level changes, and acidity of seawater may be affected by climate variability and cause changes in the asymmetry of otoliths. As a result, climate change can affect aquatic ecosystems, and FA in otoliths can be used as an indicator to understand how organisms respond to these changes. In paleoecology and paleoclimatology research, otoliths are bony structures that can be used in historical ecology and climate studies. FA of otoliths can be an important source of data for the reconstruction of populations and environmental conditions for organisms in the past. In water quality monitoring studies, otoliths can be used in water quality monitoring programs to evaluate the impact of environmental stress factors on organisms. Water quality monitoring can be carried out through FA values by measuring water pollution levels and allowing these effects to be compared with the asymmetry values in the otoliths of organisms. In genetic research, although FA is heavily associated with environmental factors, it is not possible to assume that there is no genetic relationship. For this reason, evaluating the asymmetry in otoliths by associating it with the genetic structure of the organism may help us understand how genetic factors affect the FA of otoliths. In adaptation research, FA in otoliths can be used to study the long-term environmental responses of organisms and to understand adaptation processes. In population dynamics research, FA can be used to analyze the growth, migration, and life cycle of populations of organisms. Changes in asymmetry values in otoliths may reflect the responses of populations over time and thus help us understand the dynamics of relevant populations. Fluctuating asymmetry in otoliths is an important research topic across multiple disciplines and is increasing in popularity as a valuable resource for better-understanding organisms' responses to environmental conditions, life cycles, and living conditions.

Fish are sensitive to changes in their habitats and because they react quickly to these changes, they are one of the widely preferred model organisms in the evaluation of environmental and ecological factors in aquatic systems (Bassem, 2020). Asymmetric situations caused by possible environmental stress, as in this study, may directly or indirectly cause developmental disorders in fish in the short or long term. In addition, investigating such characteristics of invasive species can provide valuable data to researchers regarding the adaptation of invasive species to the environment and their survival strategies. For this reason, it is recommended to regularly check and monitor the increase or decrease in the fluctuating asymmetry values in the otoliths of fish individuals of the *A. boyeri* in the Hirfanlı Dam Lake.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding Statement

The author does not declare any fund.

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