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Investigation of Fluctuating Asymmetry in the Four Otolith Characters of *Merlangius merlangus* Collected from Middle Black Sea

Orta Karadeniz'den Örneklenen *Merlangius merlangus*'un Dört Otolit Karakterinin Dalgalı Asimetrisinin İncelenmesi

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ABSTRACT

Fluctuating asymmetry was calculated for the otolith width, length, area and perimeter of *Merlangius merlangus* caught in the Middle Black Sea. In the present study, *M. merlangus* samples were divided nine-total length groups. All total length groups were examined for asymmetry values in the otolith characters, the highest asymmetry values were found in the Group III. The lowest asymmetry values in four otolith characters such as area, length, perimeter, and width were calculated in the Group I and Group IX. In this study, it was determined that the otolith asymmetry levels of *M. merlangus* in the Middle Black Sea were varied between the four otolith characters, the otolith area has the highest and the otolith length has the lowest asymmetric feature. In addition, there was no significant relationship between the asymmetry values of the four otolith characters and total length. The asymmetry in these otolith characters may be a result of the stress from different pollutants in the Black Sea.

Keywords: Sagittal otolith, *Merlangius merlangus*, Fluctuating asymmetry, Environmental factors, Black Sea

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ÖZET

Orta Karadeniz'den yakalanan *Merlangius merlangus*'un otolit genişliği, uzunluğu, alanı ve çevresi için dalgalı asimetri değerleri hesaplanmıştır. Bu çalışmada elde edilen *M. merlangus* örnekleri dokuz total boy grubuna ayrılmıştır. Tüm total boy grupları, otolit karakterlerindeki asimetri değerleri açısından incelenmiş ve en yüksek asimetri değerleri Grup III'de bulunmuştur. Alan, uzunluk, çevre ve genişlik gibi dört otolit karakterindeki en düşük asimetri değerleri Grup I ve Grup IX'de hesaplanmıştır. Bu çalışmada, Orta Karadeniz'deki *M. merlangus*'un otolit asimetri düzeylerinin bu dört otolit karakteri arasında farklılık gösterdiği, otolit alanının en yüksek ve otolit boyunun ise en düşük asimetrik özelliğe sahip oluğu belirlenmiştir. Ayrıca bu dört otolit karakterinin asimetri değerleri ile total boy grupları arasında anlamlı bir ilişki yoktur. Bu türün otolit karakterlerinde gözlenen asimetri Karadeniz'deki farklı kirleticilerden kaynaklı stresin bir sonucu olabilir.

Anahtar sözcükler: Sagittal otolit, *Merlangius merlangus,* Dalgalı asimetri, Çevresel faktörler, Karadeniz

1. INTRODUCTION

Otoliths are three pairs of calcareous structures and they are found in the inner ear of the Teleost fish. They can be affected the different environmental factors such as fish size, diet, stress. Because of their species-specific shapes, otoliths are very useful tools to study the various events which characterize fish's life cycles (Mérigot *et al*., 2007). In recent years, the otoliths have used as a powerful tool about age, growth, ecological interactions, life history, and environmental conditions of fishes inhabiting aquatic ecosystems such as rivers, lakes, and seas (Zenteno *et al*., 2014). Otoliths are available bony structure in marine and freshwater fish species; therefore, they are also used different studies such as taxonomy, shape analysis, prey-predator relationship and otolith asymmetry.

One of the most important problems in aquatic systems is pollution from different pollutants and the pollutants can cause stress on living things in aquatic systems. It is important that stress factors are previously identified to protect the habitat and reduction of the population. Thus, researchers are interested in monitoring sub-lethal biological responses and environmental quality (Allenbach, 2011). Many studies in this area have shown that individual morphological variability can provide a valuable early sign of genetic and environmental stress (Barton, 2002; Jawad, 2003; Jawad *et al*., 2012). Environmental degradation can be affected all aquatic organisms and their population at a level of mass mortality, fecundity, extinction (Bird *et al*., 1995; Kasuya *et al*., 2002; Koprivnikar *et al*., 2006). Moreover, other effects such as reduced growth, diseases, morphological anomalies, may be occurred (Sexton *et al*., 1992; Baumann *et al*., 1996; Bird, 1997; Schmidt, 1997; Galloway *et al*., 2004).

Fluctuating Asymmetry (FA) occurs when symmetry is the normal state and there is no tendency for one side to have a larger value than the other. FA originates from small deviations which seen on perfect bilateral symmetry (Zenteno *et al*., 2014). FA can show the specific trait of bilateral variation for an individual (Somarakis *et al*., 1997a; Somarakis *et al*., 1997b). Namely, the value of the difference between right and left otolith pairs (R-L) is zero and otolith shape has a normal form of bilateral symmetry. FA can be examined

different organisms such as plants and animals. It is also a useful bioindicator of environmental stress which may be arisen natural or anthropogenic factors (Allenbach, 2011). Fluctuating asymmetry has been shown to be a sensitive indicator of different type contaminants in aquatic systems and provides a cheap method and reliable data on lethal stress in wild fish populations (Lutterschmidt *et al*., 2016).
Symmetry and asymmetry are two important conditions that influence the life of the living creatures in water or land habitats. Asymmetry is known as the differential development of a bilateral character between the left and right sides of an organism. Fish species are influenced by ecological factors and they are a suitable organism to investigate the results of different ecological effects on bilateral characters species. Asymmetry which is generated based on environmental stress is caused drastic effects on vestibular and auditory functions of fish (Lychakov *et al*., 2006) but there is limited information about this subject for marine and freshwater species. It has been proposed that asymmetry studies can provide valuable information for adaptation in natural populations (Jones, 1987). The whiting *Merlangius merlangus* is one of the most abundant demersal fish species in the Black Sea and It is commercially important fish species in Turkish waters (Kalayci and Yesilcicek, 2014; Nedreaas *et al*., 2014). Besides, there is no information about the fluctuating asymmetry of whiting otolith dimensions in Middle Black Sea population. Therefore, the main objective of the study to calculate fluctuating asymmetry for four sagittal otolith characters such as length, width, area, and perimeter of the *M. merlangus* in the Middle Black Sea.

2. MATERIAL AND METHOD

Merlangius merlangus samples were provided from the commercial fishermen in the Middle Black Sea (Ordu, Turkey) (Figure 1). The total length of each *M. merlangus* was measured (nearest 0.1 cm) and their right and left sagittal otoliths were heedfully removed and then stored dry in the well plate until the examination. Left and right sagittal specimens which were undamaged were examined.

Figure 1. Sampling area of *Merlangius merlangus* in the Middle Black Sea.

The otolith morphometric measurements in this study were measured (nearest 0.001 mm) as the longest distance between anterior and the posterior edges for otolith length, and the longest distance from dorsal and ventral margins for otolith width (Figure 2). Otolith area (OA) and perimeter (OP) of each sagittal otolith were also recorded. All otolith measurements were performed by using the Leica Application Suit image analyzing software (Version 3.7.0**)**. The differences between right and left sagittal otolith measurements were tested using the paired t-test. Since the total length of fish samples affected otolith measurements, the fish samples were divided into total length groups and the otolith measurements were taken into consideration for the total length groups. The otolith fluctuating asymmetry was investigated for each total length groups using their otolith measurements.

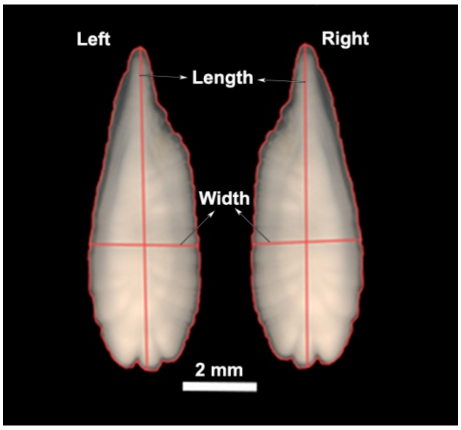

Figure 2. Left and right sagittal otoliths of *Merlangius merlangus.*

The statistical analysis included calculating the squared coefficient of asymmetry variation (CV_a^2) for otolith length, width, area and perimeter according to Valentine *et al*. (1973). Moreover, in the current study, linear regression models (y=ax+b) was used to determine the relationships; between total length groups and squared coefficient of otolith asymmetry and between total length and right-left otolith differences in *Merlangius merlangus*.

3. RESULTS

A total of 93 whiting samples which are suitable for measuring the total length were used in this study. The examined whiting specimens were ranged from 10.8-18.7 cm. The whiting samples were divided nine-total length groups. In the present study, the total length groups were arranged as I; 100-109 mm, II; 110-119 mm, III; 120-129 mm, IV; 130-139 mm, V; 140-149 mm, VI; 150-159 mm, VII; 160-169 mm, VIII; 170-179 mm and IX; 180-189 mm.

We examined 186 sagittal otoliths which are removed in pairs belonging to 93 whiting individuals from the Middle Black Sea. A total of 744 measurement values from the four otolith characters (OL, OP, OW, and OA) were tested by normality test ($P>0.05$). Right and left sagittal otolith measurement values were evaluated for each otolith character. According to the paired t-test result, the differences between the left and right sagittal otolith measurements were statistically significant ($P<0.001$) for each character (Table 1).

Average otolith measurements for length, width, area, and perimeter were found 7.446, 2.478, 13.498, and 17.763 for all otolith samples, respectively (Table 2). Fluctuating asymmetry values of the sagittal otolith characters (length, width, area, perimeter) of *Merlangius merlangus* are shown separately for all total length groups (Table 2). The asymmetry was calculated as 4.710 for length, 4.772 for width, 11.776 for the area and 8.335 for perimeter in all total length groups. It was observed that the values of all otolith characters increased in total length.

Table 1. The measurements of sagittal otolith pairs of whiting specimens

Otolith Dimensions	Left Mean ± SE (Min.-Max.)	Right Mean ± SE (Min.-Max.)
Length*	7.524±0.132	7.367±0.129
	5.379-9.973	5.336-9.731
Width*	2.511±0.036	2.445±0.035
	1.883-3.296	1.869-3.110
Area*	13.803±0.429	13.193±0.410
	7.735-22.865	7.506-21.219
Perimeter*	18.094±0.318	17.431±0.307
	13.051-24.651	12.612-23.732

*** Paired-t test; P<0.001**

According to the otolith length (OL), width (OW), area (OA) and perimeter (OP) asymmetry level results, the otolith area has the highest level of asymmetry between the four otolith characters. The otolith length was determined to be the lowest asymmetric otolith character among all otolith characters for *M. merlangus* from the Middle Black Sea. The otolith characters were sorted from highest to the lowest asymmetry levels as follows; OA > OP > OW > OL (Table 2).

In this study, all otolith characters in the nine length groups showed asymmetric features for *M. merlangus* samples. When the total length groups are examined in terms of asymmetric values among the total length groups, some differences appear between the otolith characters. When all total length groups were examined for asymmetry values in the four otolith characters, the highest asymmetry values were found in the Group III. According to the all otolith length, width, area, and perimeter results, the squared coefficient of asymmetry coefficient was calculated as a zero value for Group I and Group IX (Table 2). Moreover, it was determined that the percentage of asymmetric individuals was highest in terms of both four otolith characters and nine total length groups (Table 2).

The relationships between total length groups and squared coefficient of otolith characters asymmetry were described by a regression model and a linear equation. The equations of total length groups and squared coefficient of otolith asymmetry relationships with R^2 were $y=-2.4053x+23.648$; $R^2=0.0583$ for otolith area, $y=-0.3829x+5.5970$; $R^2=0.0409$ for otolith width, $y=-0.1433x+4.4042$; $R^2=0.0094$ for otolith length and $y=-0.0551x+6.1161$; $R^2=0.0007$ for otolith perimeter (Figure 3). The results of the asymmetry were shown that Fluctuating asymmetry was not correlated with total fish length for all otolith characters (Figure 3).

The relationships between total length and right-left otolith differences were described by the regression model and the linear equation for each otolith characters in *Merlangius merlangus*. The equations of total length group and right-left otolith differences relationships with R^2 were $y=0.0724x-0.4342$; $R^2=0.1084$ for otolith area, $y=0.0041x+0.0131$; $R^2=0.0252$ for otolith width, $y=0.0161x-0.0466$; $R^2=0.0438$ for otolith length and $y=0.0747x-0.3632$; $R^2=0.0900$ for otolith perimeter (Figure 4).

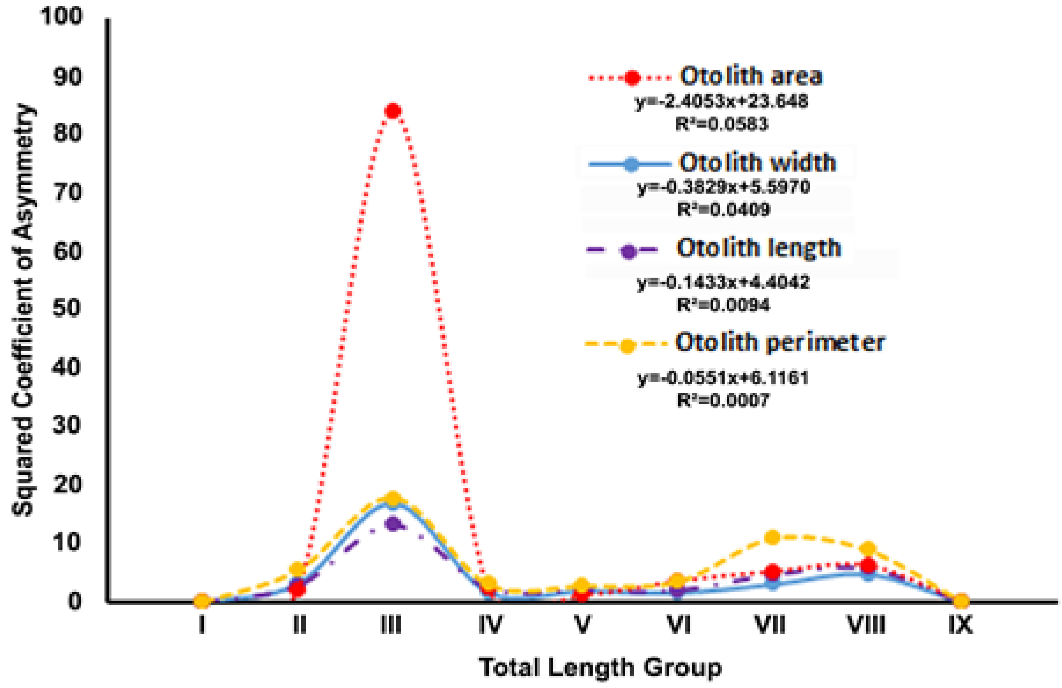

Figure 3. The relationship between total length groups and squared coefficient of otolith asymmetry.

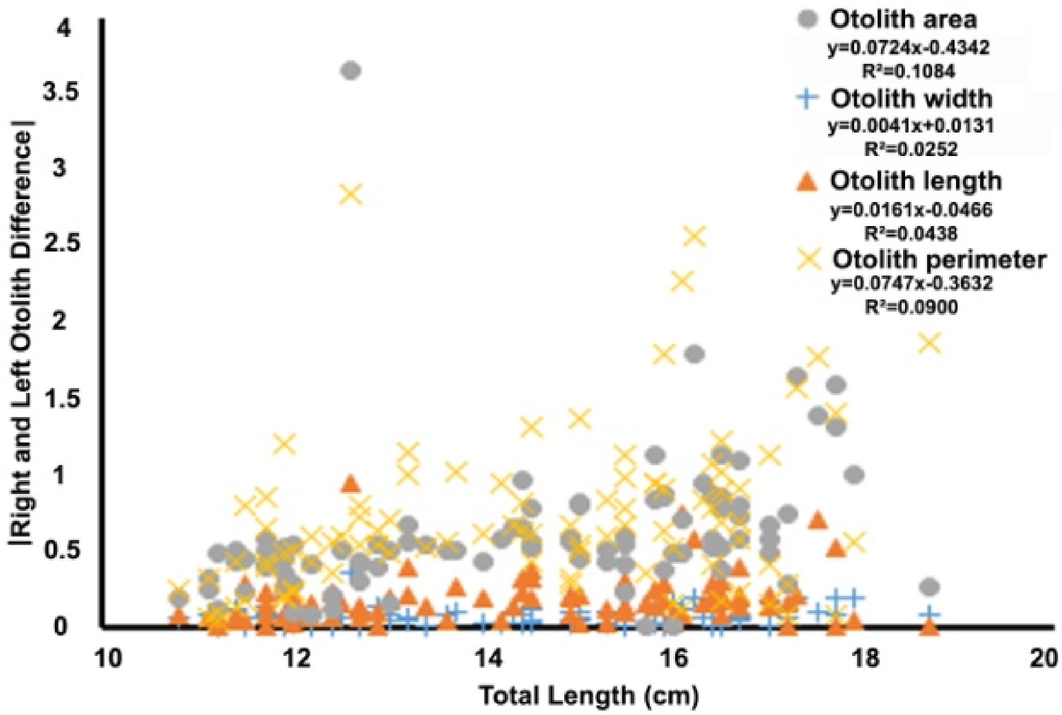

Figure 4. The relationship between total length and right-left otolith differences in *Merlangius merlangus*.

Table 2. Squared coefficient of asymmetry and character means by total length groups of *Merlangius merlangus*.

Otolith Character	Group	Total Length Range (mm)	N	CV^2_a	Character Mean	Character Min-Max	Percentage of Asymmetric individual
Otolith Length	I	100-109	1	0	5.607	5.564-5.650	100
	II	110-119	16	2.869	5.950	5.336-6.677	100
	III	120-129	14	13.216	6.354	5.764-7.576	100
	IV	130-139	7	2.468	6.697	5.636-7.376	100
	V	140-149	11	2.053	7.369	5.992-8.960	100
	VI	150-159	15	2.046	8.145	7.262-9.160	100
	VII	160-169	18	4.741	8.354	6.592-9.382	100
	VIII	170-179	10	5.794	9.289	8.289-9.973	100
	IX	180-189	1	0	9.352	9.345-9.359	100
	Total	**100-189**	**93**	**4.710**	**7.446**	**5.336-9.973**	**100**
Otolith Width	I	100-109	1	0	1.998	1.969-2.026	100
	II	110-119	16	3.172	2.050	1.869-2.297	100
	III	120-129	14	16.904	2.165	1.980-2.554	100
	IV	130-139	7	1.713	2.246	1.983-2.368	100
	V	140-149	11	1.908	2.498	2.097-2.896	100
	VI	150-159	15	1.616	2.669	2.368-2.918	100
	VII	160-169	18	3.042	2.752	2.425-3.037	100
	VIII	170-179	10	4.787	2.957	2.640-3.296	100
	IX	180-189	1	0	3.046	3.010-3.082	100
	Total	**100-189**	**93**	**4.772**	**2.478**	**1.869-3.296**	**100**
Otolith Area	I	100-109	1	0	7.812	7.718-7.906	100
	II	110-119	16	2.087	8.702	7.506-10.260	100
	III	120-129	14	84.134	9.823	8.317-13.614	100
	IV	130-139	7	2.312	10.707	8.050-12.016	100
	V	140-149	11	1.130	13.396	10.390-18.374	100
	VI	150-159	15	3.541	15.522	12.594-18.635	100
	VII	160-169	18	5.187	16.541	13.137-19.908	100
	VIII	170-179	10	6.202	19.676	15.948-22.865	100
	IX	180-189	1	0	20.287	20.159-20.415	100
	Total	**100-189**	**93**	**11.776**	**13.498**	**7.506-22.865**	**100**
Otolith Perimeter	I	100-109	1	0	13.070	12.946-13.193	100
	II	110-119	16	5.498	14.035	12.612-15.874	100
	III	120-129	14	17.906	15.172	13.801-18.242	100
	IV	130-139	7	3.049	15.864	13.362-17.232	100
	V	140-149	11	2.791	17.622	15.046-20.659	100
	VI	150-159	15	3.509	19.496	17.422-21.852	100
	VII	160-169	18	10.895	20.087	16.576-22.610	100
	VIII	170-179	10	8.918	22.101	19.154-24.447	100
	IX	180-189	1	0	22.542	22.112-22.972	100
	Total	**100-189**	**93**	**8.335**	**17.763**	**12.612-24.651**	**100**

4. DISCUSSION AND CONCLUSIONS

In marine systems, adult pelagic fish are at less risk than benthic or coastal species due to their higher mobility and the presence of higher-looking capacities against pollutants such as petroleum and hydrocarbons floating in the water (Sabljic, 2009). Pelagic fish species are less stressed than demersal fish species or pelagic fish species are more resistant to stress than demersal fish species (Simonović *et al*., 2017). In stressed ecosystems, the elimination of stress-sensitive species from the ecosystems and also the increased rates of disease and parasites can usually lead to a decrease in species richness in this system (Perry and Vanderklein, 2009).

Although bilaterally symmetric living beings are expected to exhibit symmetrical biostructures in favorable environmental conditions, however, several animals cannot have a perfect symmetry even in ideal environmental conditions. Because small inconsistencies that can arise as a result of several factors such as nutrient access, extreme temperatures, parasites, diseases, and behavioral stress in the organism can affect the developmental process of the species, may some structures of the organism asymmetrical (Palmer and Strobeck, 1992; Markow, 1995). Because fish are easily affected by their habitat, some ecological effects can be easily observed on their metabolism, growth, resistance to their disease, their reproductive potential, health situation and even their survival rate, when they are exposed to pollution or low oxygen for long periods of environmental stress (Barton *et al.*, 2002). Such stress-related effects can be transferred to population or community levels and effects at the population level can vary depending on the type, intensity, and duration of stress exposure in the species (Adams and Greeley, 2000). The pollution-induced stress can be affected the otoliths of fish to gain an asymmetric structure. Moreover, the asymmetry of morphological characters is negatively correlated with fitness of several animal taxa (Møller and Nielsen, 1997; Martin and Lopez, 2001; Bergstrom and Reimchen, 2003).

When the literature is reviewed in this area, there is no studies have been conducted on the fluctuating asymmetry of the whiting otolith area and otolith area until now. However, there are studies on the fluctuating asymmetry of otolith size and width of different fish species. In our study, the fluctuating asymmetry value of otolith width (4.772) was found higher than the otolith length (4.710). This result is similar to several studies. For instance, Jawad *et al*. (2012) reported that the asymmetry value of otolith length (2.100) was lower than otolith width (9.000) for *Sardinella sindensis* and also otolith width (21.600) was higher than otolith length (2.900) for *Sillago sihama* from the Persian Gulf Near Bandar Abbas. In addition, Jawad (2012) showed that the fluctuating asymmetry value of otolith width (10.290) higher than otolith length (5.060) in *Lutjanus bengalensis* from the Muscat City, Sea of Oman. However, in several studies, it has been reported that otolith length has a higher fluctuating asymmetry value than otolith width. For instance, Al-Mamry *et al*. (2011) reported that otolith length asymmetry (88.710) was higher than otolith width (41.750) in *Rastrelliger kanagurta* from the Muscat waters at Oman Sea. Similarly, El-Regal *et al*. (2016) noted that the asymmetry of otolith length (14.049) was higher than otolith width (10.436) in *Chlorurus sordidus* and otolith width asymmetry value (11.962) was lower than otolith length (15.190) in *Hipposcarus harid* from Hurghada, Red Sea coast of Egypt. These differences among studies may be due to the size of the sample, the species of fish, pelagic or demersal, habitat differences, and even genetic predisposition Some authors have shown that there is a positive correlation between asymmetry coefficient and the total fish length (Al-Hassan et al., 1990; Al-Hassan and Hassan, 1994; Al-Hassan and Shwafi, 1997; Jawad, 2001). However, this kind of relationship has not been observed in the present study.

The present study examines fluctuating asymmetry in the four otolith characters of the whiting in the Middle Black Sea and presents new data for the species in this habitat population. There are no studies to date on the fluctuating asymmetry of these four otolith characters of these fishes that have spread in the Black Sea. Pollution-induced stress can be one reason that causes the fluctuating asymmetry in whiting individuals. The pollution in the Black Sea is mainly influenced by the content of river waters poured into the sea. For instance, the Danube River discharges chrome, copper, nickel, mercury, lead, zinc and hydrocarbons to the Black Sea (Alkan *et al*., 2008). As a result of the present study, it is believed that fish from the coasts of the Middle Black Sea are exposed to a local environmental stress that can be detected by the FA and that this stress is most likely caused by the presence of continuous industrial discharges of heavy metals from domestic industrial activities and wastewaters.

The high asymmetry value of the otolith area might indicate the vulnerability of the individual which may develop an asymmetry in this otolith character under stress conditions. It could be assumed that otolith area is less sensitive to change of environmental variables, including pollution. On the other hand, the low asymmetry value of the otolith characters studied in this study can be explained on the basis that these characters are less vulnerable to environmental stresses. It should not be forgotten that species diversity is the best-documented indicator of environmental impact on aquatic animals, and several biotic indices have been included (Rapport, 1989; Karr, 1991). It is very important to identify and focus on factors affecting species diversity in terms of the sustainability of species diversity.

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