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Fluctuating asymmetry in otolith dimensions of *Trachurus mediterraneus* collected from the Middle Black Sea

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Abstract: Asymmetry is crucial in the natural world and assumed to reflect the developmental instability of the fish in polluted aquatic habitats. In aquatic habitats, high level asymmetry occurs in more stressed fish, and these fish consume more energy to buffer their growth than fish that are not under stress. A total of 88 specimens of *Trachurus mediterraneus* were collected in the Middle Black Sea and their total length and weight ranged from 119-164 mm and 11.14-35.09 g, respectively. There were significant differences between the right and left sagittal otolith measurements (P<0.05). There were no significant differences between males and females' otoliths measurements of the species (P>0.05). The asymmetry was calculated for the sagittal otolith characters such as area, width, length, and perimeter. The present study results indicated that otolith width has the lowest asymmetry value (4.337) while otolith length has the highest asymmetry value (8.717) among the four otolith characters obtained for the male and female of Mediterranean horse mackerel collected from the Middle Black Sea. Overall, developmental instability as fluctuating asymmetry levels within total length classes could be a relevant indicator of pollution in the habitat.

Keywords: *Trachurus,* sagittal otolith, asymmetry, environmental factors, Black Sea.

Introduction

Trachurus mediterraneus is a semipelagic and carnivore fish species, and distributed throughout the Mediterranean, Black and Marmara Seas (Fischer et al., 1987). The species is one of the most commercially important fish species in the Black Sea (Alkan et al., 2013). In marine and freshwater fish, otoliths are constituted by three pairs of bony structures, sagittal, lapillus and asteriscus, which are commonly used in age determination studies because of their species-specific dimension, morphological diversity, and chemical composition. Otolith morphology is also important for fisheries biology because it allows for studies for population determination, fish classification and phylogenetic relationship in many years (Bostanci et al., 2015).

Although symmetry can be described as a complete balance and proportionality concept in a living being, it may not always maintain this position throughout the life of living being. As a result, this change in the bilateral characters of the living beings is called asymmetry. Fluctuating asymmetry is usually characterized by small, random deviations from perfectly bilaterally symmetry (Ludwig, 1932), animals cannot compensate for some disturbances that occur during their lifetime, and some of them cause development instability (Zakharov, 1992).

Environmental factors that cause developmental instability; causing high fluctuating asymmetry, and bilaterally symmetrical deviations in the bilateral characters of the living being (Palmer, 1994; Fey and Hare, 2008). Therefore, this fluctuating asymmetry in animals can indicate specific environmental effects in the case of the organism. In fish, otolith asymmetry has been used as a bioindicator to test the condition between different populations (Grønkjaer and Sand, 2003) and it was also used to test different environmental effects in fish populations such as pollution (Hardersen, 2000), parasitism (Reimchen and Nosil, 2001), and temperature (Lu and Bernatchez, 1999).

In Turkish freshwater, several studies were conducted on otolith mass asymmetry in different fish species such as *Barbus tauricus* and *Capoeta banarescui* from Melet River (Bostanci et al., 2017), *Alburnus chalcoides* from Curi River (Kontaş et al., 2017), *Alburnus mossulensis* from Munzur River (Yedier et al., 2017a) and *Alburnus tarichi* from Lake Van (Yedier et al., 2017b). In addition, the relationship between fish status and fluctuating asymmetry has been investigated in many different marine fish species characters such as postorbital length, preorbital length, pectoral fin ray, orbital diameter and head length in *Pentaprion longimanus* from the Sea of Oman (Jawad et al., 2011), lower and upper anterior of the premaxilla, lower and upper posterior of the premaxilla, center of eye, anterior and posterior margin through the midline of the eye, isthmus, inferior margin through the midline of the eye, superior margin of the eye, dorso-lateral angle of the operculum, posterior margin of the operculum, origin of pectoral fin, insertion of pectoral fin, origin of pelvic fin, and mid-superior margin of the upper lip of *Siganus fuscescens* from five different populations in Philippines (Hermita et al., 2013), otolith length and width of *Chlorurus sordidus* and *Hipposcarus harid* from the *Egyptian Red Sea* (El-Regal et al., 2016).

Despite the fact that asymmetry characteristics of fish are important features that enable us to get information about the freshwater or sea water habitats, there is a limited number of studies on this subject in ichthyofauna of Turkey. Detailed otolith fluctuating asymmetry of *T. mediterraneus* was not studied in the Middle Black Sea. The objective of the current study was to provide some valuable information about the otolith asymmetry of Mediterranean horse mackerel in the Middle Black Sea.

Materials and Methods

All fish samples were captured on the coast of the Ordu province (40°00'-41°00' N and 37°00'-38°00' E) in the middle Black Sea region of Turkey. It has a small coastline (121 km) and many running waters such as Melet River, Turnasuyu Stream, Bolaman River, Civil Stream, Elekçi River and Akçaova Stream carry a lot of freshwater to the Black Sea.

After the samples collected, they were brought to the Hydrobiology Laboratory at Ordu University. The samples were kept in a deep freezing in there until they were examined. Specimens were measured total length to the nearest 1 mm and weighted with ± 01 g sensitiveness. In the current study, the otolith removal procedure was used according to Secor et al. (1991). Fish samples were sexed by the internal examination and their sagittal otolith pairs were removed, cleaned and stored dry before the examination. Undamaged and cleaned sagittal otolith pairs were examined and all measurements of the left and right otoliths were taken by the same research scientist to minimize the amount of error in the measurements. General measurements of fish and otolith samples were taken according to Bostanci et al. (2015).

Each otolith pairs were photographed using a Leica S8APO brand microscope with computer-connected camera system (Fig. 1a). Otolith length (OL, mm), otolith width (OW, mm), otolith area (A, mm^2) and otolith perimeter (P, mm) were measured by Leica Application Suite (Ver. 3.8) (Fig. 1b).

In the current study, four different otolith characters were used such as otolith length, width, area, and perimeter to calculate the otolith asymmetry values in the species. While Kolmogorov-Smirnov test was used for the normal distribution of all variables, Levene's test was used for the homogeneity of variances the variables. A t-test was applied in order to determine differences between males and females' otolith measurements. In addition, a paired t-test was used to determine differences between right and left sagittal otolith measurements. The statistical analysis included calculating the squared coefficient of asymmetry variation (CV^2_a) for otolith length, width, area and perimeter according to Valentine et al. (1973): $CV^2_a = (S_{r-l}*100/X_{r+l})^2$ Where S_{r-l} is the standard deviation of signed differences and 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. Minitab 17.0 statistical program was used for all necessary calculations in the present study.

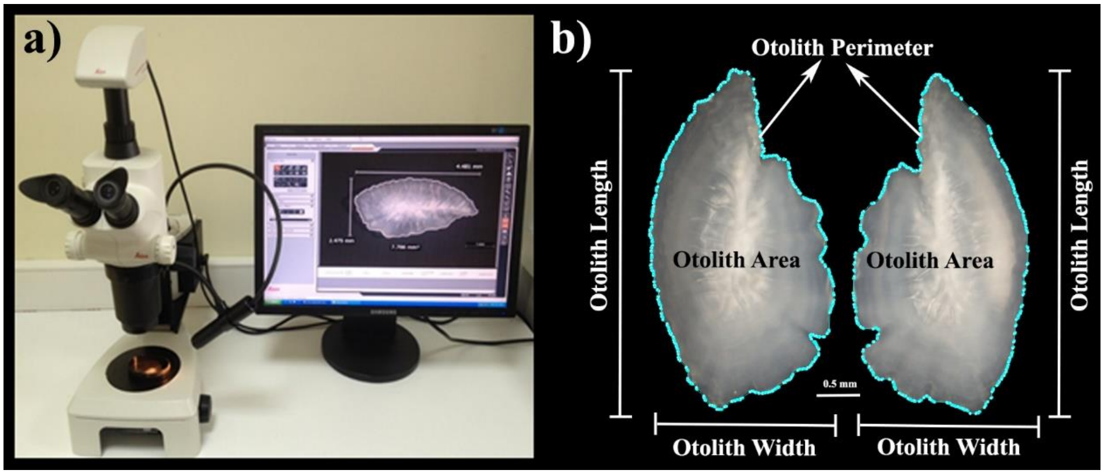

Figure 1. Leica S8APO brand microscope with computer-connected camera system (a) and measured otolith characters.

Results

A total of 88 specimens were collected and their total length and weight ranged from 119-164 mm and 11.14-35.09 g in the Middle Black Sea. The samples were divided into six total length classes (111-120; 121-130; 131-140; 141-150; 151-160; 161-170), with the differences between the size classes being equal and each class containing at least one individual. All data were passed Levene's test of homogeneity ($P>0.05$) and Kolmogorov Smirnov test of normality ($P>0.05$). Although there were no significant differences between males and females' otoliths measurements (t-test, $P>0.05$), significant differences between the right and left sagittal otolith measurements were showed (paired t-test, $P<0.05$). For these reasons, otolith measurements of the both sexes were pooled in the present study. Left and right sagittal otoliths images in male and female *T. mediterraneus* were presented (Fig. 2).

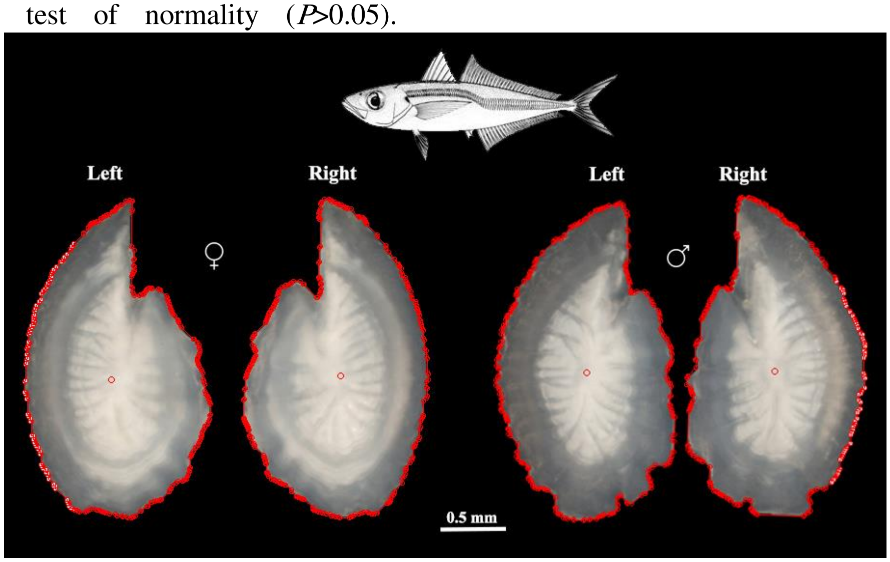

Figure 2. General view of left-right sagittal otoliths of female and male *Trachurus mediterraneus.*

Fluctuating asymmetry results were presented for the otolith length, width, area and perimeter in *T. mediterraneus* samples collected from the middle Black Sea coast of Ordu (Turkey) (Table 1). The results showed that the level of asymmetry of the otolith length was the highest among the four asymmetry values. When the otolith width contains the lowest number of asymmetric individuals, the percentage of asymmetric individuals is the highest in the otolith perimeter (Table 1). The highest asymmetry values of four otolith characters in six total length classes is 141-150 mm; however, the lowest asymmetry in the total length classes varies according to otolith characters. The asymmetry value is zero in 111-

120 mm total class for the four studied otolith characters (Table 1).

The relationships between total length classes and asymmetry of otolith characters were examined and their equations and coefficient of determinations were y=1.2515x+0.9269; R^2=0.1996 for otolith length, y=0.0708x+2.1705; R^2=0.0031 for otolith width, y=0.5191x+2.1477; R^2=0.0632 for otolith area and y=1.1971x-0.2177; R^2=0.7820 for otolith perimeter. In the present work for the four studied otolith characters, the asymmetry was not strongly correlated with total length classes (Tables 1; Fig. 3).

Table 1. The mean, min-max values of otolith characters and their fluctuating asymmetry in *Trachurus mediterraneus* by total length classes.

Otolith Character	Total length Class (mm)	N	CV^2_a	Character Mean	Character Min-Max	% of individuals with asymmetry
Otolith Length	111-120	1	0	3.980	3.952 – 4.009	100
	121-130	10	1.331	4.287	4.060 – 4.566	100
	131-140	24	6.541	4.670	4.180 – 5.222	91.67
	141-150	34	14.679	4.975	4.480 – 5.679	97.06
	151-160	17	3.401	5.156	4.723 – 5.607	100
	161-170	2	5.891	5.390	5.208 – 5.479	100
Total	**111-170**	**88**	**8.717**	**4.847**	**3.952 – 5.679**	**96.59**
Otolith Width	111-120	1	0	2.340	2.326 – 2.354	100
	121-130	10	2.607	2.463	2.310 – 2.597	100
	131-140	24	3.184	2.632	2.311 – 2.882	83.33
	141-150	34	6.447	2.814	2.525 – 3.139	91.18
	151-160	17	2.160	2.889	2.611 – 3.110	88.24
	161-170	2	0.111	2.971	2.896 – 3.053	100
Total	**111-170**	**88**	**4.337**	**2.737**	**2.310 – 3.139**	**89.77**
Otolith Area	111-120	1	0	6.566	6.459 – 6.673	100
	121-130	10	1.964	7.242	6.730 – 7.716	100
	131-140	24	5.559	8.415	6.868 – 10.082	100
	141-150	34	10.328	9.538	8.132 – 10.949	100
	151-160	17	5.196	10.154	8.772 – 11.354	94.12
	161-170	2	0.741	11.091	10.583 – 11.667	100
Total	**111-170**	**88**	**7.151**	**9.077**	**6.459 – 11.667**	**98.86**
Otolith Perimeter	111-120	1	0	10.406	10.115 – 10.698	100
	121-130	10	1.977	11.132	10.583 – 11.745	100
	131-140	24	4.333	12.131	10.676 – 13.431	100
	141-150	34	6.472	12.924	11.692 – 14.430	100
	151-160	17	4.782	13.356	12.085 – 14.594	100
	161-170	2	6.269	13.953	13.418 – 14.305	100
Total	**111-170**	**88**	**4.913**	**12.582**	**10.115 – 14.594**	**100**

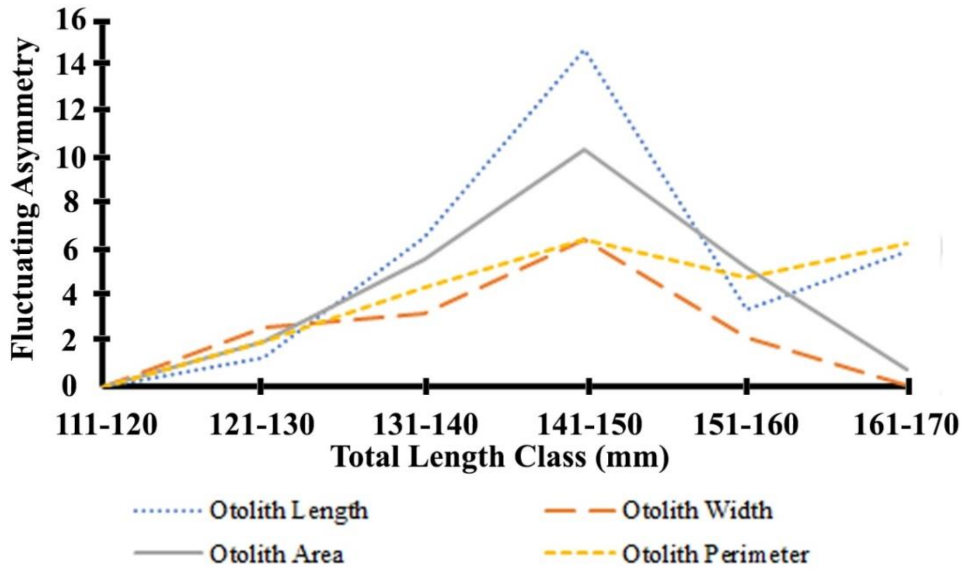

Figure 3. Total length class-fluctuating asymmetry relationship in *Trachurus mediterraneus.*

Discussion

The importance of water for living things is obvious because it has a vital role in their life and aquatic ecosystems cover a significant portion of the Earth's surface. The water on Earth is in a continuous loop. During this process, the substances that are involved in the water change the physical, chemical and biological properties of the water, creating a situation called water pollution. The pollution is a major global problem, and it is seen as the cause of many deaths and water-borne epidemics in aquatic ecosystems. *Turkey* is surrounded by sea on *three sides* and water pollution is also vital importance in there.

Sea water pollution and sediments by agricultural *and livestock wastes*, soil erosion, chemical pollution, biological and physiological contaminants, atmospheric pollution, industrial wastes, heavy metals, mining activities, insecticides and pesticides are considered the main cause of environmental stress. Animals are sensitive to environmental effects in morphometric characters, especially in the meridian characters and show a significant fitness disorder when they became exposed to environmental stress (Fowler, 1970; Osgood, 1978; Jockusch, 1997).

Direct or indirect effects of environmental stress on the animals affect the symmetry of bilateral characters of the animals and their developmental instability. The level of instability among populations and individuals is most commonly expressed by their fluctuating asymmetry levels (Ludwig 1932). Several authors stated that both environmental and genetic factors had positive effects on otolith asymmetry (Panfili et al., 2005). In the current study, some variation in fluctuating asymmetry values was observed among the four otolith characters of Mediterranean horse mackerel. Many researchers have studied many studies of otolith asymmetry of different fish species in different habitats. Fluctuating asymmetry in otolith length and width of *Rastrelliger kanagurta* (Al-Mamry et al., 2011) *Chlorurus sordidus,* and *Hipposcarus harid* (El-Regal et al., 2016) and they indicated that otolith length has the highest fluctuating asymmetry among the otolith characters which are examined in the studies. The results are correlated the current study result and the otolith length was identified as the most asymmetric otolith character with 8.717 asymmetric value among the four otolith characters of Mediterranean horse mackerel in the middle Black Sea region. However, some authors stated that otolith width is the most asymmetric otolith character for *Liza klunzingeri* (Sadighzadeh et al., 2011), *Sardinella sindensis* and *Sillago sihama* (Jawad et al., 2012a), *Lutjanus bengalensis* (Jawad, 2012) and *Carangoides caeruleopinnatus* (Jawad et al., 2012b).

Unlike the present study, the otolith length has the lowest fluctuating asymmetry value and otolith area is the most asymmetric character of the *Brevoortia tyrannus* (Fey and Hare, 2008). When the number of individuals exhibiting asymmetry in the four otolith characters of the current study was taken into account, otolith perimeter was the highest among the percentages obtained for the four otolith characters. In the other otolith asymmetry studies, the characters with the highest percentages of asymmetric individual of the fish species are as follows; otolith width for *Liza klunzingeri* (Sadighzadeh et al., 2011) and *Carangoides caeruleopinnatus* (Jawad et al., 2012b), otolith length for *Hipposcarus harid* (El-Regal et al., 2016).

Total length and asymmetry in morphological characters of the fish species have shown to be correlated as indicated by several authors (Al - Hassan and Hassan, 1994; Al - Hassan and Shwafi, 1997; Jawad, 2001); however, the current study results did not support their results. The otolith fluctuating asymmetry is not correlated with the total length of the *T. mediterraneus* in the current study. This difference between studies may cause several reasons such as difference in fish species, the number of total size groups, the pollution level of the sampling areas and number of individuals in length classes.

In the Edremit Bay, North Aegean Sea, fluctuating otolith asymmetry values of *T. mediterraneus* were calculated 1.7277 for the otolith length, 2.1304 for the width, 2.8425 for the area and 1.5931 for the perimeter (Bostanci et al., 2018). We can see that the calculated fluctuating otolith asymmetry values are well below the values obtained in our study. *T. mediterraneus* otoliths from the Black Sea population showed more fluctuating asymmetry than the North Aegean Sea population. When these two populations of horse mackerel fish are compared, it is concluded that the Black Sea population is under more stress.

Environmental factors are increasing day by day and they threaten the natural populations and put them under stress. There are several effects of the asymmetry on fish species, the asymmetry causes abnormal swimming activity (Helling et al., 2003), hearing problems (Lychakov and Rebane, 2005) and some adaptations problems (Jorgensen and Fiksen, 2010). Therefore, conservationists need simple tools to measure the effects of these stresses before they are irreversibly affected (Lens et al., 2002a) and protect these populations. The measurement and analysis of fluctuating asymmetry are simple, the method is not destructive and requiring inexpensive equipment; therefore, it has become a popular measure of the quality and health of individuals and populations in their habitats (Møller and Thornhill, 1998; Lens et al., 2002a). In many studies, developmental instability was measured using the fluctuating asymmetry values of the samples and the asymmetry could be considered as a possible indicator of environmental stress on the population (Parsons, 1990; Alados et al. 1993; Lens et al. 2002b).

In Turkish water, the consumption of Mediterranean horse mackerel and their economic values is high. Therefore, it is very important to ensure sustainability of the fish populations. According to current study results, the middle Black Sea population of the Mediterranean horse mackerel is under more environmental stress. One of the main reasons for this stress can be water pollution because the Black Sea is becoming increasingly polluted by many rivers contaminated with shallow and mixed surface waters, agricultural, industrial and mine waste containing nitrogen and phosphorus (Boran and Altınok, 2010) even the Black Sea is known as the most polluted sea in the Turkey (Ökmen, 2011). Heavy metal pollution is one of the *major* environmental concerns in the Black Sea (Tuzen, 2003) as it is in most marine habitats (Balkas et al., 1982; Tarıq et al., 1991). The pollutants are potentially accumulated in not only marine fish but also freshwater species and they subsequently transferred to people through the food chain. Therefore, water pollution can affect not only marine organisms but also higher organisms through the food chain. The conditions of the aquatic animals and their habitats need to be well known by researchers. For these reasons, asymmetry could be used as an alternative and inexpensive method to determine environmental stress from the heavy metals and other pollutants in aquatic ecosystems.

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