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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Investigation of otolith mass asymmetry in three stocks of European sardine, *Sardina pilchardus* (Walbaum, 1792) from Türkiye

Türkiye'den üç sardalya, *Sardina pilchardus* (Walbaum, 1792) stoğunun otolit kütle asimetrisinin incelenmesi

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Abstract: It was aimed to investigate sagittal otolith mass asymmetry *Sardina pilchardus* sampled from Aegean, Marmara and Mediterranean seas of Türkiye in present study. In this study, differences between right and left otoliths were statistically significant for Marmara and Mediterranean seas (P<0.05) not significant for Aegean Sea, (P>0.05). The mean values of otolith mass asymmetry (X) were found between 0.0393 and 0.0144 according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively. In addition, absolute mass asymmetry $|X|$ were calculated as 0.03226±0.00514, 0.02057±0.00439 and, 0.05141±0.00755 for *S. pilchardus* samples according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively. The present study showed that the otolith mass asymmetry in *S.pilchardus* does not depend on fish length and otolith growth. Also, when there were no significant differences between localities for otolith mass asymmetry (P>0.05), there were significant differences for absolute otolith mass (P<0.05). The value of the otolith mass asymmetry can reveal information about pollutants such heavy metals, pesticides, stressors, and changes in the physico-chemical characteristics of water in relation to the environment of fishes. This is the first study about otolith mass in three stocks of *S. pilchardus* (Walbaum, 1792) from Türkiye.

Keywords: *Sardina pilchardus*, otolith, mass asymmetry, stock, Türkiye

Öz: Bu çalışmada, Türkiye'nin Ege, Marmara ve Akdeniz kıyılarından örneklenen *Sardina pilchardus*'un sagittal otolit kütle asimetrisi çalışılmıştır. Bu çalışmada, sağ ve sol otolitler arasındaki fark Marmara ve Akdeniz için istatistiksel olarak önemli (P<0,05), Ege Denizi için önemsiz (P>0,05) olarak bulunmuştur. Ortalama X değerleri sırasıyla Ege Denizi, Marmara Denizi ve Akdeniz'e göre 0,0393 ile 0,0144 arasında bulunmuştur. Ayrıca, *S. pilchardus* örneklerinin $|X|$ değerleri Ege Denizi, Marmara Denizi ve Akdeniz'e göre sırasıyla 0,03226 ±0,00514, 0,02057±0,00439 ve 0,05141± 0,00755 olarak hesaplanmıştır. Bu çalışmanın sonuçlarına göre, *S. pilchardus*'ta otolit kütle asimetrisinin (X) balık boyu ve otolit büyüme hızına bağlı olmadığı görülmüştür. Ayrıca, otolit kütle asimetrisi lokaliteler arasında anlamlı farklılık göstermezken (P>0,05), mutlak otolit kütle asimetrisi lokaliteler arasında farklılık göstermiştir (P<0,05) Otolit kütle asimetri değeri; ağır metaller, pestisitler, stres faktörleri, suyun fiziko-kimyasal özellikler gibi balıkların yaşadığı ortamla ilgili değişiklikler ve kirlilik faktörleri hakkında bilgi verebilir. Bu çalışma, Türkiye'den üç *S. pilchardus* (Walbaum, 1792) stokunda otolit kütlesi ile ilgili ilk çalışmadır.

Anahtar kelimeler: *Sardina pilchardus*, otolit, kütle asimetrisi, stok, Türkiye

INTRODUCTION

The fish otoliths, often known as "ear bones," are calcareous structures found in the inner ear of vertebrates that serve as sound receptors and balance organs (Schulz-Mirbach and Ladich, 2016; Tuset et al., 2021). In most species the sagitta is large, but in some species, the asteriscus is larger like Cypriniformes, Siluriformes, Characiformes and Gymnotiformes (Berra and Aday, 2004). These calcareous structures, in which the life histories of fishes are hidden, have been the most basic element of many different studies until today (Yu et al., 2014; Yilmaz et al., 2015; Bostanci and Yedier, 2018; Chesalin, 2021; Ozpicak et al., 2021; Pavlov, 2022; Jawad et al., 2023). The morphology of otoliths is extremely unique and varies greatly throughout fish families, however, it can also be quite species-specific (Maisey, 1987). Many studies published in recent years have revealed that otolith mass asymmetry (OMA) is a very important study area because of playing a major role in acoustic functionalities (Lychakov et al., 2006; Jawad et al., 2011; Jawad et al., 2017; Yedier et al., 2018; Bouriga et al., 2021). Because the otoliths of teleost fishes are easily quantified, they make an excellent biological model for analyzing the physiological significance of OMA (Lychakov et al., 2006).

In fishes, otoliths should be bilaterally symmetrical, but in some circumstances, weight discrepancy between the left and right otolith masses is seen, which is known as otolith mass asymmetry (Yedier et al., 2018). When fishes are subjected to weightlessness during parabolic or space flight, OMA may be at least partly responsible for anomalous behavior in fish (Lychakov et al., 2006). In order to analyze otolith displacements with respect to OMA and otolith mass under sound stimulation or gravity, mathematical models were used (Lychakov and Rebane, 2005). According to various studies on OMA, X values range between -0.2 and +0.2 (Jawad, 2013; Bouriga et al., 2021). In this concept, if X has an absolute value greater than 0.2, a fish's ability to produce sound may change (Lychakov et al., 2006). Therefore, OMA is a very important field of study both for the environment in which the fish live and for the adaptation of the fish to their environment.

Pilchard or sardine, *Sardina pilchardus* (Walbaum, 1792) is one of the most popular sea fish worldwide. Sardine is an economically important and short-lived pelagic fish (Silva et al., 2019), widely distributed throughout the Mediterranean Sea (Jemaa et al., 2015), Northeast Atlantic, from the Celtic and North Seas to Mauritania and Senegal (ICES, 2018), with smaller populations in the Archipelagos of Azores, Madeira, and the Canary Islands and, is also found in the Mediterranean, Marmara, and Black seas (Parrish et al., 1989). Commercially valuable marine pelagic fish populations are frequently being driven to extinction due to excessive overfishing (Pauly et al., 2003; Atarhouch et al., 2006). European sardine catches have decreased in several areas during the past ten years, and nearly all of its geographic range has been identified as having totally or extensively exploited (FAO, 2018, 2019; ICES, 2018). There are many different studies on the genetics (Atarhouch et al., 2006;), ecology (Chouvelon et al., 2014; Castalago et al., 2015), fishing (Marçalo et al., 2006; Molina-Fernandez et al., 2015), feeding (Garrido et al., 2008; Costalago and Palomera, 2014), age and growth (Dahel et al., 2016; Baldè et al., 2022), and morphology (Silva, 2003; Baibai et al., 2012) of this fish species, which is very important in economically. Although the increase in OMA research throughout the world, there is no study about OMA of *S. pilchardus* in Türkiye.

According to literature, the harvested sardines from northern coasts such as Marmara and Aegean seas, are smaller than samples from Mediterranean Sea (Sarmasık et al., 2008). The temperature effect on development rate (hot Mediterranean waters versus cooler Aegean and Marmara seas) may be the only explanation for this, although size disparities may also be influenced by the genetic background of different stocks. Therefore, these differences can also affect the otolith characteristics. In addition, this study is aimed to investigate otolith mass asymmetry and compare total length-OMA and total length-absolute otolith mass asymmetry relationships in three stocks of *S. pilchardus* from Aegean, Marmara and Mediterranean seas.

MATERIALS AND METHODS

Study material and sampling

Samples of sardine were obtained from commercial fishermen from Aegean Sea (AS) (n=50), Marmara Sea (MS) (n=49), and Mediterranean Sea (MEDS) (n=50) in Türkiye. Specimens were defrosted for laboratory analysis about one month later to ensure that all fish were analyzed after a similar period of being frozen. The total length (L_T) of each sample was measured to ± 0.1 cm.

Otolith extraction and mass asymmetry

Sagittal otolith pairs were removed by making right and left distinctions. Otoliths were dried and kept in eppendorf tubes after cleaned with distilled water. Otolith pairs were weighted (± 0.0001g). Otolith mass asymmetry (X) was determined by using the following formula:

$$X = (M_R - M_L) / M,$$

M_R and M_L are the otolith masses of cleaned right and left otoliths and M is the average mass of M_R and M_L. Theoretically, otolith mass asymmetry (X) could vary from -2 to +2. 'Zero' ($M_R = M_L$) value denotes the absence of the mass asymmetry, whereas '-2' or '+2' values imply greatest asymmetry. When the value of X is positive, the right otolith mass is greater than the left otolith mass, and when it is negative, the opposite is true.

Additionally, the formula X=a×TL+b was used to compute the relationship between (X) and total length, as well as absolute otolith mass asymmetry ($|X|$) and total length. The paired t-test was used to examine the left and right otoliths and examine any variations between variables across all samples. Also, otolith mass asymmetry was compared with ANOVA between localities. Statistical analyses were performed in SPSS 21.0, Minitab 17.0 software, and the Microsoft Excel packages.

RESULTS

Sardina pilchardus samples were collected from Aegean Sea (12.93±0.13 cm TL), Marmara Sea (13.02±0.10 cm TL) and Mediterranean Sea (22.84±0.17 cm TL). Descriptive statistics of otolith weight in *S. pilchardus* individuals were in Table1.

Table 1. Descriptive statistics of *S. pilchardus* otolith weights according to localities

Locality	N	Side	Mean	±SE	±SD	Minimum	Maximum	*P*
Aegean Sea	50	R	0.0013	0.00004	0.0003	0.0006	0.0019	0.077
		L	0.0013	0.00004	0.0003	0.0006	0.0020	
Mediterranean Sea	49	R	0.0032	0.00012	0.0008	0.0022	0.0051	0.000*
		L	0.0033	0.00012	0.0009	0.0020	0.0051	
Marmara Sea	50	R	0.0012	0.00004	0.0003	0.0007	0.0021	0.007*
		L	0.0013	0.00004	0.0003	0.0007	0.0021	

*There are statistically differences between the right and left otolith pairs; *n*, number of samples R, Right; L, Left; SE, Standard Error; SD, Standard Deviation; Mean, Average otolith weight; *P*, Significance

Otolith mass asymmetry was calculated as $-0.10081 \leq X \leq +0.10081$, $-0.10081 \leq X \leq +0.10081$, and $-0.20161 \leq X \leq +0.10081$ for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Table 2). According to results, right otoliths are heavier than left otoliths (70% for Aegean Sea, 65% for Marmara Sea and 51% for Mediterranean Sea). In addition, the mean values of $|X|$ were calculated as 0.03226 ±0.00514, 0.02057±0.00439 and, 0.05141± 0.00755 for *S. pilchardus* samples according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively.

Table 2. Descriptive statistics of otolith mass asymmetry of *S. pilchardus* according to localities

Locality	Mean	SE	SD	Minimum	Maximum
Aegean Sea	-0.0121	0.0067	0.0473	-0.1008	0.10081
Mediterranean Sea	-0.0393	0.0089	0.0630	-0.20161	0.10081
Marmara Sea	0.0144	0.0049	0.0341	-0.10080	0.10081

Mean, Average otolith mass asymmetry; SE, Standard Error; SD, Standard Deviation; *P*,Significance

Moreover, when there were no significant differences between localities for otolith mass asymmetry (Kruskal-Wallis test, P>0.05), also, there were significant differences for absolute otolith mass (P<0.05). In addition, the correlation coefficients r^2 and regression equations were calculated for all of the localities (Figure 1, Figure 2). Correlation coefficients and regression equations were y = -0.0094x + 0.1091; r^2 = 0.0306, y = -0.0005x-0.0079; r^2= 0.0001 and, y = -0.0005x-0.0079; r^2= 0.0917 for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Figure 1a, b and c).

Based on the results of regression analysis of total length-absolute otolith mass asymmetry, the correlation coefficients and regression equations were calculated as y = 0.0076x - 0.0663; r^2= 0.0342, y = -0.0022x + 0.0492; r^2 = 0.0026, and y = 0.013x -0.2447; r^2 = 0.0809 for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Figure 2a, b and c). The results do not support the hypothesis since no significant correlation between X-total length (0.0001 ≤ r^2≤ 0.0917) and $|X|$-total length was found (0.0026≤ r^2≤ 0.0803).

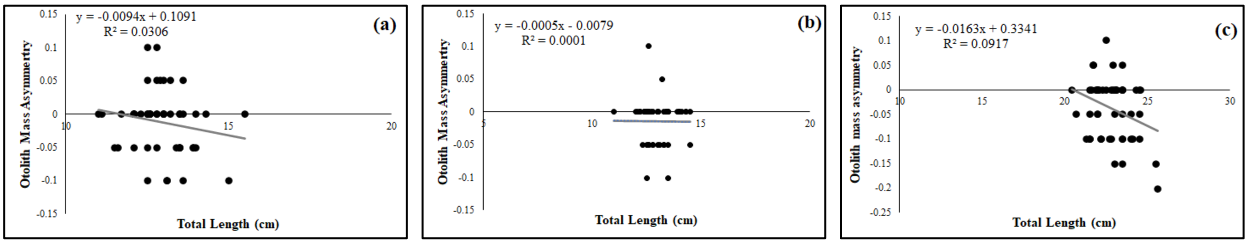

Figure 1. Sagittal OMA (X) and total length relationship in *S.pilchardus* (a) Aegean Sea, (b) Marmara Sea, (c) Mediterranean Sea

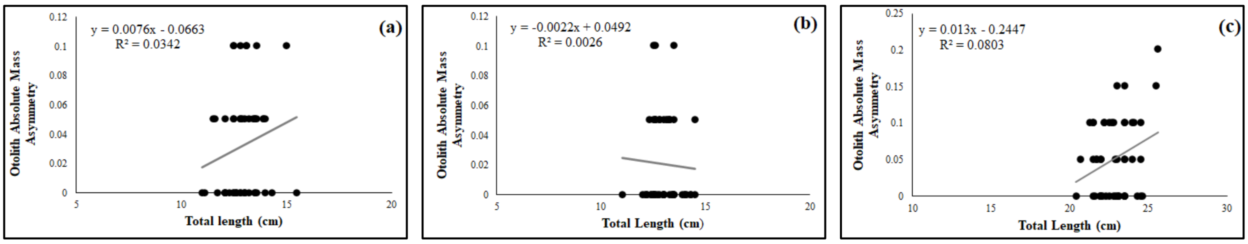

Figure 2. IXI and total length relationship in *S.pilchardus* (a) Aegean Sea, (b) Marmara Sea, (c) Mediterranean Sea

DISCUSSION

In fisheries science there are many investigations on OMA, and the otolith weight asymmetry values were found to be in the range of -0.2<X<+0.2 for marine and freshwater species (Lychakov, 1992; Lychakov and Rebane, 2005; Yedier et al., 2018; Kontas et al., 2019; Jawad and Quasim, 2020; Bouriga et al., 2021; Jawad et al., 2021; Jawad and Adams, 2022). Our results showed to fall within that range (-0.20161≤ X ≤+0.10081). In addition, the otolith weight asymmetry was less than 0.06, which was lower than the value found for many marine species (Lychakov et al., 2006) and was unaffected by the otolith growth stage. Reduced acoustic and vestibular functionality in fish ears is thought to be a result of OMA (Lychakov and Rebane, 2005). Bouriga et al. (2021) were calculated X between –0.3636 ≤ X≤ 0.1538 for *S. pilchardus* from Gulf of Tunis. Differences in OMA may be affected by the ecological conditions, physiological state of species and its habitat (Grønkjær, 2016). Both genetic and environmental conditions have an impact on the morphological variability of sagitta (Lombarte et al., 2010; Annabi et al., 2013). Additionally, changes in otolith mass asymmetry can have a deleterious impact on other aspects of fish life, particularly their ability to hear and balance. In accordance with other fish species' conditions, the otolith weight difference grows with fish length. (Lychakov and Rebane, 2004).

Additionally, Lychakov and Rebane (2005) demonstrated that only fish with big otoliths and $|X|$ >0.2 may, in principle, experience difficulty with sound handling as a result of improper and inconsistent movement of the fish's two otoliths on either side of its head. As a result, because their otolith weight non-symmetry is below acute levels, the majority of fish species can flee with effective incapacity. According to Lychakov and Rebane (2005), only fish with the biggest otoliths and $|X|$ >0.2 might theoretically experience issues with sound processing because of the disparity and peculiar movement of the fish's otoliths on either side of its head. In the present study, otolith absolute mass asymmetry is very low, $|X|$ was found between 0.02057±0.0043 and 0.05141±0.00755 according to localities. There are similar results in the literature, too (Jawad et al., 2017; Bouriga et al., 2021; Jawad et al., 2021).

In several investigations, the relationship between the total length and the otolith mass asymmetry has been examined. Although, sagittal otolith mass disparities rise with fish length, this is a phenomena in bottom fish more than pelagic fish (Lychakov et al., 2006). However, results of present study do not support the hypothesis since no significant relation between mass asymmetry-total length ($0.0001 \leq r^2 \leq 0.0917$) and absolute otolith mass asymmetry-total length was found ($0.0026 \leq r^2 \leq 0.0803$) (Jawad, 2013; Yedier et al., 2018; Bouriga et al., 2021). Similiar to the literature, there is no correlation between fish size and otolith mass asymmetry in *S.pilchardus* which is a pelagic fish species. Fish length and otolith mass asymmetry are thought to be related in a complicated trend.

Numerous research conducted throughout the world examined the otolith mass and shape asymmetries. In Türkiye, otolith mass asymmetry studies are limited (Yedier et al., 2018; Kontaş et al., 2019). However, in Türkiye, which is surrounded by seas on three sides, it plays a very important role in fisheries studies to examine the lives of fishes and to obtain maximum efficiency in them. Exposure to domestic, industrial, and agricultural wastes causes ongoing degradation of the aquatic environment, and the harm that pollution is doing to the ecosystem is getting worse (Turgut and Özgül, 2009). Stress is brought on in aquatic animals by pollution in their surroundings. In fish, this stress may lead to developmental instability. The fact is that, according to earlier research in otolith mass asymmetry, there is a connection between environmental stress and pollution-related asymmetry (Jawad et al., 2012). Currently, otolith mass asymmetry may be a result of environmental stress.

Somatic development and otolith accumulation are indirectly impacted by environmental variables. Additionally, the otolith mass asymmetry is a low-cost method to assess the environmental health state, and as a suggestion, populations of other fish species should be checked for this kind of bilateral asymmetry. It should be evident that there is a special physicochemical mechanism for paired otolith formation that keeps the otolith mass a disparity as low as possible. This mechanism is still not fully understood. However, contrary to other data, the otolith weight does not appear to play a role in the feedback regulation of its growth (Lychakov, 2002).

Future studies on otolith mass asymmetry in marine fish are expected to use the results of the current study as a baseline, enabling researchers to compare the otolith mass asymmetry of *Sardina pilchardus* populations from Türkiye and overseas.

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AUTHORSHIP CONTRIBUTIONS

Melek Ozpicak: Conceptualization; Investigation, methodology, resources, software, validation, writing – original draft, writing – review and editing. Semra Saygin: Investigation, methodology, resources, software, validation, writing – original draft, writing – review and editing.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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