

# Relationship between Sagittal Otolith Size and Fish Size in *Engraulis encrasicolus* and *Sardina pilchardus* (Osteichthyes: Clupeiformes) in the Southern Aegean Sea, Turkey

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## ABSTRACT

The objective of this study was to determine the regressions between otolith size (length and height), otolith weight vs. fish length, and weight of European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) (n=360) and European pilchard *Sardina pilchardus* (Walbaum, 1792) (n=360), living off Güllük Bay, Turkey. Fish were caught using a purse seine between January and March 2014 in the southern Aegean Sea. No differences were found between the size and weight of the left and right otoliths. Equations were used to reconstruct the original dimensions of prey from the size of hard structures found in food samples of piscivorous predators living in or in the vicinity of the aquatic habitat. A linear regression model was used to determine the relationship between fish length and otolith size, whereas an exponential regression model was used to describe the relationships between lengths and weights of otoliths and fish for both species. All regressions yielded high coefficients of determination ( $r^2$ ) of 0.78–0.93 for *E. encrasicolus* and 0.80–0.95 for *S. pilchardus*. We conclude that otolith length and otolith weight are good indicators of the length and weight of the two species.

**Keywords:** European anchovy, European pilchard, Güllük Bay, otolith morphometry

## INTRODUCTION

Otoliths are small opaque structures composed of calcium carbonate in an organic matrix and they also have vestibular and sound detection function in fishes other than lampreys, sharks, and rays (Campana, 2004). Otoliths also have a distinctive shape, which varies widely among fish families, yet can be highly species-specific (Maisey, 1987). Although they are composed of protein and calcium carbonate crystals, they are situated in the skull and therefore protected from digestion. Thus, several identification guides and keys have been published for South Africa by Smale et al. (1995), for the northeast Atlantic Ocean by Härkönen (1986), the Bering Sea by Morrow (1976), the northwest Atlantic Ocean by Campana (2004), the western Mediterranean, north and central eastern Atlantic by Tuset et al. (2008), and fossil fishes by Nolf (1985).

Otoliths can be used in diet studies of piscivorous animals, providing the whole fish is consumed or at least if the head is not discarded to such an extent that the results of the study are heavily biased (Härkönen, 1986). During feeding studies, the identification and quantification of this prey is often a difficult task: in most cases specimens are already partially or totally digested and the hard remains in the stomach, intestines, and faeces are the only diagnostic features that can be considered (Battaglia et al., 2010). Otoliths are somewhat resistant to digestion and may be used as an important tool for prey classification in several feeding studies (Pierce and Boyle, 1991; Pierce et al., 1991; Granadeiro and Silva, 2000; Battaglia et al., 2010). Furthermore, the relationship between fish length and otolith size and weight has been used with several fish species to draw conclusions on the body size and biomass of prey species (Frost and Lowry, 1980; Al-Mamry

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et al., 2010). Thus, marine biologists frequently depend on the morphology and size of conserved otoliths to examine the species and size composition of the diet of piscivore animals (Campana, 2004).

In the present study, the relationships between fish size and otolith size were studied in two marine species in the southern Aegean Sea: the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) and the European pilchard *Sardina pilchardus* (Walbaum, 1792). *E. encrasicolus* is a pelagic-neritic fish species forming large schools and it is a unique member of the Engraulidae family distributed in the Black Sea, the Sea of Marmara, the Aegean and the Mediterranean and estuaries of adjacent watersheds in Turkish waters (Fricke et al., 2007). The conservation status of this species was reported as vulnerable (VU) and the threats were FIT (a species that is commercially exploited as a target species) and FIB (a species that is not regularly commercially exploited, but frequently caught as bycatch in fisheries) in Turkey (Fricke et al., 2007). *Sardina pilchardus* is a pelagic-neritic species forming schools and it is a member of the Clupeidae family distributed in the Black Sea, the Sea of Marmara, the Aegean and the Mediterranean including adjacent estuaries and lower reaches of watersheds in Turkey (Fricke et al., 2007). The conservation status of this species was stated as near threatened in Turkey (NT) and the threats on the species were reported as FIT and as FIB by Fricke et al. (2007). Both keystone species, which have medium priority for conservation action, are sensitive to human activities (Fricke et al., 2007) and they are also economically one of the most important fish species for Turkish waters. In 2012, the total marine fish catch was 315636.5 tones in Turkey; of the catch 163981.9 tones were *E. encrasicolus* (51.95% of the total catch) and 28248 tones were *S. pilchardus* (8.95% of the total catch). A total of 34784.1 tones, *E. encrasicolus* (11141.4 tones, 32.03 % of total catch) and *S. pilchardus* (9973.5 tones, 28.67 % of total catch) have the highest ratio of catch of marine fishes for the Aegean Sea, where the present study was conducted (TUIK, 2013). Başçınar & Atılğan (2016), Zengin et al. (2015a, b) studied otolith morphometry and shape analysis of *E. encrasicolus* in the Black Sea and the Sea of Marmara. The aim of this study was to provide new data on the morphology and the relations between the otolith size and the fish size for the researchers studying the stomach contents and trophic interactions among marine animals in the Aegean Sea.

## MATERIAL AND METHOD

The fish were collected in monthly intervals during the period of January-March 2014 off Güllük Bay (southern Aegean Sea) (Figure 1) using commercial purse seine boats. The total length (TL) of the fish was measured to the nearest mm. Fish weight (W) was determined to the nearest 0.01 g on a digital balance. Sagittae (Figure 2) (total of 360 individuals, i.e., 720 otoliths per species) were removed with forceps through a cut in the cranium. Otoliths were then cleaned with 10% NaOH solution and dried, and the left and right otoliths were considered separately. Each sagitta was placed with the sulcus acusticus oriented upwards and otolith length (OL) was measured in mm through an eye-piece micrometer under a stereo zoom microscope (Olympus SZX-16). It was defined as the longest dimension between the rostrum and postrostrum axis (nomenclature of Smale et al., 1995; Tuset et al., 2008) through the focus of the otolith (Al-Mamry et al., 2010). Otolith height (OH) was measured in mm as the longest dimension between the ventral and dorsal surfaces of each sagitta. The image was taken of the internal side (medial or proximal) of the otolith as this side presents the sulcus acusticus (Tuset et al., 2008). Otolith weight (OW) was determined in mg. The paired t-test was applied to examine any dissimilarities between sagittae. When there is no significant difference ( $p < 0.05$ ), the  $H_0$  hypothesis ( $b_{right} = b_{left}$ ) was used. A single regression was used for each parameter (OL, OW, and OH). Linear regression equations ( $y = ax + b$ ) and exponential regression equations ( $y = ax^b$ ) were fitted to determine which equations (TL-OL, TL-OH, TL-OW, W-OL, W-OH, W-OW, OW-OL, OH-OL and OW-OH) are best describing various relations between otolith and fish size (Tarkan et al., 2007). The highest  $r^2$  scores were used to determine which type of regression model (Linear or exponential) was used between the parameters. Moreover, some otolith shape indices were calculated: aspect ratio (OH/OL:%) and OL/TL:% (Tuset et al., 2008) for both species to compare with other studies on the same species.

## RESULT AND DISCUSSION

The sagittal otoliths of 360 *Engraulis encrasicolus* and 360 *Sardina pilchardus* specimens were examined. Table 1 shows the descriptive statistics regarding length and weight of both species and their sagittal otoliths (with otolith width): In *E. encrasicolus*

**Table 1.** Descriptive statistics of length and weight data of specimens and their otoliths obtained from the Southern Aegean Sea; Values given are the mean  $\pm$  standard deviation (SD) and range in brackets

Species	N	Fish		Otolith		
		TL Mean ( $\pm$ SD) Min.-Max.	Weight Mean ( $\pm$ SD) Min.-Max.	Length Mean ( $\pm$ SD) Min.-Max.	Height Mean ( $\pm$ SD) Min.-Max.	Weight Mean ( $\pm$ SD) Min.-Max.
<i>E. encrasicolus</i>	360	109.07 $\pm$ 22.96 [57-150]	8.61 $\pm$ 5.26 [1.21-21.21]	2.68 $\pm$ 0.41 [1.7-3.4]	1.17 $\pm$ 0.17 [0.8-1.5]	8 $\pm$ 4 [1-21]
<i>S. pilchardus</i>	360	126.70 $\pm$ 25.39 [67-177]	17.44 $\pm$ 9.25 [2.29-42.67]	2.39 $\pm$ 0.49 [1.2-3.4]	1.22 $\pm$ 0.18 [0.7-1.5]	17 $\pm$ 9 [2-37]

N: sample size; TL: Total Length; Min.: Minimum; Max.: Maximum  
All lengths in mm, fish weight in g, otolith weight in  $10^4$  g

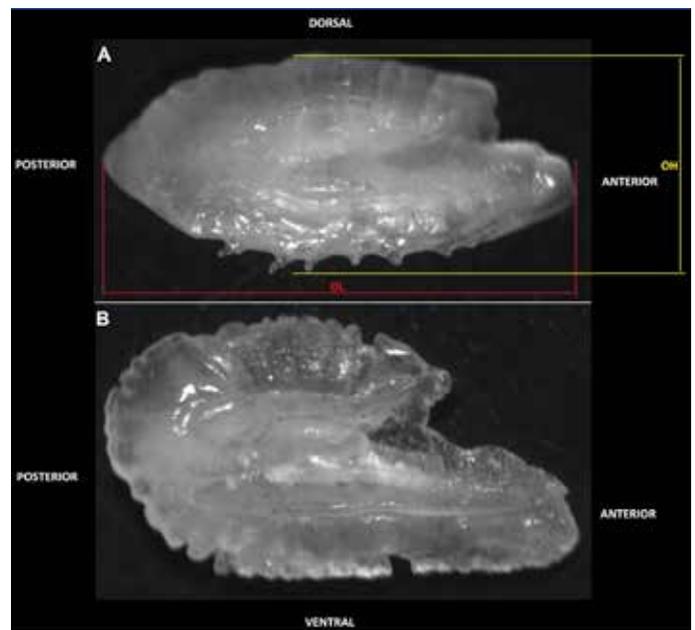
**Table 2.** Intercept values (a), regression slope (b) and coefficients of determination ( $r^2$ ) for linear (L) and exponential (E) relationships between otolith morphometric parameters, fish length and weight of *Engraulis encrasicolus* and *Sardina pilchardus*

	Relationship	Regression	a	b	$r^2$	Significance
<b><i>Engraulis encrasicolus</i></b>						
Fish Length	TL vs. OL	L	55.427	- 39.467	0.93	$p < 0.05$
	TL vs. OH	L	137.28	- 51.382	0.86	$p < 0.05$
	TL vs. OW	E	1587.4	0.3714	0.83	$p < 0.05$
Fish Weight	W vs. OL	E	0.1033	4.3187	0.86	$p < 0.05$
	W vs. OH	E	3.4932	4.6922	0.78	$p < 0.05$
	W vs. OW	L	12647	- 15877	0.85	$p < 0.05$
Otolith	OW vs. OL	E	2E-05	3.6356	0.84	$p < 0.05$
	OH vs. OL	L	0.3987	0.1002	0.80	$p < 0.05$
	OW vs. OH	E	0.0004	3.9387	0.81	$p < 0.05$
<b><i>Sardina pilchardus</i></b>						
Fish Length	TL vs. OL	L	52.028	2.2924	0.95	$p < 0.05$
	TL vs. OH	L	140.2	- 44.851	0.84	$p < 0.05$
	TL vs. OW	E	880.69	0.2962	0.83	$p < 0.05$
Fish Weight	W vs. OL	E	1.1935	2.9498	0.82	$p < 0.05$
	W vs. OH	E	6.6862	4.0985	0.80	$p < 0.05$
	W vs. OW	L	9880	1.1075	0.84	$p < 0.05$
Otolith	OW vs. OL	E	8E-05	3.2972	0.87	$p < 0.05$
	OH vs. OL	L	0.3455	0.3972	0.82	$p < 0.05$
	OW vs. OH	E	0.0006	4.5842	0.80	$p < 0.05$



**Figure 1.** Map of the study area

us, the mean total length was 109.07 mm (57–150 mm), and the length of otoliths ranged from 1.7 to 3.4 mm, height from 0.8 to 1.5 mm, and weight from 0.0001 to 0.0021 g; in *S. pilchardus*, the mean total length was 126.70 mm (67–177 mm), and the length of the otoliths 1.2–3.4 mm, their height 0.7–1.5 mm, and their weight 0.0002–0.0037 g. Statistically no significant difference was detected (Student's t-test for paired comparisons,  $p > 0.05$ ) between otolith pairs. So, measurements of left sagittae were used for detecting fish and otolith size relations. The relations between fish and otolith measurements are given in Table 2. All



**Figure 2. a, b.** Left sagittal otoliths of (a) *Engraulis encrasicolus* (TL=150 mm, OL=3.4 mm), (b) *Sardina pilchardus* (T=150 mm, OL=2.8 mm) from proximal side. OL=Otolith length, OH=Otolith height

of them yielded a high coefficient of determination ( $r^2$ ) between 0.78 and 0.93 for *E. encrasicolus* and between 0.80 and 0.95 for *S. pilchardus* (Table 2). % (OH/OL) and % (OL/TL) ratios were calculated with the ranges of 39.6-48.1 and 2.2-3.1 for *E. encrasicolus* and 42.0-61.6 and 1.8-2.0 for *S. pilchardus*, respectively.

Both species are of great economic importance. Furthermore, they are significant for the trophic level in the marine environment because they are consumed by several piscivorous fishes: *E. encrasicolus* are consumed by other *E. encrasicolus* (Valdés et al., 1987), *Seriola dumerili* (Matallanas et al., 1995), *Trachurus mediterraneus* (Santic et al., 2003), *Huso huso* (Berg, 1962), *Alosa fallax* (Assis et al., 1992), *Coryphaena hippurus* (Palko et al., 1982), *Etmopterus spinax* (Macpherson, 1979), *Merluccius merluccius* (Cabral and Murta, 2002), *Ophichthus rufus* (Casadevall et al., 1994), *Scomber scombrus* (Cabral and Murta, 2002), *Thunnus thynnus* (Sanz Brau, 1990), *Galeus melastomus* (Macpherson, 1979), *Saurida undosquamis* (Golani, 1993), *Uranoscopus scaber* (Sanz, 1985), *Xiphias gladius* (Cavaliere, 1963), *Ciliata mustela* (Costa, 1988), *Oblada melanura* (Pallaoro et al., 2004) and *Elops lacerta* (Hie Dare, 1980). For *E. encrasicolus*, our % ratio relationships between fish (57–150 mm TL,  $n=360$ ) and sagitta sizes were calculated as % (OL/TL)= 2.2-3.1 and % (OH/OL)= 39.6-48.1 for the Southern Aegean Sea; Tuset et al. (2008) reported these ratios as % (OL/TL)= 2.1-2.5 and % (OH/OL)= 42.5-46.3 for three specimens (134, 155 and 177 mm TL) from the Western Mediterranean Sea and the Atlantic Ocean. In the present study, OL/TL ratios were found to be similar to those of Tuset et al. (2008). Başçınar and Atılğan (2016) calculated otolith length and width (height) ratios ( $A_R$ , please see for the Method to Başçınar and Atılğan, 2016) of *E. encrasicolus* ( $n=54$ ) for the Black Sea coast of Ukraine, Rize and Samsun as: 1.69-2.18, 1.82-2.23 and 1.76-2.26. They calculated the equations between otolith height and length:  $y=0.3224x+0.7619$  ( $r^2=0.39$ ) in Ukraine,  $y=0.41x+0.31$  ( $r^2=69$ ) in Rize and  $y=0.3296x+0.5985$  ( $r^2=56$ ), with the linear regression model. According to their results, otoliths in Ukraine are different from the other two regions. Zengin et al. (2015a) were used otolith shape analyses and calculated dimensions of *E. encrasicolus* in the Black Sea ( $n=137$ ) and Marmara Sea ( $n=126$ ). According to their data, they could not find any difference between localities and between the left and right otoliths from the same locality. In addition, they offered that, otolith length was the best index for estimating fish length (please, see Zengin et al. (2015a) for the detailed data). Although Zengin et al. (2015a) noticed that the linear regression model was preferred for the examination of the relationship between fish length and otolith characteristics in most of the studies, in the present study, the exponential regression model gave higher  $r^2$  scores than the linear regression model, especially, for the length-weight relations. Therefore, an exponential regression model was used to explain relations between length-weight variables. Zengin et al. (2015b) also investigated some morphometric and otolith features of *E. encrasicolus* caught in the Black Sea and the Marmara Sea. Their results showed that there are statistical differences between the Black Sea and the Marmara Sea for otolith length and otolith weight.

*Sardina pilchardus* are eaten by *Seriola dumerili* (Matallanas et al., 1995), *Seriola rivoliana* (Barreiros et al., 2003), *Trachurus*

*mediterraneus* (Santic et al., 2003), *Trachurus trachurus* (Cabral and Murta, 2002), *Alosa fallax* (Assis et al., 1992), *Coryphaena hippurus* (Massutí et al., 1998), *Merluccius merluccius* (Cabral and Murta, 2002), *Sarda sarda* (Yoshida, 1980), *Scomber scombrus* (Kyrtatos, 1992), *Thunnus thynnus* (Sanz Brau, 1990), *Lepidorhombus whiffiagonis* (Morte et al., 1999), *Serranus cabrilla* (Labropoulou and Eleftheriou, 1997), *Serranus hepatus* (Labropoulou and Eleftheriou, 1997), *Synodus saurus* (Soares et al., 2003), *Chelidonichthys lucernus* (Morte et al., 1997), *Uranoscopus scaber* (Sanz, 1985), *Xiphias gladius* (Cavaliere, 1963), *Zeus faber* (Silva, 1999), *Oblada melanura* (Pallaoro et al., 2004), *Trisopterus luscus* (Costa, 1988), and *Dicentrarchus labrax* (Costa, 1988). Tuset et al. (2008) reported a % ratio relationship between the length of *S. pilchardus* (138, 175 and 214 mm TL,  $n=3$ ) and sagitta sizes as OL/TL=1.8-2.0 and OH/OL=45.5-48.7; in the present study (67–177 mm TL,  $n=360$ ) these ratios were calculated as OL/TL=1.8-2.0 and OH/OL=42.0-61.6. In the present study, OL/TL ratios were found to be higher than those of Tuset et al. (2008), but again this may be related to the small sample size of Tuset et al. (2008).

OH/TL ratios have larger ranges in the present study than those of Tuset et al. (2008) for both species. The largest specimen examined by Tuset et al. (2008) was larger than the specimens in this study for both species. However, in the present study, the number of specimens examined was higher than those of Tuset (2008). Tuset et al. (2008) described the sagittal otoliths of both species: having an elliptic shape and a funnel like ostium which is longer than the cauda. The cauda is tubular, straight, ending far from the posterior margin in both species. The Sulcus acusticus is heterosulcoid in *E. encrasicolus* but pseudo-archaeulcoid in *S. pilchardus*. The anterior region is peaked in both species, but the rostrum is short, broad, and pointed while the antirostrum is short, broad, peaked or poorly defined in the larger otoliths of *E. encrasicolus*. *S. pilchardus* has a broad, long and pointed rostrum; and it has a larger antirostrum with otolith growth.

Many otolith atlases, such as Härkönen (1986), Smale et al. (1995) or Tuset et al. (2008), were prepared for large geographic areas. Even though they include many fish species, but with fewer sample sizes. This paper supplies information about the TL–OL, TL–OH, TL–OW, W–OL, W–OH, W–OW, OW–OL, OH–OL and OW–OH relationships for *E. encrasicolus* and *S. pilchardus* in the Southern Aegean Sea. According to the data of the present study, otolith length is the best index for estimating fish length ( $r^2>0.93$ ) for both species.

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