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The Relationships Between Otolith Dimensions-Total Length of Chub (*Squalius cephalus,* L.1758) Sampled from Some Inland Waters of the Middle Black Sea Region

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ABSTRACT

In this study, the relationships between otolith dimensions and total length of chub (*Squalius cephalus,* L.1758) sampled from a few inland waters of Black Sea was researched. Chub samples were obtained from the four different localities (Abdal, Akçay, Terme and Yedikır). Power models were applied to estimate the relationships between the otolith measurements and total length (*TL*). Paired t-test, independent t test and ANOVA were done to test the data statistically. The average total length of individuals sampled from Abdal, Akçay and Terme Streams, Yedikır Dam Lake varies between 11.49 ± 0.79 cm, 10.31 ± 0.52 cm, 10.33 ± 0.29 cm and 11.11 ± 0.33 cm, respectively. When all the data were evaluated according to localities, it was found that there was no difference in terms of otolith breadth (*OB*) and otolith length (*OL*) for asteriscus and lapillus otoliths. There were differences in terms of otolith weight of lapillus in localities. There were no differences of asteriscus otolith weight of chub between localities. The relationships between *TL* and *OB, OL* and *OW* were determined using the power regression equation and best fit was obtained between TL and OW for Terme (r^2 =0.936) and Yedikır (r^2 =0.912), *OL* for Akçay and Abdal Streams (r^2=0.973).

Keywords: Chub, otolith morphometrics, total length, population, Black Sea

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Orta Karadeniz Bölgesi'ndeki Bazı Akarsulardan Örneklenen Tatlısu Kefali (*Squalius cephalus,* L. 1758)'nin Otolit Özellikleri-Total Boy Arasındaki İlişkiler

Öz: Bu çalışmada Karadeniz Bölgesi'ndeki bazı içsularda yaşayan tatlısu kefalinin (*Squalius cephalus,* L.1758) total boy ile otolit özellikleri arasındaki ilişkiler araştırılmıştır. Tatlısu kefali örnekleri 4 farklı lokaliteden (Abdal Çayı, Akçay, Terme Çayı ve Yedikır Baraj Gölü) yakalanmıştır. Otolit ölçümleri ile total boy arasındaki ilişkilerin hesaplanması için power model kullanılmıştır. İstatistiksel analizler Paired t-test, Independent t test, ANOVA ile test edilmiştir. Abdal, Akçay, Terme Çayı ve Yedikır Baraj Gölü'nden elde edilen bireylerin ortalama total boyları sırası ile 11,49±0,79 cm, 10,31±0,52 cm, 10,33±0,29 cm ve 11,11±0,33 cm arasında değişmektedir. Lokalitelere göre tüm veriler birlikte değerlendirildiğinde asteriskus ve lapillus otolitlerinin otolit enleri (*OB*) ve otolit boyları (*OL*) arasında bir farklılık bulunmamaktadır. Fakat dört lokalite birlikte değerlendirildiğinde lapillus otolit ağırlıkları arasında fark mevcuttur. Lokalitelere göre asteriskus otolit ağırlıkları bakımından bir farklılık mevcut değildir. *TL* ve *OB, OL* ile *OW* arasındaki ilişkiler power regresyon modeli kullanılarak belirlenmiştir. Terme (r^2 =0,936) ve Yedikır için *OW* (r^2 =0,912), Akçay ve Abdal Çayları için *OL* (r^2 =0,973) değerlerinin *TB* ile en kuvvetli ilişkileri gösterdiği belirlenmiştir.

Anahtar kelimeler: Tatlısu kefali, otolit özellikleri, total boy, populasyon, Karadeniz

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Introduction

Cyprinidae is one of the widespired fish family all over the world (Bănărescu and Coad 1991; Kottelat and Freyhof 2007). *Squalius cephalus* (chub) (L., 1758) is one of the most common freshwater fish species in Cyprinidae. The chub is distributed in the whole of Europe, the Black Sea, the Azov Sea, the Caspian Sea, and Anatolian inland

waters (Bulut et al. 2012). *S. cephalus* is an opportunistic species and common in almost all running waters in Turkey (Bogutskaya 1997). There are 21 species belongs to genus *Squalius* in freshwaters of Turkey (Froese and Pauly 2017). Chub prefers fresh, clean, and fast-flowing waters, as well as dam lakes (Kottelat and Freyhof 2007). *S. cephalus* have fusiform body covered with large-sized cyloid scales and larger mouth. The lips are weakly developed and not keratinized. Ventral and anal fins are orange-yellow color and pigment-free (Polat and Uğurlu 2011). There is a row of black pigments along the free margin of each flank scale and no or very few pigments on central parts of scales (Kottelat and Freyhof 2007). There are lots of studies about genetic, biological characteristic, population parameters and systematics of chub (Altındağ 1996; Laroche et al. 1999; Arlinghaus and Wolter 2003; Vlach et al. 2005; Koç et al. 2007; Turan et al. 2007; Bostancı and Polat 2009; Dehais et al. 2010; Innal 2010; Bulut et al. 2012; Demirol et al. 2016; Cejko and Krejszeff 2016; Gouskov 2016; Kılıç and Becer 2016; Özcan et al. 2017). However, studies about the otolith features of chub are limited (Tarkan et al. 2007; Bostancı 2009).

Otoliths continuously accumulate layers of calcium carbonate and trace elements, creating daily and seasonal records of age (Campana 1999; Hart et al. 2015). Morphological and morphometric characteristics of otoliths comprise an important instrument for species identification (Tuset et al. 2008). The particularity about the fish otoliths was first observed by Aristotle in the third century (Stinton 1975), their taxonomic utility was recognized by Cuvier (Cuvier and Valenciennes 1836). Otolith morphology varies between species, however separete stocks of the same species, often identical physically can sometimes be discriminated through subtle differences in otolith morphometrics (Bolles and Begg 2000; Tuset et al. 2003; Zengin et al. 2015; Ibáñez et al. 2017; Mapp et al. 2017). Futhermore, the relationship between the fish size and otolith dimensions have several benefits in estimating the size of the prey. Fish size and/or weight can be functionally related to an appropriate otolith measurement (width, length, or weight) and the resulting relationships can subsequently be used for size estimation (Nolf 1985; Pierce et al. 1991; Tollit et al. 1997; Granadeiro and Silva 2000). When the relationships between otolith dimensions and total length in a species is determined, the total length or standard length of a fish from its otolith dimensions can be estimated, or vice versa (Sen et al. 2001; Battaglia et al. 2010; Başusta et al. 2013; Yilmaz et al. 2014; Saygin et al. 2017; Yazicioğlu et al. 2017; Zengin et al. 2017).

The aim of this study was to detect the relationships between the total length and otolith characteristics of chub sampled from the four different localities (Abdal Stream, Akçay Stream, Terme Stream and Yedikır Dam Lake) along inland waters of the Middle Black Sea Region. This study is first study that examined the relationships between total length and otolith morphometrics of *S. cephalus* sampled from Abdal, Akçay, Terme Streams and Yedikır Dam Lake.

Materials and Methods

S. cephalus samples obtained from the four different localities (Abdal Stream (n=44), Akçay Stream (n=57), Terme Stream (n=55) and Yedikır Dam Lake n=62) (Figure 1). SAMUS 725 MP shocker were used to capture fish samples. Samples were collected between October 2015-April 2017.

The systematic positions of the samples were determined using various identification keys (Geldiay and Balık 2007; Kottelat and Freyhof 2007; Polat and Uğurlu 2011). All captured fish were measured to the nearest 0.1 cm for total length (*TL*) and weighted to the nearest 0.01 g. The sex was determined by macroscopic examination of the gonads. Utricular (lapillus) and lagenar (asteriscus) otoliths were removed by making left and right distictions. Otoliths were weighted using Precisa precision scales (*OW*) (± 0.0001 g). All otoliths were photographed on the distal side with a Leica DFC295 digital camera. Otolith breadth (*OB*) and length (*OL*) (± 0.001 mm) were determined by Leica Application Suit Ver. 3.8 Imaging Software. OL was defined as the greatest distance between anterior and posterior edges, and OB was defined as the greatest distance from dorsal to ventral edges (Battaglia et al. 2010) (Figure 2).

Linear and nonlinear models were applied to estimate the relationships between the otolith measurements (*OL, OB, OW*) and *TL*.

$y=ab^x$ and $y=a+bx$

where y is otolith measurement and x is fish length (Zar 1999).

However, in evaluating the relationships between *TL* and otoliths dimensions, the power model is preferred because of its higher r^2 values. All data were tested by Kolmogorov-Smirnov if the data is normally distributed (P>0.05). Data were analysed statistically by Paired t-test, Wilcoxon test, Independent Two Sample t test, Mann-Whitney U test and ANOVA-Tukey test. SPSS 20, Minitab 17.0 and the Excel software were utilized in the evaluation of data.

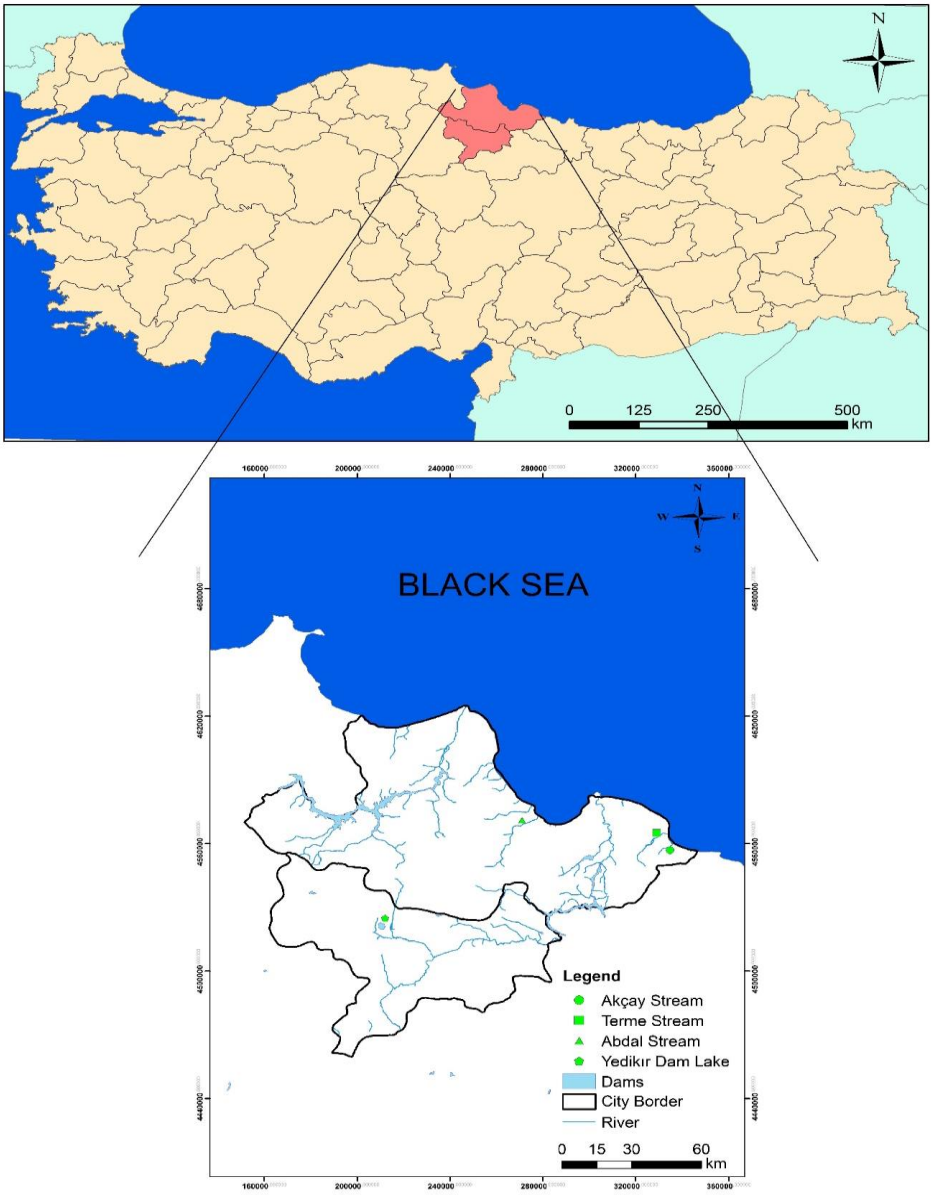

Figure 1. The map of sampling area

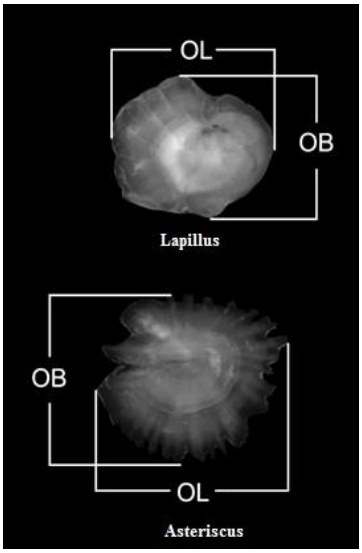

Figure 2. *OB* and *OL* measurements of lapillus and asteriscus otoliths.

Results

The descriptive statistics of chub samples from four locality indicated in Table 1. There is no differences in terms of *TL* or *W* between localities (P>0.05).

When the asteriscus otoliths of female and male were compared there is no difference in terms of *OB, OL* and *OW* in Abdal, Akçay and Terme samples (P>0.05). However, *OL* and *OB* of Yedikır samples were different from each other (P<0.05) for asteriscus otoliths of female and male. In the same way, no difference could be determined *OB, OL* and *OW* for Abdal and Terme whereas the *OL* of Akçay samples and *OL* and *OB* of Yedikir samples were different in the lapillus otoliths between female and male individuals (Table 2).

The differences between otolith parameters between the right and left otolith pairs of asteriscus and lapillus were determined using appropriate tests. Wilcoxon test were used for this comparisons. Right and left asteriscus otoliths were compared there was no significant differences in terms of otolith length, breadth and weight for all localities (P>0.05) (Figure 3). But when right and left lapillus otoliths were compared, there was a statistically significant difference in terms of otolith length of Abdal, Akçay and Terme Streams and otolith breadth and weight of Yedikır Dam Lake Samples (Table 2).

Table 1. Descriptive statistics of *S. cephalus*

Locality	Coordinates	Parameter	N	Mean	S.d	Min.	Max.	S.e.
Abdal Stream	41°08'59.56"N 36°39'34.88" E	Total Length	44	11.49	5.26	5.60	29.40	0.79
		Weight	44	33.59	64.36	1.82	328.1	9.70
Akçay Stream	41°05'30.99"N 37°07'20.89" E	Total Length	57	10.31	3.91	5.80	18.00	0.52
		Weight	57	18.81	19.37	1.79	67.57	2.57
Terme Stream	41°09'34.03" N 36°53'28.48" E	Total Length	55	10.33	2.15	6.40	15.60	0.29
		Weight	55	13.18	8.61	2.39	46.99	1.16
Yedikır Dam Lake	40°47'11.00" N 35°33'47.55" E	Total Length	62	11.11	2.58	6.70	17.70	0.33
		Weight	62	20.18	16.75	3.39	71.30	2.13

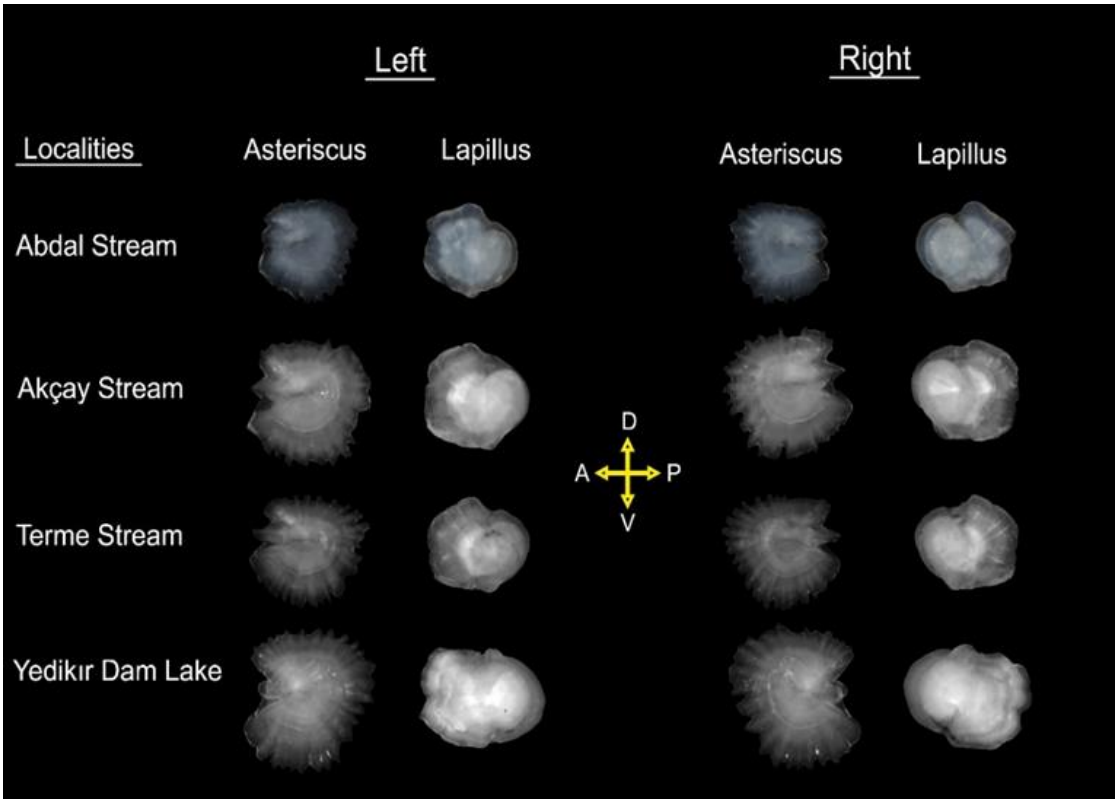

Figure 3. Right and left otolith pairs of *S.cephalus* from different localities (*D*: Dorsal, *V*: Ventral, *P*: Posterior, *A*: Anterior)

Table 2. Statistical comparisons between right and left otolith pairs of asteriscus and lapillus

Localite	Variable	Mean±S.e	Significant
Abdal Stream	Right-left asteriscus otolith length	1.60±0.61	P> 0.05
	Right-left asteriscus otolith breadth	1.49±0.51	P> 0.05
	Right-left asteriscus otolith weight	0.0023±0.002	P> 0.05
	Right-left lapillus otolith length	1.83±0.53	P<0.05*
	Right-left lapillus otolith breadth	1.24±0.041	P> 0.05
	Right-left lapillus otolith weight	0.0036±0.004	P> 0.05
Akçay Stream	Right-left asteriscus otolith length	1.53±0.58	P> 0.05
	Right-left asteriscus otolith breadth	1.43±0.50	P> 0.05
	Right-left asteriscus otolith weight	0.0019±0.002	P> 0.05
	Right-left lapillus otolith length	1.35±0.46	P<0.05*
	Right-left lapillus otolith breadth	1.14±0.46	P> 0.05
	Right-left lapillus otolith weight	0.0030±0.002	P> 0.05
Terme Stream	Right-left asteriscus otolith length	1.44±0.31	P> 0.05
	Right-left asteriscus otolith breadth	1.36±0.28	P> 0.05
	Right-left asteriscus otolith weight	0.0016±0.0008	P> 0.05
	Right-left lapillus otolith length	1.32±0.27	P<0.05*
	Right-left lapillus otolith breadth	1.14±0.14	P> 0.05
	Right-left lapillus otolith weight	0.0023±0.0012	P> 0.05
Yedikır Dam Lake	Right-left asteriscus otolith length	1.55±0.36	P> 0.05
	Right-left asteriscus otolith breadth	1.46±0.32	P> 0.05
	Right-left asteriscus otolith weight	0.0016±0.0001	P> 0.05
	Right-left lapillus otolith length	1.37±0.33	P> 0.05
	Right-left lapillus otolith breadth	1.10±0.26	P<0.05*
	Right-left lapillus otolith weight	0.0022±0.0014	P<0.05*

*Statistically different

Table 3. Equations of relationships between TL and otolith characteristics of *S. cephalus*

Locality	Asteriscus			Lapillus		
	Formula	r^2	P	Formula	r^2	P
Abdal Stream	$OL=0.188TL^{0.882}$	0.973	<0.001	$OL=0.210TL^{0.819}$	0.966	<0.001
	$OB=0.212TL^{0.806}$	0.965	<0.001	$OB=0.184TL^{0.788}$	0.929	<0.001
	$OW=1.238E\text{-}0.05TL^{2.061}$	0.945	<0.001	$OW=1.841E\text{-}0.05TL^{2.078}$	0.948	<0.001
Akçay Stream	$OL=0.157TL^{0.974}$	0.975	<0.001	$OL=0.171TL^{0.887}$	0.955	<0.001
	$OB=0.180TL^{0.893}$	0.971	<0.001	$OB=0.114TL^{0.987}$	0.946	<0.001
	$OW=7E\text{-}0.06TL^{2.279}$	0.940	<0.001	$OW=7E\text{-}0.06TL^{2.409}$	0.963	<0.001
Terme Stream	$OL=0.231TL^{0.784}$	0.609	<0.001	$OL=0.249TL^{0.715}$	0.562	<0.001
	$OB=0.249TL^{0.727}$	0.584	<0.001	$OB=0.166TL^{0.818}$	0.652	<0.001
	$OW=4E\text{-}0.06TL^{2.557}$	0.936	<0.001	$OW=7E\text{-}0.06TL^{2.501}$	0.9222	<0.001
Yedikır Dam Lake	$OL=0.278TL^{0.719}$	0.526	<0.001	$OL=0.216TL^{0.760}$	0.722	<0.001
	$OB=0.237TL^{0.746}$	0.597	<0.001	$OB=0.152TL^{0.823}$	0.843	<0.001
	$OW=6E\text{-}0.06TL^{2.320}$	0.912	<0.001	$OW=9E\text{-}0.06TL^{2.234}$	0.933	<0.001

Discussion

Many studies have been carried out in order to separate the stocks of the same species living in different localities using various methods. In recent years, studies using phenotypic, genetic methods and various bony structures for stock seperations have increased (Renán et al. 2004; Ibáñez et al. 2007; Ramíréz- Pérez et al. 2010; Kohestan-Eskandari et al. 2013; Verma et al. 2014; Renán et al. 2016; Saygin et al. 2017; Ibáñez et al. 2017). The shape and morphometrics of otoliths reflects phenotype and development stage and is

influenced by factors such as sex, body condition, age, year-class, and stock as well as local environmental conditions (Mérigot et al. 2007; Vignon and Morat 2010).

Otoliths are an indirect method for studying fish populations and assessing the relationship between the environment and organisms. Relationships between bony structure dimensions and fish length are commonly used in fisheries science. These mathematical associations enable the back-calculation of fish length in previous ages (Casselman 1990). In addition, otolith studies particularly have a very important place in species identification from the discovery of fossiliferous layers in archaeological sites and prey-predator relations (Tuset et al. 2008).

Chub belongs to Cyprinidae family and prefers fresh, clean, and fast-flowing waters, as well as dam lakes (Kottelat and Freyhof 2007). This fish species were especially prefered for sport fishing. There are some studies about genetics, age, growth, feeding, length-weight relationships and reproduction features of the chub inhabiting Europen and Turkish waters (Turan et al. 2007; Sen and Saygın 2008; Stefanova et al. 2008; Innal 2010; Özuluğ and Freyhof 2011; Cejko and Krejszeff 2016; Özcan et al. 2017). It is a widely distributed fish species in Turkey. Particularly fish length-otolith biometry studies are important evidence that can be used to determine the size distributions of fish consumed by predators. In this study, otolith dimensions and total length relationships of chub form four different localities were investigated. Linear and nonlinear functions are preferred to describe otolith size-fish size relationships (Şen et al. 2001; Morley and Belchier 2002; Waessle et al. 2003; Tarkan et al. 2007; Battaglia et al. 2010; Kumar et al. 2012; Škeljo and Ferri 2012; Basusta et al. 2013; Felix et al. 2013; Yilmaz et al. 2014; Jawad et al. 2017). In this study, nonlinear equation was prefered for total length and otolith dimensions relationships because of higher r^2 values.

Fish size-otolith size relationships will be useful for researchers examining food habits of piscivores and size of fish in archaeological samples (Harvey et al. 2000). Bostanci (2009) was investigated relationships between fork length and otolith charachteristics of chub and found strong relationships, too. In conclusion, when the relationships between fish length and otolith measurements were evaluated, otolith weight for Terme and Yedikır and otolith length for Abdal and Akçay Streams were found to be the best indicator for estimating the length of fish.

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