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Research Article

Light and Scanning Electron Microscopic Observations on *Grillotia erinaceus* (Cestoda: Trypanorhyncha)

Ahmet Özer[1], Türkay Öztürk[1], Sevilay Okkay[1,2], Violetta Yurakhno[3], Julia Kornyychuk[3]

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ABSTRACT

In the present study, the plerocercoids of *Grillotia erinaceus* were obtained from the wall of the anterior oesophagus, stomach, pyloric caeca and liver of teleost Black Sea whiting *Merlangius merlangus* and adults were collected from the intestine of elasmobranch thornback rays *Raja clavata* caught by commercial fishing vessels off Sinop, Turkey. Standard parasitological investigation methods were applied and morphological diagnostic features of the whole parasite, bothria, scolex, tentacular armatures and tentacles were studied in detail using a light and Scanning Electron Microscope (SEM). The plerocercoids of this parasite had a total length of 5.96 mm on average and this was 21.6 mm on average in adults. The measurement data of all morphological diagnostics are provided and photomicrographs of each part of the parasite are presented. This study also provides the detailed morphological features of both plerocercoids and adults of *G. erinaceus* in *M. merlangus* and *R. clavata* for the first time in the Turkish coasts of the Black Sea.

Keywords: *Grillotia erinaceus*, Merlangius merlangus, raja clavata, black sea

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INTRODUCTION

Metazoan trypanorhynch cestodes, including the members of the genus *Grillotia* with approximately 277 valid species, are common parasites of marine fish and while sharks and rays host the adults, a wide variety of marine invertebrates and teleosts are infected by larval forms with low host specificity and a wide zoogeographical, or even cosmopolitan, distribution (Palm, 2004; Palm & Klimpel, 2007; Palm & Caira, 2008; Palm, Waeschenbach, Olson, & Littlewood, 2009). The cestode order Trypanorhyncha Diesing, 1863 is characterized by a scolex bearing 2 or 4 bothria and a tentacular apparatus, consisting of four retractile tentacles adorned with hooks as extensions of tentacle sheaths that are attached to four bulbs (Dollfus, 1942; Jones, Beveridge, Campbell, & Palm, 2004). These cestode parasites have a unique and complex attachment apparatus enabling their attachment and movement in the host organs through antagonistic bulbs and retractor muscles with the ability of invagination and retraction of them (Palm, Waeschenbach, Olson, & Littlewood, 2009). Larval and adult trypanorhynch cestodes have the same scolex morphology which makes accurate taxonomic diagnosis (Palm, 2004).

Grillotia plerocercoids are easily visible due to their white spherical or ovoid cysts and occur attached to the serosal surface or embedded in the wall of the oesophagus, stomach, pyloric caeca or intestine of their host fishes (Lubieniecki, 1976). Gadoid fishes are involved as the second intermediate hosts (Lubieniecki, 1976) and *Merlangius merlangus* has been reported to be the host of *Grilloia erinaceus* plerocecoids (Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno, 2012; Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno, 2014; Tepe, Oğuz, &

Heckmann, 2014). On the other hand, the adult trypanorhynchs including *G. erinaceus* are found in the spiral intestine of sharks and rays (elasmobranchs) (Palm, Yulianto, & Piatkowski, 2017). This species was reported as adults from 24 elasmobranch species and as pleurocercoids from 62 teleost fish species (see Menoret & Ivanov, 2012). Deardorff, Raybourne, & Mattis (1984) reported a decrease in the commercial value of affected stock caused by metacestodes (postlarvae and plerocerci) in the musculature of fishes.

The aim of the present study is to provide light and ultrastructural observations of a trypanorhynch plerocercoid, *Grillotia erinaceus* in whiting, *Merlangius merlangus* and adults from thornback rays *Raja clavata* collected from the Sinop coasts of the Black Sea, thus providing the first detailed observations on this species in Turkey.

MATERIAL AND METHOD

Specimens of *Grillotia erinaceus* were obtained from the wall of the anterior oesophagus, stomach and pyloric caeca of teleost Black Sea whiting *Merlangius merlangus* and from the intestine of elasmobranch thornback rays *Raja clavata* caught by commercial fishing vessels in the Black Sea off Sinop (N 42° 05' 68" E 35° 10' 55") in the period between May 2011 and April 2014. These fish were then examined for cestode parasites using standard methods. Cestode worms obtained from the mesenteries and stomach wall of whiting were either studied fresh or fixed in 10% formalin for morphological observations; subsequently, the formalin was replaced by 70% ethanol, then the tentacles of several worms were detached from scolexes and mounted in glycerine jelly (Chubb, Pool, & Veltkamp, 1987). Photographs of the mounted specimens and detached tentacles were taken using an Olympus BH2 microscope attached with a DP25 digital camera operated with digital imaging software. The measurements are in millimetres (mm), as is the range followed in parentheses by the mean. For SEM imaging, the worms were dehydrated in a graded ethanol series, placed in hexamethyldisilazane and allowed to dry (Shively & Miller, 2009). They were mounted on stubs and coated with gold and then SEM micrographs were taken using a Jeol JSM-6510LV scanning electron microscope at an accelerating voltage of 10kV. The terminology for the morphological characteristics and their measurements of trypanorhynchs follows Beveridge & Campbell (2007).

RESULTS AND DISCUSSION

Grillotia erinaceus plerocercoids were found in the form of white ovoid blastocysts approximately 6 mm long and were easily visible and occur attached to the serosal surface or embedded in the wall of the anterior oesophagus, stomach, pyloric caeca and liver of host fish *M. merlangus* (Figure 1). Free and encapsulated plerocercoids of *G. erinaceus* with a general view of scolex and bothria with 4 tentacles, external, internal and antibothridial surfaces of tentacular armatures as well as profiles of hooks on each tentacle are provided in Figure 2A-U.

Specimens of pregravid to mature *G. erinaceus* were found in the spiral intestine of the thornback rays *Raja clavata*. Adult worms were 21.6 mm long on average and the number of acraspedote proglottids was up to 32 per worm (Figure 3A). The Scolex were elongated and 6.2. mm long and 1.0 mm wide on average (Figure 3B). Tentacles elongated without basal swelling, measuring about 80 µm in diameter without hooks at base, about 60 µm without hooks in the metabasal region, the hook arrangements on tentacular apparatus are given in Figure 3C-E. All measurement data are provided in Table 1.

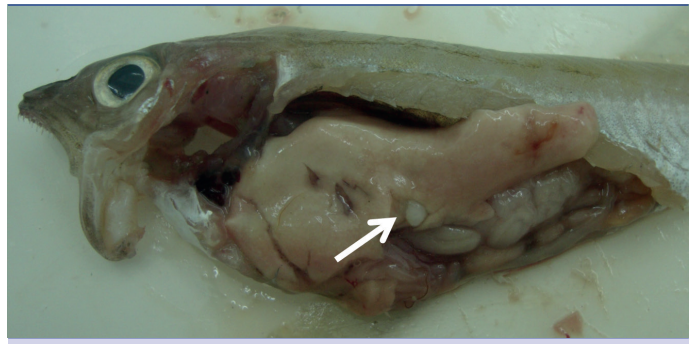

Figure 1. Whitish ovoid approximately 6mm diameter blastocyst (arrowed) attached to the wall of the *Merlangius merlangus* liver.

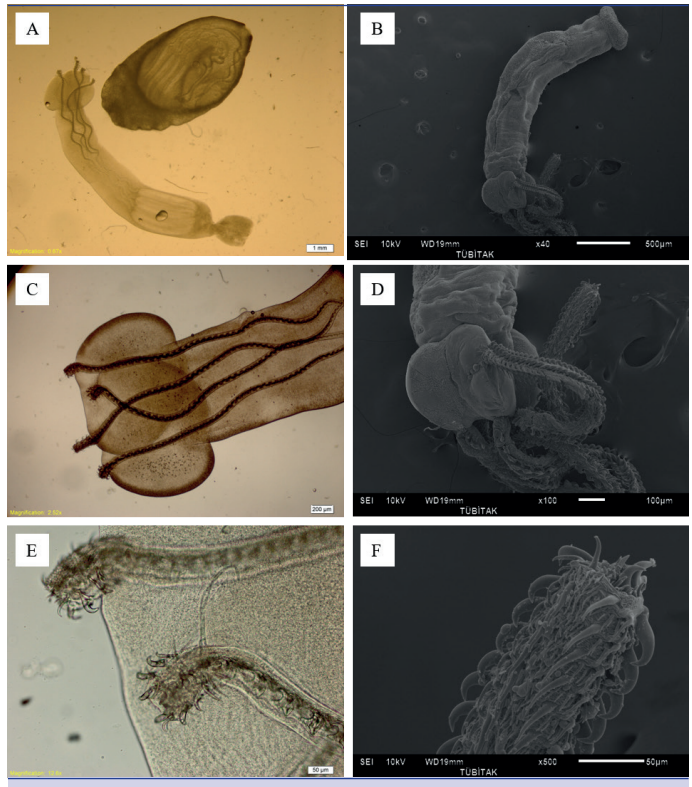

Figure 2. **A)** Free and encapsulated plerocercoids in *M. merlangus*, **B)** A general view of the scolex in SEM, **C)** Bothria with 4 tentacles, **D)** Bothria with fully exerted tentacles, **E)** A closer look at the tentacles, some parts of hooks outside the tube at tip and rest still inside, **F)** SEM observation of some exerted hooks at the tip of a tentacle.

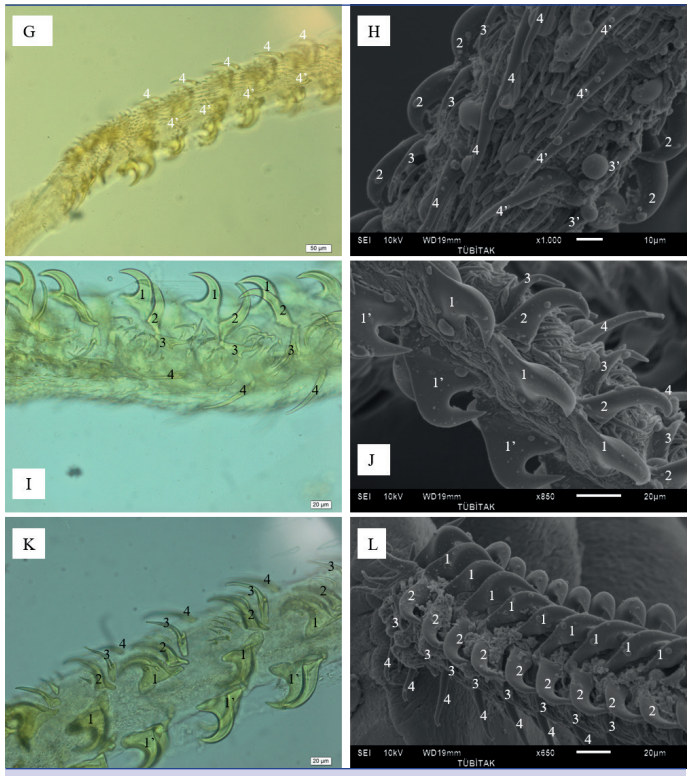

Figure 2. **G)** External surface, **H)** SEM observation of external surface, **I)** Antibothridial surface, **J)** SEM observation of the antibothridial surface and internal surface on the left hand side, **K)** Internal surface, **L)** SEM observation of internal surface.

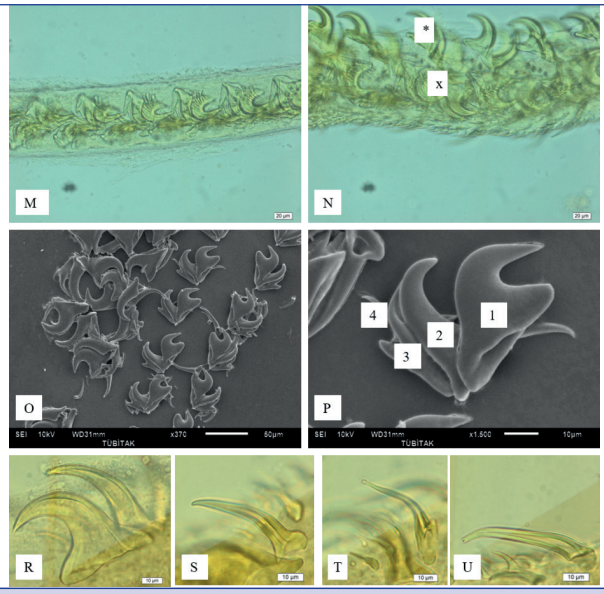

Figure 2. **M)** Hooks inside tentacle tube. **N)** Some parts of hooks outside (*) and some inside (x) the tentacle tube. **O)** SEM observation of hooks arrangements. **P)** Closer look at hook profiles 1-4 (SEM). **R)** Profile of hook 1. **S)** Profile of hook 2. **T)** Profile of hook 3. **U)** Profile of hook 4.

Grillotia erinaceus is one of the well-known species of the genus and the description here was made according to the morphometric data and figures presented by Lubieniecki (1976), Kornyushin & Solonchenko (1978), Beveridge & Campbell (2007). *Grillotia erinaceus* plerocercoids were found in the form of white spherical or ovoid blastocysts approximately 6 mm long and occurred attached to the serosal surface or embedded in the wall of the oesophagus, stomach, and pyloric caeca as was reported by Lubieniecki (1976) and Brickle, MacKenzie, & Pike (2006). This species was described initially from species of *Raja* Linnaeus, 1758 from the coast of Belgium but was subsequently reported from various species of rays on both sides of the north Atlantic (Dollfus, 1942) and it was first reported with only morphometric data by Kornyushin & Solonchenko (1978) in a cartilaginous fish *Raja clavata* in the Black Sea. Later, Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno (2014) provided detailed information about its seasonal and host related occurrence at two southern and northern locali-

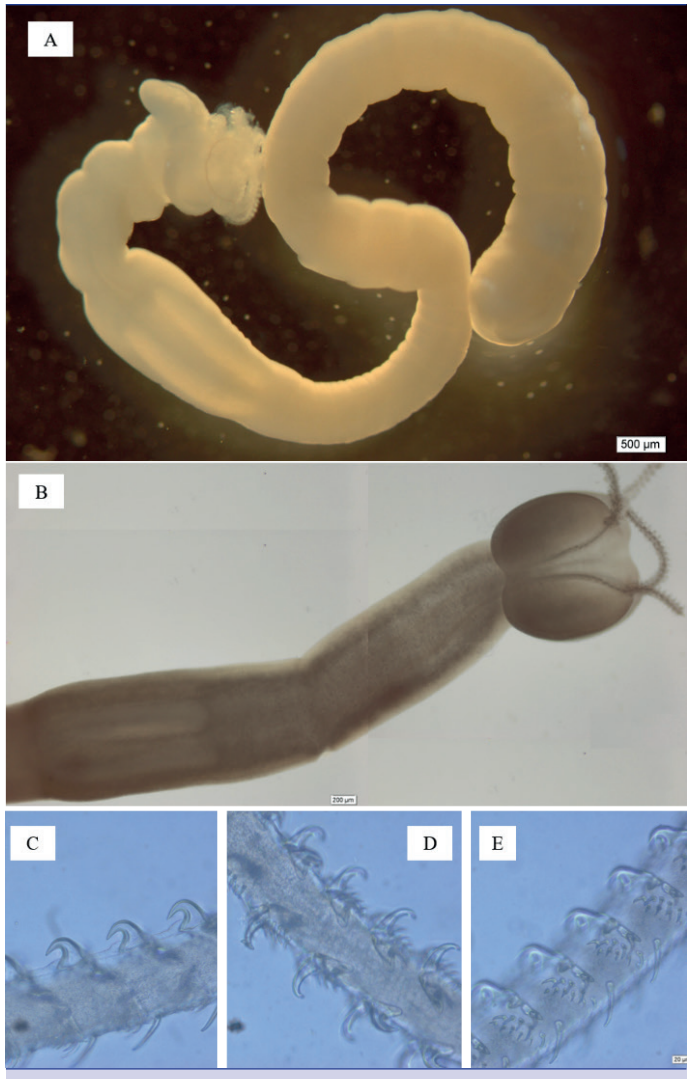

Figure 3. **A)** Mature individual of *Grillotia erinaceus* from *Raja clavata*, **B)** Scolex with four tentacular tubes and bulbs of mature cestode, **C)** Profile hook 1 tentacular tube, **D)** Profile of hook 2, **E)** Profile of hook 3 and 4.

Table 1. Measurements (mm) of *Grillotia erinaceus* (van Beneden, 1858) provided by different authors.

Measurements	Beveridge and Campbell (2007)	Kornyushin and Solonchenko (1977)	This study	This study
Host	*Raja clavata*	*Raja clavata*	*Raja clavata*	*M. merlangus*
	n=10	-	n=10	n=10
Stage	Gravid - mature	Gravid-mature	Mature	Plerocercus
Length of scolex	2.56-4.32 (3.50)	3.5-7.0	4.55-7.21 (5.96)	9.50-11.45 (10.93)
Width of scolex	0.39-0.83 (0.60)	-	1.11-1.55 (1.35)	1.90-3.15 (2.69)
Pars bothrialis	0.44-0.73 (0.60)	0.65-1.20	0.73-0.88 (0.81)	1.80-2.45 (2.18)
Pars vaginalis	1.46-2.74 (2.11)	1.30-3.70	2.22-3.34 (2.98)	5.92-7.84 (6.87)
Bulb length	0.94-1.63 (1.34)	0.90-2.00 (1.50)	0.88-1.61 (1.16)	3.18-4.35 (3.87)
Bulb width	0.16-0.25 (0.18)	0.18-0.37 (0.25)	0.18-0.23 (0.21)	0.40-0.62 (0.50)
Pars post-bulbosa	0-0.37 (0.13)	-	0.72-1.38 (1.01)	1.10-1.93 (1.56)
Hook 1 (length)	42-68 (57)	45-50	32-38 (34.65)	36.5-49.15 (43.3)
Hook 1 (base)	34-53 (45)	40-42	40-48 (42.00)	36.0-49.10 (45.3)
Hook 2 (length)	46-67 (55)	45-50	28-32 (30.50)	28.5-36.2 (33.2)
Hook 2 (base)	21-29 (26)	26	19.5-20.5 (20.05)	25.7-33.1 (28.0)
Hook 3 (length)	49-61 (54)	60	37-41 (38.70)	38.6-49-4 (43.4)
Hook 3 (base)	10-14 (13)	-	6.9-7.5 (7.06)	18.2-24.2 (21.7)
Hook 4 (length)	49-61 (54)	60	37-40 (38.10)	39.5-46.2 (43.4)
Hook 4 (base)	10-14 (13)	-	10-12 (10.55)	9.10-12.3 (10.8)

ties in the Black Sea. General features of the parasite are all in accordance with Kornyushin & Solonchenko (1978) and Beveridge & Campbell (2007) with some differences in measurement data of several parts of the scolex and tentaculate armatures as a result of possibly different environmental and host factors (Table 1).

CONCLUSION

In the present study, we provided the first comprehensive data on both the light and ultrastructural observations of *Grillotia erinaceus* plerocercoids infecting the Black Sea whiting, *Merlangius merlangus*, and adults infecting the thornback ray *R. clavata* off the Turkish coasts of the Black Sea. All the illustrations and morphometric data presented here make further contributions to our current knowledge and will also provide a base for further studies.

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Ethics Committee Approval: This study was carried out in accordance with animal welfare and trial ethics. All procedures were performed in accordance with the Law on Veterinary and Medical Activities and National Animal Welfare Act.

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Conflict of interest: The authors declare that they have no conflicts of interest.

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Research Article

Relationships between Fish Sizes and Otolith Sizes of Whiting (*Merlangius merlangus* Linnaeus, 1758) from the Western Black Sea

Taner Yıldız[1]

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ABSTRACT

The objective of this study was to determine the regressions between otolith sizes and shape indices vs. fish length, and weight of whiting, *Merlangius merlangus* (Linnaeus, 1758), from the Black Sea. Samples were collected randomly from commercial bottom trawlers between November 2017 and January 2018 in the western Black Sea. No differences were found in otolith size and indices by means of otolith position while a distinct difference by sexes was detected. Strong relations with high descriptive coefficients were found between otolith sizes and weight and fish length and weight. However, the regression relationships between otolith shape indices and fish length and weight were defined as very weak. As a conclusion, it can be emphasized that the otolith sizes and weight of whiting can be used for the determination of the size and weight of the fish.

Keywords: Otolith dimensions, fish size, whiting, Black Sea

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INTRODUCTION

Whiting has a wide distribution including the Black Sea, the Azov Sea, the Marmara, the Aegean Sea and the Adriatic Sea (Whitehead et al., 1986). Whiting, one of the two members of the Gadidae family distributed in the Black Sea, is one of the most important target species in the Black Sea bottom trawl fishery.

In the Black Sea, whiting is quite dominant in terms of catch composition of demersal species (Genç et al., 2002). It is caught by bottom gillnets as well as bottom trawl nets in the central and eastern part of the Black Sea. Due to the high commercial value and the traditional consumption behavior of the public, the market is always in high demand.

In all fish except sharks, stingrays, and lampreys (Campana, 2004) on both sides of the head, behind the eyes, adjacent to the brain, in the channels of the inner ear (Smale et al., 1995) otoliths, small and white structures (Campana, 2004) are formed as a result of regular accumulation of calcium carbonate crystals during the life of the fish (Furlani et al., 2007). Annual growth rings in otoliths during fish growth are similar to age occurrences in trees (Casselman, 1983). As the fish grow, the otoliths continue to grow, and there is always a strong relationship between otolith size and fish size (Hunt, 1992). Studies on this relationship have increased in different aspects of fish and fisheries biology studies in recent years. Otoliths vary in size and shape from one fish group to another. It is even characteristic for the genus and species of fish (Demir, 1965). Otolith morphology is used in studies in many different areas for fish biology; anatomy of fish species, identification of new fish species, taxonomic revisions of fish taxons, determination of phylogenetic relationships, studies of eco-morphology, determination of similarities between fish growth and otolith growth (Campana, 1999; Bostancı et al., 2012). The relationship between fish size and otolith size has been utilized to calculate the size or age of prey obtained from the

stomach content of several fishes (Pitcher, 1980; Bailey & Ainley, 1982; Jobling & Breiby, 1986; Granadeiro & Silva, 2000; Javor et al. 2011). In addition, otolith shape can be described in many ways, one of the simplest being manual distance measurement. Such measurements can be used in a series of mathematical equations that calculate shape indices (Burke et al., 2008)

Due to ecological and economic importance of the Black Sea whiting, although there have been many studies on the distribution and biomass (Çiloğlu et al., 2001; Genç et al., 2002; Gönener & Bilgin, 2006; Gönener & Bilgin, 2010), population parameters (Düzgüneş & Karaçam, 1990; Samsun et al., 1994; İşmen, 2002; Özdemir et al., 2006), age and growth (Polat & Gümüş, 1996; Yildiz and Karakulak, 2019), reproduction biology (Reşat, 2013; Mazlum & Bilgin, 2014), feeding regime and diet (Samsun et al., 2011; Mazlum & Bilgin, 2014) and length-weight relationship (Kalaycı et al., 2007; Ak et al., 2009; Van et al., 2019; Yıldız et al., 2018) no publication has been found on the relationship between otolith sizes and fish sizes. However, only one study revealed the otolith asymmetry levels of whiting in the Middle Black Sea (Kontaş et al., 2018). In the light of the above-mentioned motivations, the aim of this study was to determine the relationship between the length and weight of whiting and various dimensions of otolith.

MATERIAL AND METHOD

The whiting samples used in the study were randomly sampled from the bottom trawler vessels engaged in commercial fishing in the western Black Sea between November 2017 and January 2018. Total length, total weight, and sex of each individual were recorded in the laboratory. Sex determination was made macroscopically using color and structural differences in the gonads. The significance of sex-related difference in length distribution between male and female individuals was checked with the Kolmogorov-Smirnov test (α=0.05). 260 sagittal otoliths from 130 whiting specimens measured by biometric measurements were removed and fixed in a dry manner. Images of otoliths were recorded using a Leica DC 500 camera system connected to a Leica S8 APO stereo microscope and image analysis program (Leica Application Suite Version 4.3.0). Morphometric measurements such as length (OL), width (OW), perimeter (OP) and area (OA) of the otoliths were performed on these images (Figure 1). Otolith area (OA) was automatically calculated using the Leica Application Suite. Using these measurements, the otolith shape indices were calculated using the formulas given in Table 1 (Tuset et al. 2003). The right and left otoliths were weighed separately on a digital balance (Kern ABJ) with a precision of ±0.0001 g, and the otolith weights (OWE) were recorded.

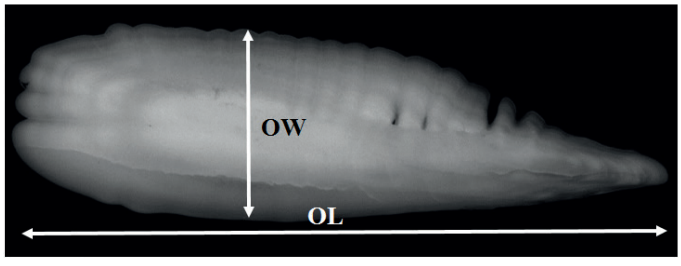

Figure 1. Whiting otolith and two basic morphometric measurements.

Descriptive statistics (mean, minimum and maximum values and standard deviation) of fish length and weight, otolith dimensions and shape indices were calculated. The dimensions of the otolith were correlated with the length and weight of the fish using linear and non-linear (exponential) regression analyses. The relationships between the otolith dimensions were determined using regression analysis (Zar, 2010). The results of regression analysis, relationship types obtained, equation constants and descriptive coefficients showing the strength of the relationship were calculated. The significance of the difference in otolith size and shape indices depending on otolith position (right-left) and sex (male-female) was tested using Multivariate Analysis of Variance (MANOVA). Before the analysis, the Levene test was applied for the assumption of homogeneity of the variances. The non-homogeneous data were adapted to the homogeneous distribution with the $\log_{x+1}$ converter. All statistical tests were performed using R Programming (R Development Core Team, 2018).

Table 1. The formulas of otolith shape indices.

Otolith shape indices	Formula
Circularity	OP/OA^2
Rectangularity	OA/(OL×OW)
Form factor	$(4OA)/OP^2$
Roundness	$(4OA)/(OL^2)$
Ellipticity	(OL−OW)/(OL+OW)
Aspect Ratio	OL/OW

RESULTS

Descriptive statistics of total length and weight values of male (n=60) and female (n=70) individuals are given in Table 2. While the average length was 17.5 cm for female and 13.4 cm for male, the mean weight was 44.37 g for females and 21.55 g for males (Table 2; Figure 2). The difference between the length-frequency distributions of the sexes was found significant ($p<0.05$).

According to the results of the MANOVA test, the difference in morphometric values due to otolith position (right-left) was not statistically significant ($p>0.05$), but it was found to be significant due to sex (male-female) ($p<0.05$). For this reason, regression relationships between fish length and weight and otolith dimensions (OL, OW, OA, OWE, OP) were calculated separately for sexes by combining the right-left otolith values with 260 otoliths (both right and left together). Considering the descriptive statistics of the otolith dimensions, it is seen that the mean values of the female individuals are greater than the males in all otolith sizes (Table 3).

Table 2. Descriptive statistics of fish length and weight by sex.

	TL		TW	
	♀	♂	♀	♂
Min	10.7	8.7	9.12	3.74
Max	22.9	21.1	95.54	75.49
Mean	17.5	13.4	44.37	21.55
SD	±2.53	±3.18	±18.49	±15.22

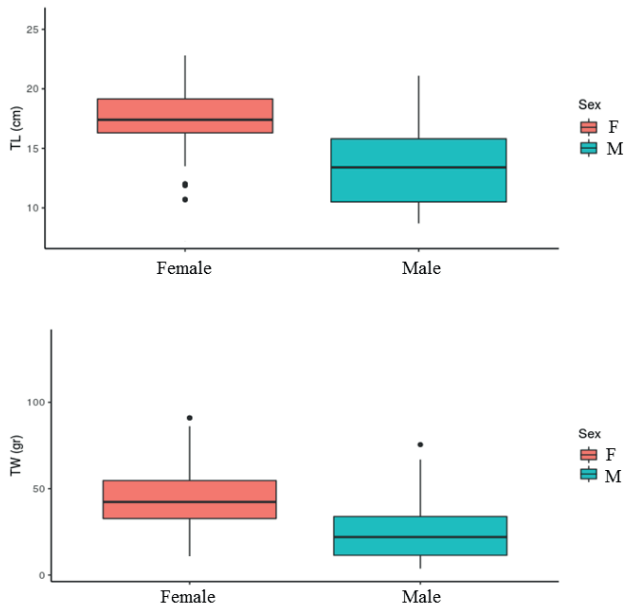

Figure 2. Boxplot of the total length (upper) and weight (lower) distributions by sex.

Table 3. Descriptive statistics of otolith morphometric values by sex.

	OL	OW	OA	OWE	OP
			♀		
Min	5.517	1.887	7.443	0.0085	12.395
Max	12.894	4.137	38.108	0.0763	30.867
Mean	9.061	2.842	19.136	0.0325	20.426
SD	±1.431	±0.364	±5.321	±0.0128	±3.235
			♂		
Min	3.816	1.366	3.925	0.0032	8.685
Max	10.787	3.514	26.08	0.0481	24.185
Mean	6.937	2.279	12.021	0.0173	15.695
SD	±1.715	±0.493	±5.288	±0.0101	±3.899

Descriptive statistics of the otolith shape indices calculated on the basis of the measured dimensions of the otoliths are given in Table 4 for the sexes separately. According to the MANOVA results, the difference due to otolith position (right-left) is not statistically significant ($p>0.05$). The values of shape indices calculated for the sexes were also very close to each other and no statistical difference was observed ($p>0.05$).

As a result of regression analysis used to determine the relationship models between otolith dimensions with fish length and weight; the relationship types obtained (linear (L) or exponential (E)), equation constants (*a* and *b*) and the coefficient of determination (R^2) indicating the strength of the relationship are shown in Table 5. In both sexes, regressions between total length and weight with otolith morphometry were defined with a high coefficient of determinations. As it can be seen from the table, the descriptive coefficient of regression relations in male individuals was always higher than female individuals. While the relationships between male individuals are completely linear, the relationships between fish length and otolith dimensions in female individuals are mostly exponential.

The results of the regression analysis used to determine the equations between the otolith dimensions in the explanation of the otolith morphometry are shown in Table 5. Except for five relationships, regression relationships were defined linearly. The highest relationship was found between the otolith length and otolith perimeter for males.

The regression relationships between the shape indices and the fish length resulted in descriptive coefficients that were too low to be correlated with the fish length. Otolithic rectangularity does not show a significant relationship with fish length ($p>0.05$; $R^2=0.01$).

DISCUSSION

In the otolith atlas of Tuset et al. (2008), the whiting otolith was described as; Shape: lanceoated, anterior region more globose than the posterior, margins lobed in the smaller otoliths. Sulcus acusticus: heterosulcoid, pseudo-ostiocaudal, median. Ostium: elliptic, broad, shorter than the cauda. Cauda: tubular, straight, as wide as the ostium, separated from the ostium by a solid bridge-like collum. Anterior region: round to irregular. Posterior region: sharply lanceolated. In this study, the whiting otoliths

Table 4. Descriptive statistics of otolith shape indices by sex.

	Circularity	Rectangularity	Aspect ratio	Roundness	Form factor	Ellipticity
Value			♀			
Min	18.87	0.63	2.61	0.25	0.40	0.45
Max	31.22	0.80	3.63	0.38	0.67	0.57
Mean	22.23	0.73	3.18	0.29	0.57	0.52
SD	±1.75	±0.02	±0.21	±0.02	±0.04	±0.02
			♂			
Min	18.62	0.67	2.60	0.26	0.37	0.44
Max	30.71	0.78	3.57	0.36	0.67	0.56
Mean	21.37	0.73	3.02	0.31	0.59	0.50
SD	±1.58	±0.02	±0.19	±0.02	±0.04	±0.02

Table 5. Regression relationship parameters and descriptive coefficients between fish length and weight with otolith dimensions and among otolith dimensions by sex (E: exponential, L: linear).

	♀				♂			
Variables	***a***	***b***	***R²***	**Regression type**	***a***	***b***	***R²***	**Regression type**
TL-OL	3.3712	0.55	0.8529	E	0.5209	-0.074	0.9334	L
TL-OW	0.1275	0.6011	0.8597	L	0.1489	0.2753	0.9208	L
TL-OA	3.1461	0.1005	0.9018	E	1.6213	-9.8026	0.9519	L
TL-OWE	0.0026	0.1398	0.9047	E	0.0031	-0.0245	0.9419	L
TL-OP	7.5228	0.0561	0.8867	E	1.1768	-0.1452	0.9221	L
TW-OL	0.0619	6.2887	0.7634	L	0.1025	4.7278	0.8271	L
TW-OW	0.0163	2.1136	0.8140	L	0.0293	1.647	0.8174	L
TW-OA	0.2438	8.2102	0.8584	L	0.3274	4.966	0.8876	L
TW-OWE	0.0006	0.0063	0.8584	L	0.0006	0.0037	0.8977	L
TW-OP	0.1439	13.978	0.8087	L	0.2318	10.7	0.8181	L
OL-OW	0.2318	0.7421	0.8287	L	0.2803	0.3346	0.9489	L
OL-OA	3.5945	-13.434	0.9354	L	1.5853	0.2766	0.9698	E
OL-OWE	0.0029	0.260	0.9123	E	0.001	0.389	0.9577	E
OL-OP	2.2229	0.284	0.9677	L	2.2551	0.0504	0.9843	L
OW-OA	14.151	-21.091	0.9401	L	10.556	-12.035	0.971	L
OW-OWE	0.0018	1.0016	0.878	E	0.0007	1.3382	0.938	E
OW-OP	8.1976	-2.8764	0.8533	L	7.6539	-1.7479	0.9387	L
OA-OWE	0.0023	-0.0118	0.9272	L	0.0019	-0.0054	0.9679	L
OA-OP	0.591	9.1176	0.9446	L	0.7217	7.02	0.9577	L
OWE-OP	242.44	12.526	0.9234	L	367.9	9.319	0.9231	L

(TL: Fish length, TW: Fish weight, OL: Otolith length, OW: Otolith width, OP: Otolith perimeter, OA: Otolith area, OWE: Otolith weight)

sampled in the western Black Sea have similar characteristics. Hehir (2003) stated that whiting otoliths have a thinner and flatter structure than other gadoid otoliths. Atılgan et al. (2010) also reported that the whiting otoliths have a relatively large and thickened structure compared to the body size. In this study, although the thickness of the whiting otoliths was not measured, a thickening was observed in the central part of the large otoliths.

Tuset et al. (2008) stated that the average otolith width of whiting is between 33.8% and 35.6% of the average otolith length. In this study, it was calculated that the ratio of average otolith width to average otolith length was between 27.5% and 38.5%. Tuset et al. (2008) reported that the circularity is between 19.4 and 24.0 and the rectangularity is 0.5. In this study, the circularity was calculated between 18.6 and 31.2 and the rectangularity was calculated between 0.66 and 0.79. Unfortunately, Tuset et al. (2008) did not give the length values of the specimens so a comparison of otolithic properties cannot be made.

When the right and left region otolith pairs are examined in terms of otolith dimensions, the absence of a statistically significant difference shows that otoliths can be used without distinguishing them from each other and that the choice of right or left otoliths can be made. For this reason, it can be said that the otolith morphometry studies with whiting can be evaluated without considering right-left otolith differences.

However, the difference between male and female individuals in otolith sizes is significantly different. It has been emphasized in many studies that female individuals in the whiting population always have bigger lengths than males (İşmen, 1995; Yıldız & Karakulak 2019). Otolith dimensions are related to fish length, as fish length increases, so do otolith dimensions. Otolith sizes are also larger than males because female individuals always reach big lengths. In addition, it has been emphasized in many studies that the growth in male and female individuals is different, that females always reach higher asymptomatic length (L_{inf}) and that there is an increase in the proportion of female individuals parallel to the increase in age and length in the population (İşmen, 1995; Çiloğlu, 1997; Samsun, 2005; Yıldız & Karakulak, 2019).

According to the results of the regression analysis, there is a strong relationship between fish size and otolith dimensions of whiting. However, this relationship is exponential, not linear, especially in female individuals. In other words, it can be said that the linear relationship between otolith dimensions and total length is disrupted at a certain point in the life cycle of female individuals. This may be due to thickening of the whiting otoliths in the central region in older ages. Mineral accumulation in otoliths occurs more in the width of otoliths than otolith length. There is a strong relationship between fish weight and otolith dimensions. In contrast to fish size, these relationships are defined linearly in males and females. Researchers, working with organisms that

feed on fish such as predator fish and marine mammals, try to determine the prey composition of the species they work in, using the shape and size of undigested otoliths in the stomach contents of these species (Campana, 2004). Owing to these studies, it is possible to understand the food chain in the sea by using otoliths (Smale et al., 1995). Moreover, by using the fish length-otolith size relationships, the prey size can be estimated from the otolith length obtained from the stomach contents. According to the results of this study, whiting otolith dimensions and weight can be used to determine fish length and weight in future studies.

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Ethics committee approval: This study was performed in accordance with ethical standards of animal experiments.

Conflict of Interests: The author declares that there are no conflicts of interest.

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Research Article

Some Growth Parameters of Five Fish Species in the Lower Sakarya River, Turkey

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ABSTRACT

In this study, a total of 1283 samples of five fish species belonging to two families, Cyprinidae and Leuciscidae, were collected from the Lower Sakarya River between June 2017 and May 2018 in order to determine some growth parameters.The samples were collected monthly with trammel net, fykenets, and electro shocker. The age of the fish was determined from the scales. The von Bertalanffy's growth model was calculated Lt = 92.18(1-e$^{-0.054(t+0.040)}$) for *A. brama*, Lt = 69.40(1-e$^{-0.040(t+0.030)}$) for *B. bjoerkna*, Lt = 51.09(1-e$^{-0.114(t+0.024)}$) for *C. gibelio*, Lt = 48.11(1-e$^{-0.088(t+0.023)}$) for *R. rutilus* and Lt = 41.74(1-e$^{-0.104(t+0.035)}$) for *V. vimba*. The phi-prime growth performance index (Φ') value was computed as 2.628, 2.268, 2.474, 2.307 and 2.260 for *A. brama, B. bjoerkna, C. gibelio, R. rutilus* and *V. vimba*, respectively. This study provides basic information on some growth parameters of five fish species living in the Lower Sakarya River. The results of this study are useful for fishery managements and stock assessment in the Sakarya River.

Keywords: Age and growth, cyprinidae, leuciscidae, fishery management, Sakarya River

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INTRODUCTION

Rivers and natural lakes are important ecosystems of our world and cover approximately 2.5% of the earth's surface (Shiklomanov, 1999). Turkey has important freshwater resources and one of these freshwater resources is the Sakarya River. The Sakarya River basin (58000 km^2), one of the majör rivers pouring into the Black Sea, covers approximately 7 % of Turkey's surface area (783000 km^2). Its average flow rate is about 190 m^3 per second. The water temperature changes between 7 to 24 °C through out the year. The river basin is divided into three regions named Lower, Mid and Upper Basin (Şengörür & İsa, 2001).

Cyprinidae is found in North America (from northern Canada to southern Mexico), Africa, and Eurasia. Cyprinidae is the largest family of freshwater fish with 346 genera and 3,170 species in the world. Leuciscidae is the other important freshwater fish with 90 genera and 672 species (Eschmeyer, Fricke & van der Laan, 2017). Various researches have been carried out on the fish species living in the Sakarya River and its tributaries. (Ölmez, 1992; Emiroğlu, 2011; Kahraman, Göktürk & Aydın, 2014; Korkmaz & Zencir Tanır, 2016; Memiş, Tosun, Yamaner, Tunçelli & Gessner, 2019; Reis, Cerim & Ateş, 2019).

Age and growth are related with each other. Age gives a knowledge about sexual maturity, spawning period, fish size, growth rate and lifespan. Knowledge of all these parameters are important data for fisheries management and vary among populations. Accurate age determination and estimates of growth parameters are fundamental requirements for understanding population dynamics and maintaining sustainable yields in fisheries biology (Campana & Thorrold, 2001).

In this study, some growth parameters were determined for *Abramis brama* (Linnaeus, 1758),

Blicca bjoerkna (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758) and *Vimba vimba* (Linnaeus, 1758) that were caught in the lower Sakarya River. These datas contribute to the sustainable management of the Sakarya River fisheries.

MATERIALS AND METHODS

This study was carried out between June 2017 and May 2018 in the 159.5 km section of the Sakarya River within the borders of Sakarya province. The aforementioned section includes Mekece in the south of Pamukova and Karasu Yenimahalle, where it deposits into the Black Sea.

The samples were collected monthly with trammel nets (inner panel: 52-72-88 mm, outer panel: 300 mm; stretched mesh sized), fyke net (140 mm stretched mesh sized, 5 m leader net) and electro shocker (SAMUS 1000; 500W) from the three stations (Pamukova, Adapazarı and Karasu) identified in the lower Sakarya River Basin (Figure 1). The sampling areas were sandy-muddy substrates and depths were between 1.5-10 meters.

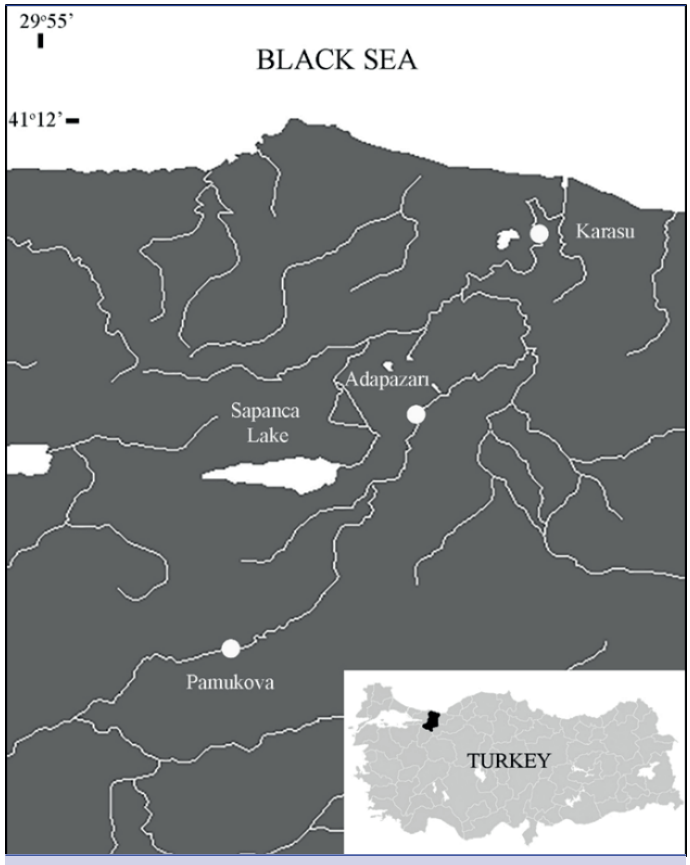

Figure 1. Study area.

The samples were brought to the laboratory and the fish species were determined according to their diagnostic characteristics (Kottelat & Freyhof, 2007). Total lengths and weights of samples were measured with measuring boards (0.1 cm) and precision balance (0.01 g). Scales were used to determine the age of the fish. The scales were taken from the area between the dorsalfin and the lateral line on the left side of the fish by forceps and placed in numbered envelopes (Lagler, 1966). Scales were removed from the envelopes and placed in petri dishes, containing 3% NaOH solution, in order to be purified from foreign bodies. Randomly selected scales were examined under a binocular microscope (Chugunova, 1963).

Growth parameters were investigated by applying the von Bertalanffy growth function. The von Bertalanffy growth function was calculated as follows: $L_t = L_\infty (1\text{-}e^{-k(t\text{-}to)})$ (von Bertalanffy, 1957), where L_t is length at age t, L_∞ is asymptotic length, k is the growth coefficient, and t_0 is the hypothetical age at which length is equal to zero (Ricker, 1975).

The growth performance index was calculated by the equation of Pauly & Munro (1984):

$\phi' = Log\ k + 2\ Log\ L\infty$

RESULTS AND DISCUSSION

In this study, all samples were analyzed to estimate age and growth parameters, including five fish species from the Lower Sakarya River, Turkey. The parameters shown in Table 1 included sample size (n), range of total length (TL) and body weight (W), and standard error (SE).

The von Bertalanffy's growth model was calculated $Lt = 92.18(1\text{-}e^{-0.054(t+0.040)})$ for *A. brama*, $Lt = 69.40(1\text{-}e^{-0.040(t+0.030)})$ for *B. bjoerkna*, $Lt = 51.09(1\text{-}e^{-0.114(t+0.024)})$ for *C. gibelio*, $Lt = 48.11(1\text{-}e^{-0.088(t+0.023)})$ for *R. rutilus* and $Lt = 41.74(1\text{-}e^{-0.104(t+0.035)})$ for *V. vimba*. The asymptotic length ($L\infty$), growth coefficient (k), hypothetical age (t_0) and growth performance index (ϕ') were shown in Table 2.

This study is the first assessment of the age and growth of *A. brama, B. bjoerkna, C. gibelio, R. rutilus* and *V. vimba* in the lower Sakarya River. The growth parameters ($L\infty$, K, t_0, ϕ') studied by different authors are given from other water areas (Table 3).

It was determined that the age composition of *A. brama* individual sex tend to 2^+-9^+ages. The age composition results of different researches were given in Table 4 for *A. brama.* Asymptotic length value was higher when compared to previous studies for *A. brama* (Table 3). In contrast, k value is lower than other studies. According to the growth performance index results, it can be said that *A. brama* showed average development in conditions of the lower Sakarya River.

The maximum age of *B. bjoerkna* in this study was 10^+ years which is higher than that of reported studies in Table 4. These differences may be due to the variations in sampling method and period, potential aging errors, and overfishing. The $L\infty$ value obtained in this study was higher than that in earlier researches. In the present study, the k value was found to be lower than that found by other researchers (Table 3). Ma, Xie, Huo, Yang & Huang,(2010) reported that different size distributions in different study may be the causes of differences among all of the estimated parameters.

The age composition of *C. gibelio* individuals was between 1^+-7^+ in the present study. Some differences were observed in age groups of *C. gibelio* when compared to previous researches

Table 1. Mean total length (ML. cm). mean weight (W. g). number of sample (n) and standard error (SE) for different age groups of five fish species.

		Age									
		1+	2+	3+	4+	5+	6+	7+	8+	9+	10+
A. brama	n		5	14	37	55	17	8	2	3	
	ML±SE		18.3±2.32	21.2±1.30	24.6±1.88	30.0±1.17	33.1±0.73	36.1±1.75	39.1±0.51	41.4±1.16	
			(14.3-20.3)	(19.1-23.5)	(21.1-28.0)	(26.7-31.9)	(31.8-34.6)	(34.1-39.6)	(38.8-39.5)	(40.3-42.6)	
	MW±SE		66.92±20.21	104.64±23.91	167.60±38.44	299.39±46.61	416.40±53.25	560.22±73.34	762.81±20.49	872.23±45.03	
			(33.35-87.52)	(69.02-145.0)	(101.8-232.8)	(194.9-442.2)	(348.2-557.8)	(484.4-717.5)	(737.3-748.3)	(820.3-900.2)	
B. bjoerkna	n	32	43	37	82	91	80	59	71	44	8
	ML±SE	11.3±1.23	13.9±0.83	16.4±0.84	18.3±0.76	19.6±0.57	21.1±0.60	22.3±0.53	23.9±0.82	26.1±0.85	28.3±1.14
		(6.2-12.7)	(12.5-15.6)	(15.1-17.6)	(16.7-19.4)	(17.0-20.5)	(18.5-22.5)	(20.2-23.5)	(21.1-25.2)	(22.7-27.4)	(27.1-30.4)
	MW±SE	17.82±5.15	30.58±6.88	54.39±13.68	80.40±10.58	93.86±12.54	115.11±18.35	139.45±21.65	177.85±31.16	236.59±31.70	302.43±46.49
		(3.15-30.49)	(16.71-46.98)	(31.35-85.89)	(53.37-106.1)	(71.45-138.0)	(76.47-168.3)	(87.18-188.1)	(121.3-246.2)	(185.4-317.6)	(198.6-347.4)
C.gibelio	n	45	51	15	30	23	12	3			
	ML±SE	12.7±0.99	15.9±1.64	21.3±0.99	24.3±0.90	26.6±0.78	28.8±0.91	31.8±0.54			
		(9.3-13.7)	(13.7-19.5)	(19.6-22.7)	(22.6-25.9)	(24.9-28.1)	(27.8-30.9)	(31.4-32.4)			
	MW±SE	40.37±8.97	77.10±24.92	191.76±37.34	254.18±49.50	325.37±61.90	425.02±73.72	499.65±80.76			
		(13.76-55.10)	(42.59-137.6)	(130.1-276.5)	(183.1-434.1)	(232.7-463.8)	(298.6-557.6)	(448.5-592.7)			
R.rutilus	n	25	32	28	13	15	12	13	8	6	
	ML±SE	12.4±0.57	15.3±1.35	18.1±1.62	20.6±0.78	22.9±1.23	25.1±1.35	27.2±1.47	29.1±1.26	30.4±1.26	
		(11.2-13.4)	(13.4-17.8)	(14.5-20.3)	(18.8-21.4)	(20.6-24.0)	(23.2-26.9)	(24.8-29.6)	(27.6-30.9)	(28.7-32.2)	
	MW±SE	22.79±3.53	34.34±10.94	62.76±20.22	105.89±27.48	128.03±20.55	166.25±44.87	218.58±55.49	295.58±87.46	382.39±73.87	
		(14.46-30.28)	(23.44-64.25)	(26.25-96.67)	(60.53-158.9)	(92.02-179.0)	(108.1-248.8)	(122.6-305.4)	(167.7-449.6)	(282.1-496.5)	
V.vimba	n	3	68	37	20	72	46	18			
	ML±SE	13.0±0.59	14.7±1.04	18.8±1.31	21.1±0.89	22.6±1.02	24.8±0.68	26.8±0.89			
		(12.3-13.4)	(12.7-16.8)	(15.6-20.9)	(19.2-23.3)	(20.6-24.3)	(23.1-26.5)	(25.8-29.1)			
	MW±SE	26.20±4.66	31.95±8.97	79.95±24.35	116.74±14.12	132.86±16.81	156.84±22.19	209.07±41.89			
		(20.86-29.46)	(20.79-63.63)	(32.5-135.48)	(78.19-141.9)	(93.4-182.34)	(111.8-238.2)	(172.3-322.6)			

Table 2. Growth parameters (L_{∞}, k, t_0) and growth performance index (Ø') for five fish species.

Species	N	L_{∞}	k	t_0	Ø'
Abramis brama	141	92.18	0.054	-0.040	2.628
Blicca bjoerkna	547	69.40	0.04	-0.030	2.268
Carassius gibelio	179	51.09	0.114	-0.024	2.474
Rutilus rutilus	152	48.11	0.088	-0.023	2.307
Vimba vimba	264	41.74	0.104	-0.035	2.260

Table 3. Growth parameters (L_{∞}, k, t_0) and growth performance index (Ø') for five fish species studied by different authors.

Species	Location	N	L_{∞}	k	t_0	ø'	References
Abramis brama	Dąbie Lake	290	44.62***(TL)	0.175	0.23	2.542	Kompowski, 1988
	Volvi Lake	443	50.7*(FL)	0.094	-0.41	2.383	Valoukas & Economidis, 1996
	Rubikiai Lake	209	65.7***(SL)	0.085	0.482	2.565	Žiliukienė & Žiliukas, 2011
	Sakarya River	141	92.18***(TL)	0.054	-0.01	2.628	This study
Blicca bjoerkna	Berounka River		23.4***(SL)	0.270	-0.27	2.169	Hanel, 1991
	Balaton Lake	127	35.9***(SL)	0.098	-0.639	2.101	Specziár et al., 1997
	Sapanca Lake	350	31.91*(TL)	0.122	-1.087	2.10	Okgerman et al., 2012
	Ladik Lake	434	32.85***(FL)	0.11	-2.64	2.074	Yilmaz et al., 2015
	Sakarya River	547	69.4***(TL)	0.04	-0.02	2.268	This study
Carassius gibelio	Lysimachia Lake		32.5***(FL)	0.282	-0.51	2.47	Leonardos et al., 2001
	Egirdir Lake	616	33.3***(FL)	0.346	-0.302	2.58	Balık et al., 2004
	Aksu River	128	36.86***(TL)	0.244	-0.791		İnnal, 2012
	Seyhan River	317	32.30***(TL)	0.307	-0.526	2.505	Ergüden, 2015
	Sakarya River	177	51.09***(TL)	0.11	-0.02	2.458	This study
Rutilus rutilus	Volvi Lake	233	33.3***(TL)	0.081	-1.30	1.95	Papageorgiou, 1979
	Berounka River		28.5***(SL)	0.169	-0.17	2.14	Hanel, 1991
	Balaton Lake	112	31.9***(SL)	0.160	0.026	2.21	Specziár et al., 1997
	Sapanca Lake	136	31.87**(TL)	0.195	-0.034	2.297	Okgerman et al., 2009
	Sakarya River	152	48.11***(TL)	0.09	-0.02	2.318	This study
Vimba vimba	Berounka River		27.8***(SL)	0.212	-0.22	2.214	Hanel, 1991
	Caspian Sea coast	845	26.1***(FL)	0.280	-0.65	2.280	Chaichi et al., 2011
	Sapanca Lake	217	24.70*(FL)	0.205	-1.464	2.097	Okgerman et al., 2011
	Sakarya River	264	41.74***(TL)	0.10	-0.04	2.241	This study

*Female, **Male, ***Combined

(Table 4). These differences may be due to the sampling method, fishing activity, feeding habitats, population density and the ecological condititions of water bodies.

The ages and lengths of the *R. rutilus* ranged between 1^+ - 9^+ years, 11.2 to 32.2 cm in the lower Sakarya River (Table 1). In other research on *R. rutilus*, age distribution was reported to be 1^+ - 6^+ (Sedaghat & Hoseini, 2012) in the Southern Caspian Sea, 1^+ - 4^+ in Seyhan Dam Lake (Ergüden, Ergüden, & Göksu, 2008). Due to the maximum size obtained in the sampling, asymptotic length value calculated for *R. rutilus* was found higher compared to the research in Table 3.

Despite wide distribution of *V. vimba* individuals, information on the biology of this species in Turkey is scarce. The age composition of this species was between 1^+ - 7^+ in the present study. The growth rate for length and weight in this research was generally high in comparison with populations from other studies (Table 4). The growth performance index of *V. vimba* (ø') in the lower Sakarya River was similar to that previously reported, apart from 2.097 (ø') *V. Vimba* caught in the Sapanca Lake (Okgerman, Elp, & Yardımcı, 2011). The differences in the growth of Vimba between regions might have been because of the ecological condititions of the Sakarya River, competition for food between Vimba and the other fish species and differences on condition, length, age, sex, and gonadal development of *V. vimba* (Ricker, 1975).

Growth can be evaluate when age and size information are combined. Growth provides us with some indication of resource utilization and the effectiveness of our management strategies.

Table 4. Mean length and mean weight for different age groups of five fish species studied by different authors.

Species	Location	Sex		Age										References
				1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	
Abramis brama	Solina dam reservoir	Combined	TL				25.9	30.8	33.5	34.0	35.2	36.9	38.1	Epler et al., 2006
			W				214	364	457	457	496	548	598	
	Rubikiai Lake	Combined	SL	5.6	10.0	13.6	17.0	21.5	24.3	27.6	30.2	33.2	35.9	Žiliukienė & Žiliukas, 2011
			W	3	18	50	97	196	296	403	578	767	957	
	Ladik Lake	Combined	FL	12.72	16.14	18.08	23.9	28.75	34.09	39.53	42.16			Yılmaz et al., 2015
			W	32.41	65.14	95.54	258.3	418.6	749.2	1167	1435.2			
	Sakarya River	Combined	TL		18.3	21.2	24.6	30.0	33.1	36.1	39.1	41.4		This study
			W		66.92	104.64	167.60	299.39	416.40	560.22	762.81	872.23		
Bilicca bjoerkna	Balaton Lake	Combined	SL	8.4	10.9	13.0	15.0	16.8	18.8	20.5	22.0			Specziár et al., 1997
			W	12.0	28.4	50.9	79.9	117	166	220	274			
	Sapanca Lake	Female	TL	6.9		12.99	14.96	16.73	17.80	20.01	20.77	23.2		Okgerman et al., 2012
			W	3.35		23.32	39.23	58.28	70.02	109.73	110.0	159.4		
	Uluabat Lake	Combined	FL	8.35	10.55	12.34	13.80	15.32	16.31	17.85				Şaşı & Berber, 2012
			W	9.56	13.73	31.02	80.17	90.32	104.93	122.68				
	Aras Dam Lake	Female	TL	17.07	20.14	21.18	23.49	23.65						Jamali et al., 2015
			W	58.7	112.2	126.1	157.9	161.7						
	Sakarya River	Combined	TL	11.3	13.9	16.4	18.3	19.6	21.1	22.3	23.9	26.1	28.3	This study
			W	17.82	30.58	54.39	80.40	93.86	115.11	139.45	177.85	236.59	302.43	
Carassius gibelio	Egirdir Lake	Combined	FL	11.9	18.1	22.9	25.5	27.4	29.6					Balık et al., 2004
			W	42.0	145.2	297.0	451.4	602.1	857.5					
	Aksu River	Combined	TL	12.16	17.91	21.19	24.18	26.93	29.43					İnnal, 2012
			W	40.71	109.41	179.43	285.38	452.21	540.3					
	Seyitler Reservoir	Combined	FL	15.35	17.74	21.02	23.79	25.56		31.75				Bulut et al., 2013
			W	46.15	130.6	214.11	300.38	348.46		755.4				
	Rozov Klade-nets Reservoir	Combined	SL	11.64	15.78	17.86	19.78	21.48	23.91					Zhelev et al., 2015
			W	42.82	90.79	118.24	160.55	198.26	263.40					
	Sakarya River	Combined	TL	12.7	15.9	21.3	24.3	26.6	28.8	31.8				This study
			W	40.37	77.10	191.76	254.18	325.37	425.02	499.65				

Table 4. Continue.

Species	Location	Sex		Age										References
				1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	
Rutilus rutilus	Volvi Lake	Combined	TL	7.6	11.3	12.3	13.9	15.1	16.0	17.2	18.1	19.3	20.5	Papageorgiou, 1979
			W	2.93	13.59	19.30	24.86	39.98	48.74	63.85	71.92	77.24	101.35	
	Solina Reservoir	Combined	TL			15.8	19.9	21.3	22.3	23.5	25.0			Epler et al., 2005
			W			47.3	101.2	125.7	152.5	201.5	210.0			
	Seyhan Dam Lake	Combined	FL	14.72	17.40	19.25	22.04							Ergüden et al., 2008
			W	47.23	75.12	94.50	185.62							
	Sapanca Lake	Female	TL	7.25	12.79	16.26	18.67	20.10	21.66	24.13	28.58	30.97		Okgerman et al., 2009
			W	5.19	25.38	47.56	81.14	99.01	122.96	183.59	315.34	412.53		
	Sakarya River	Combined	TL	12.4	15.3	18.1	20.6	22.9	25.1	27.2	29.1	30.4		This study
			W	22.79	34.34	62.76	105.89	128.03	166.25	218.58	295.58	382.39		
Vimba vimba	Sariyar Dam Lake	Combined	FL		10.45	14.61	16.98	19.12	20.97	23.12				Ekmekci & Erk'akan, 1992
			W		13.50	42.02	74.55	112.05	161.07	235				
	Kirmir Stream	Combined	FL	10.62	13.01	15.01	17.78	19.54						Tutucu, 2002
			W	15.64	31.89	51.52	88.70	124.42						
	Barycz River	Female	TL	6.2	12.2	17.9	23.5							Łuszczek-Trojna- ret al., 2008
			W	2.25	17.1	67.6	145.9							
	Sapanca Lake	Female	FL		12.62	14.58	17.03	17.87	19.43					Okgerman et al., 2011
			W		27.47	46.49	71.38	82.75	103.58					
	Sakarya River	Combined	TL	13.0	14.7	18.8	21.1	22.4	24.8	26.8				This study
			W	26.20	31.95	79.95	116.74	132.86	156.84	209.07				

When we evaluate age and growth in combination, the relationship between population size and biomass can be easier to understand. This understanding is the basis of modern fisheries resource allocation and management.

CONCLUSION

In conclusion, this study provides basic information on age and growth of *A. brama, B. bjoerkna, C. gibelio, R. rutilus* and *V. vimba* living in the lower Sakarya River. The results of this study are useful for evaluating the relative condition of fishery managements and stock assessment in the Sakarya River. Also, this study will contribute to further scientific studies in the same area.

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Research Article

Antibacterial Activity of Cyanobacteria *Dolichospermum affine* Isolated from Freshwater

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ABSTRACT

Cyanobacteria are known for their potential for antibacterial activity against a variety of pathogens, which are of medicinal importance in drug development. In addition, Cyanobacterial species produce various secondary metabolites that are used as dye and pigmentation and food additives. Cyanobacteria *Dolichospermum affine* (Lemmermann) Wacklin, L. Hoffmann & Komárek was isolated from freshwater resources and its antimicrobial effect was studied. Chloroform, methanol and water extracts of *D. affine* were tested to investigate their efficiency against five pathogenic bacterial strains [*Pseudomonas aeruginosa* (ATCC 27853), *Shigella dysenteriae* (ATCC 11835), *Escherichia coli* (ATCC 25924), *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 6633)]. The antimicrobial test was determined using the disk diffusion method. The antimicrobial activities of *D. affine* extracts were measured using the diameter of the inhibition zone (DIZ) of the pathogen microorganisms. The results showed that *B. subtilis* and *E.coli* were more sensitive, while *S. aureus* and *P. aeruginosa* showed more intermediate results. The highest antimicrobial activity was measured against *E. coli* (DIZ=13.9±0.05 mm - methanol), followed by *B. subtilis* (DIZ=13.6±0.05 mm - methanol). The lowest antibacterial effect of *D. affine* extracts were observed against *P. aeruginosa* (DIZ=11.7±0.02 mm - chloroform) and *S. aureus* (DIZ=12.2±0.03 mm - chloroform). The Gram-negative bacteria *S. dysenteria* exhibited no zone of inhibition. The aqueous extract showed poor activities against the tested pathogenic bacteria. Therefore, this study revealed that *D. affine* extracts would be a promising natural resource for new antibiotics and further research would be needed.

Keywords: Cyanobacteria, *Dolichospermum affine*, antibacterial activity, zone of inhibition

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INTRODUCTION

Algae are organisms that live in both aquatic and terrestrial environments and in a wide variety of habitats, there are also numerous species and they are the primary producers of ecosystems. The components produced by algae are antioxidants and pigments (including fucoxanthine, carotenoids, lutein, b-carotene, astaxanthin and phycobilliproteins), long chain polyunsaturated fatty acids (LC-PUFA) and proteins (essential amino acids methionine, threonine and tryptophan). These secondary metabolites have wide applications in food, feed, agricultural and pharmaceutical industries (Gouveia, 2014; Walker, Purton, & Becker, 2005; Brennan & Owened, 2010). Today, the use of biomedical and pharmacological potentials of secondary algal metabolites in algal biotechnology is a relatively new trend (Lorenz & Cysewski, 2000; Walker et al., 2005). Naturally active compounds found in algae biomass have different biological properties such as cytotoxic, antibiotic, antioxidant, antifungal, anti-inflammatory and antihelminthic (Pulz & Gross, 2004; Gouveia, Batista, Sousa, Raymundo, & Bandarra, 2008; Plaza, Santoyo, & Jaime, 2010; Patil, Patil, Mahajan, & Mahajan, 2011). Algae are also used as biomolecule and biomass sources in fish farming, which can increase the nutritional value of foods or provide

additional health benefits (Mulbry, Kondrad, & Buyer, 2008). They are also used in bioremediation applications and as a biofertilizer because of their nitrogen fixation (Demir, 2011). Today, the most important future use of biomass obtained from algae is thought to be production of biofuels as a renewable energy source (Converti, Casazza, Ortiz, Perego, & Borghi, 2009; Demirbas, 2010).

Cyanobacteria were the first photosynthetic organisms living in the seas 3.5 billion years ago, capable of photosynthesis and having prokaryotic cell structure. Due to their physiological flexibility and long evolutionary backgrounds, they are found in a wide variety of ecosystems (Weis & Pang, 2010). They show distinct morphological differences for species as well as single-celled or filamentous forms. The cell size of cyanobacteria is in the range of 0.5-1 µm to 40 µm. It is capable of synthesizing chlorophyll-a and at least one phycobiline as a pigment. Phycocyanin, which belongs to the phycobilin group, is blue in color and is the cause of the blue-green color of most cyanobacteria with chlorophyll-a (Madigan, Martinko, Stahl, & Clark, 2012). An important feature of some species of cyanobacteria is the ability to fix atmospheric nitrogen (N_2). Cyanobacteria species that fix nitrogen are generally in filament form and make N_2 fixation by a small number of specialized cells called "Heterocyst" (Whitton, 2000).

In recent years, interest in biologically active substances from cyanobacteria has increased. Various studies have demonstrated that cyanobacterial secondary metabolites have hypocholesterolemic properties, enzyme inhibitor and other pharmacological effects (Abobaker & Elsalhin, 2019). Various types of cyanobacteria are known to produce intracellular and extracellular metabolites with antibacterial and antifungal properties (Kreitlow, Mundt, & Lindequist, 1999). These natural products are used in the production of raw pharmaceutical materials and as structural models in the synthetic molecules (Gault & Marler, 2009).

Bacterial infections cause major diseases worldwide, leading to high mortality rates in humans and animals. Antimicrobial agents are widely used in the treatment of bacterial infections, but bacteria can become resistant to existing drugs. For this reason, researchers have begun to search for natural compounds in order to discover new antibacterial compounds (Taskin, Ozturk, Taskin, & Kurt, 2007). Cyanobacteria are seen as promising biological resources in this field. Previous studies on antimicrobials obtained from natural sources have focused on *Spirulina platensis, Chrococcus* sp., *Oscillatoria* sp., *Synechocystis aquatilis, Anabaena* sp., *Oscillatoria limosa, Pseudoanabaena limnetica, Phormidium tenue* and *Spirulina platina* species (Özdemir, Karabay, Dalay, & Pazarbaş, 2004; Demiriz, Çökmüş, & Pabuçcu, 2011). As can be seen above, the antimicrobial properties of cyanobacteria have been studied in different species, but a study on *Dolichospermum affine* has not been found in the literature. This research investigated the antibacterial activity of cyanobacteria *D. affine* extracts against five selected pathogenic bacteria.

MATERIALS AND METHODS

Sample isolation

In our previous studies, *D. affine* was isolated in samples collected from various freshwater resources in Ankara, Turkey. The one-cell growth technique was used for the isolation of strains (Parvin, Zannat, & Habib, 2007). Taxonomic identification of the isolate was based on morphological features and species keys (Prescott 1973; John, Witton, & Brook, 2002; Guiry & Guiry 2018). The subcultures were prepared by putting 30 ml BG-11 nutrient media into 50 ml Erlenmeyer flasks and adding approximately 20% culture depending upon the intensity of cells (Hur, Bae, Youn, & Jo, 2015). BG-11 medium contained (in g/L) $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA, 0.001; Na_2CO_3, 0.02. This medium was amended with 1 ml trace solution of composition (in g/L) H_3BO_3, 2.86; $MnCl_2$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2\ H_2O$, 0.39; $CuSO_4 \cdot 5\ H_2O$, 0.079; and $Co(NO_3)_2 \cdot 6H_2O$, 0.0494 (UTEX, 2016). All the chemicals were obtained from Merck, Germany. The pH was adjusted to 6.8 (Andersen & Kawachi, 2005). Those containing 30 ml cultures were incubated at 25°C under fluorescent lamps at a photon flux density of 50 µmol photons m^{-2} s^{-1} with a photocycle of light for 16 hours and darkness for 8 hours (Guillard, 2005).

Cyanobacteria culture

D. affine was cultivated in BG-11 culture medium and the experiments were carried out in 500 ml Erlenmeyer flasks containing 200 ml of medium and 50 ml suspended culture at room temperature. Light was provided by cool-white fluorescent lamps at photon flux density of 50 µmol photons m^{-2} s^{-1} with a photocycle of a light for 16 hours and darkness for 8 hours for 14 days (Guillard, 2005). After culturing, the cells of *D. affine* were centrifuged at 5000 rpm for 20 min (Nüve NF 200), the supernatant was discarded, and the remaining pellets were then used to test the effect of the algal extracts on some bacteria strains.

Preparation of algal extracts

Approximately one gram of dried powder of *D. affine* pellets was extracted with chloroform, methanol and water (10 ml) and shaking overnight for complete extraction. The extract was filtered and the filtrate was concentrated under reduced pressure at 37-40°C and stored in a refrigerator till further use. The concentration was adjusted to 1mg/ml using the same solvent used for extraction was assayed for antibacterial activity (Malathi, Ramesh Babu, Mounika, Snehalatha, & Digamber Rao, 2014; Deshmukh & Puranik, 2012).

Test microorganisms

In vitro antibacterial studies were carried out against the 5 human pathogen bacteria as shown in Table 1. Nutrient Broth was used to grow these cultures and incubated at 30±1°C overnight.

Table 1. Test organisms.

Human Pathogen Bacteria	Code	Type
Pseudomonas aeruginosa	ATCC 27853	gm negative
Shigella dysenteria	ATCC 11835	gm negative
Escherichia coli	ATCC 25924	gm negative
Staphylococcus aureus	ATCC 29213	gm positive
Bacillus subtilis	ATCC 6633	gm positive

Antibacterial assay

The antibacterial activity test was done using the agar well diffusion method (Perez, Pauli, & Bazerque, 1990). 0.1 ml of diluted inoculum (10^5 CFU ml^{-1}) of the bacterial strains were swabbed on agar plates, and 5.0 mm size diameter wells on agar plates were made with a sterile cork borer (5.0 mm). Using a micropipette, 100 µl of algal extract was added to the wells made on each plate. The plates were allowed to incubate at 37±2°C for 24 to 48 h. Antibacterial activity was assessed by measuring the zone of inhibitions (mm) against the bacterial strains. Negative controls were prepared using the same solvents employed to dissolve the obtaining extracts. Gentamycin (10 µg) and Ampicillin (10 µg) antibiotic discs were used as a positive reference standard to determine the sensitivity of one strain from each bacterial species. The tests were performed in triplicate. The following antimicrobial index formula was used to compare the antimicrobial activity of the sample with the activity of the standard (Malathi et al., 2014):

Antimicrobial Index = (Extract inhibition zone/Antibiotic inhibition zone) × 100

Statistical analysis

The results were presented as mean values ± standard deviation. The standard deviations were calculated using Microsoft Excel.

RESULTS AND DISCUSSION

Dolichospermum affine (Lemmermann) Wacklin, L. Hoffmann & Komárek is a Cyanobacteria which belongs to the family Aphanizomenonaceae of class Cyanophyceae and this is a freshwater species. Fig. 1 presents the taxonomic classification (left) of *D. affine* (Guiry & Guiry, 2018) and its appearance under a microscope (right).

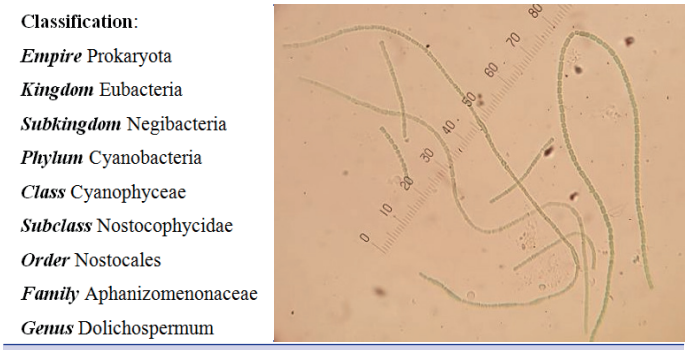

Figure 1. Classification (left) and microscopic images (right) of D. affine.

Cyanobacteria produce different bioactive compounds with antibacterial, antifungal, antiviral, and anti-inflammatory properties of industrial, therapeutic and agricultural importance (Sethubathi & Prabu, 2010). The discovery that extracts from cyanobacteria have antimicrobial activity has shown that Cyanobacteria can be an important source in obtaining new bioactive compounds in the pharmaceutical field (Thajuddin & Subramanian, 2005). In the study of Sethubati & Prabu (2010) with the extracts they obtained from *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscule*; *Oscillatoria* sp. showed the highest antibacterial activity and *L. majuscule* the lowest. In the study of Abd El-Aty et al., (2014) with *Anabaena sphaerica* and *Oscillatoria agardhii,* they found that these species showed antibacterial activity against some Gram-negative and Gram-positive bacteria. In this study, the results of antibacterial activity of the *D. affine* against two Gram-positive and three Gram-negative bacterial strains are shown in (Table 2). It is obvious that the diameter of the inhibition zone (DIZ) depends on the type of algal species, the kind of solvent used and the tested pathogenic microorganisms. In the present study, extracts of *D. affine* were used with three different solvents, namely methanol, chloroform and aqueous extracts. The mean values of three replicates of the DIZ (in millimeters) around each well with different extracts are also given in Table 2. *D. affine* extracts confirmed antibacterial activities against four tested pathogenic bacteria out of five pathogenic bacterial strains. Methanol extracts exhibited better antibacterial activities than chloroform extracts. According to DIZ results, *Bacillus subtilis* and *Escherichia coli* were more sensitive, while *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed more intermediate results. The highest antimicrobial activity was measured against *E. coli* [DIZ=13.9±0.05 mm (methanol) and DIZ=13.8±0.01 mm (chloroform)], followed by *B. subtilis* [DIZ=13.6±0.05 mm (methanol) and DIZ=13.5±0.06 mm (chloroform)] (Fig. 2). *D. affine*'s chloroform extract showed the lowest antibacterial effect in both *P. aeruginosa* (DIZ=11.7±0.02 mm) and *S. aureus* (DIZ=12.2±0.03 mm). The Gram-negative bacteria *S. dysenteria* exhibited no zone of inhibition. The aqueous extract showed poor activities against the tested pathogenic bacteria. Chloroform, Methanol, Distilled water negative control also showed no inhibitory effect, while the positive control (Gentamicin and Ampicillin) showed inhibition diameters ranging from 13.0 to 27.0 mm and 13.0 to 26.0 mm respectively.

The antibacterial effects of the *D. affine* extracts were compared with commercial antibiotics and the results of this comparison are given in (Table 3) as the antimicrobial index. According to the index data, the efficacy of the chloroform and methanol extracts obtained from *D. affine*, especially on *E. coli*, were 63% and 66% similar to the efficacy of currently used antibiotics. It can be concluded that *D. affine* is an alternative to current commercial applications as an antibacterial agent in phytotherapy.

Halder (2015) tested the antibacterial effects of *Anabaena variabilis* extracts in different solvents against the eight pathogenic bacterial strains out of which three are Gram positive (*Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus*) and five are Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri and Vibrio cholerae*) using the agar well diffusion method. It was found that the results obtained from the same solvent and bacteria used in the study by Halder (2015) were similar to the results in this study. In the study conducted by Abobaker & Elsalhin (2019), extracts of *Anabaena circinalis* in different concentrations (25, 50, 75 and 100%) were tested to determine the efficacy against four bacterial strains (*Achromobacter xylosoxidans, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae*). Results showed that the highest level of antimicrobial activity was recorded against *S. dysenteriae* at

Table 2. The diameter of inhibition zone (DIZ) of the different solvent extracts of *D. affine*.

Human Pathogen Bacteria	Chloroform DIZ (mm)	Methanol DIZ (mm)	Water DIZ (mm)	Antibiotic Positive Control DIZ (mm)	
				GEN[1]	AMP[2]
P. aeruginosa (ATCC 27853)	11.7±0.02	12.3±0.01	8.4±0.03	27	26
S. dysenteria (ATCC 11835)	NCD[a]	NCD[a]	NCD[a]	13	13
E. coli (ATCC 25924)	13.8±0.01	13.9±0.05	8.0±0.02	21	22
S. aureus (ATCC 29213)	12.2±0.03	13.5±0.07	7.3±0.05	26	25
B. subtilis (ATCC 6633)	13.5±0.06	13.6±0.05	8.2±0.04	24	25
Negative Control[b]	0	0	0	0	

[a]NCD= No Culturable Cells Detected; [b]Negative Control= Distilled water; (GEN)[1]: Gentamicin 10 µg; (AMP)[2]:Ampicillin 10 µg; Data are given as mean ± standard deviation of triplicates. Mean values, n = 3.

Table 3. Antimicrobial index of *D. affine* extracts.

Human Pathogen Bacteria	Antibiotics	Antimicrobial Index in Percentage		
		Chloroform	Methanol	Water
P. aeruginosa (ATCC 27853)	GEN	43	46	31
	AMP	45	47	32
S. dysenteria (ATCC 11835)	GEN	NCD	NCD	NCD
	AMP	NCD	NCD	NCD
E. coli (ATCC 25924)	GEN	66	66	38
	AMP	63	63	36
S. aureus (ATCC 29213)	GEN	47	52	28
	AMP	49	54	29
B. subtilis (ATCC 6633)	GEN	56	57	34
	AMP	54	54	33

NCD= No Culturable Cells Detected - (GEN) Gentamicin 10 µg; (AMP) Ampicillin 10 µg

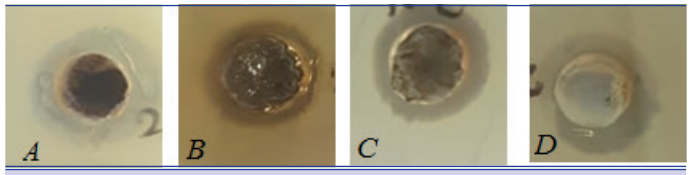

Figure 2. Inhibition zone of some pathogen bacteria (methanol extracts) [**A.** *P. aeruginosa* (ATCC 27853); **B.** *E. coli* (ATCC 25924); **C.** *S. aureus* (ATCC 29213); **D.** *B. subtilis* (ATCC 6633)].

100% concentration. However, in this study, the extracts in different solvents of *D. affine* against *S. dysenteriae* did not cause any antibacterial effect. It is thought that this may be caused by the species used in the studies and the different solvents.

Compounds obtained from cyanobacterial extracts with antimicrobial and antitumoral activity worldwide are attracting a great deal of attention. Mtolera & Semesi (1996) stated that these components include amino acids, terpenoids, fluorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulfides and fatty acids. Cyanobacter extracts are usually obtained with organic solvents such as methanol, hexane, chloroform, ethanol, acetone, diethyl ether, butanol and DMSO (Shamchi, 2016). For substances extracted by means of these solvents, Cowan (1999) suggests that these substances can be either terpenoid or flavonoid. In this study, it can be thought that the active substance acting on test bacteria may be terpenoid or flavonoid, however, further analysis is required to say this.

Antibiotic-resistant bacterial species are serious threats to animal and human health and cause serious damage. Clinical studies on the resistance mechanism of bacteria have enabled the identification of clinical uses of all antimicrobial agents (Helms, Vastrup, Gerner-Smidt, & Molbak, 2002). Due to increased bacterial resistance to commercial standards and reserve antibiotics, it is important to search for new active substances with antibacterial activity (Abobaker & Elsalhin, 2019). In this context, Cyanobacteria have started to be seen as promising sources in the production of antimicrobial substances due to their biologically active substances. Extracts of different Cyanobacteria species obtained by different solvents exhibited different degrees of antimicrobial activity on pathogenic microorganisms and have been studied by different researchers. In the study conducted by Tiwari & Sharma (2013), cyanobacterial extracts of *Anabaena variabilis* and *Synechococcus elongates* showed a significant antibacterial ratio against *Enterococcus* sp., *Klebsiella* sp. and *E. coli*. Malathi et al., (2014) observed significant antibacterial activities of *Anabaena variabilis* in chloroform and methanol crude extracts against *B. subtilis* and *P. aeruginosa*. Rania & Taha (2008) reported that *Spirulina platensis* extracts from different solvents show different degrees of antimicrobial activity on both Gram-positive and Gram-negative microorganisms.

CONCLUSION

In this study, it was found that extracts of *D. affine* strain obtained using different solvents have pharmaceutically interesting bioactive compounds. The cyanobacterial extracts obtained showed antibacterial properties against tested pathogenic bacteria, except *S. dysenteria*. The present research has shown that the antimicrobial activity of cyanobacterial strains is dependent on the solvents used to make the extracts and the effect of these solvents. Therefore, it is suggested that more detailed studies should be conducted to confirm the effect of antimicrobial activity of crude extracts prepared from different solvents. The future studies aim to identify the bioactive components responsible for antimicrobial effect from cyanobacterial strains by purification. It is thought that the findings obtained from this study can be used for future research and for the production of antibacterial drugs of cyanobacterial origin.

Conflict of interest: The author declares no conflict of interest.

Ethical approval: This article does not contain any studies with animals.

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Short Communication

Length-Weight Relationships for Three Deep Sea Fish Species in North Eastern Mediterranean, Turkey

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ABSTRACT

In the present study, length–weight relationships (LWRs) were estimated for three deep sea fish species, namely, *Nettastoma melanura* Rafinesque, 1810; *Lampanyctus crocodilus* (Risso, 1810); and *Chauliodus sloani* Bloch & Schneider, 1801 in the north-eastern Mediterranean Sea. A total of 102 fish samples were collected from Mersin Bay (Erdemli coast). Their length–weight relationship b values ranged between 2.458 and 3.496, and all regressions were found to be significant for all three species ($p<0.001$). This study is the first reference on length–weight relationships for these three deep-sea fish species from the North-eastern Mediterranean Sea coast of Turkey. Besides, Length–weight relationships for *C. sloani* and *L. crocodilus* were not yet available in Fishbase for the Eastern Mediterranean, and hence these results obtained from this study will be useful to researchers and fisheries biologists in the field.

Keywords: Deep sea fishes, Length-weight parameters, Blackfin sorcerer, Jewel lanternfish, Sloane's viperfish, Mersin Bay

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INTRODUCTION

In fisheries biology and fisheries management, length-weight relationships (LWRs) data are useful to determine the weight of an individual fish of known length or total weight from the length-frequency distribution (Garcia, Buarte, Sandoval, Von Schiller, & Mello, 1989; Froese, 1998; Koutrakis & Tsikliras, 2003). Besides, these relationships are an important component of FishBase (Froese & Pauly, 2019).

To date, there are a limited number of studies on the population of three deep-sea fish species length-frequency distributionin the western Mediterranean (Merella, Quetglas, Alemany, & Carbonell, 1997 Porcu et al., 2013) and eastern Mediterranean (Bílge, Yapici, Fíliíz, & Cerím, 2014; Deval, Güven, Saygu, & Kabapçioğlu, 2014).

The present study shows the first-time results of an investigation of length-weight relationships of three deep-sea fish species: Blackfin sorcerer, *Nettastoma melanura* Rafinesque, 1810; Jewel lanternfish, *Lampanyctus crocodilus* (Risso, 1810); and Sloane's viperfish *Chauliodus sloani* Bloch & Schneider, 1801from Mersin Bay (N.E. Mediterranean, Turkey).

Although biological studies on the deep sea fish fauna are limited in the Mediterranean Sea, this paper provides the first information on the length-weight relationships of three deep sea fish species in the North eastern Mediterranean Sea coast of Turkey. Besides, Length–weight relationships for *C. sloani* and *L. crocodilus* were not yet available in Fishbase for the Eastern Mediterraenan.

MATERIALS AND METHODS

Study area

The present study recorded deep sea fish specimens from the Mersin Bay Erdemli coast, Tur-

key) (Figure 1). Mersin Bay is an important fishing area of the Northeastern Mediterranean Sea due to its nutrient-rich fresh water inputs.

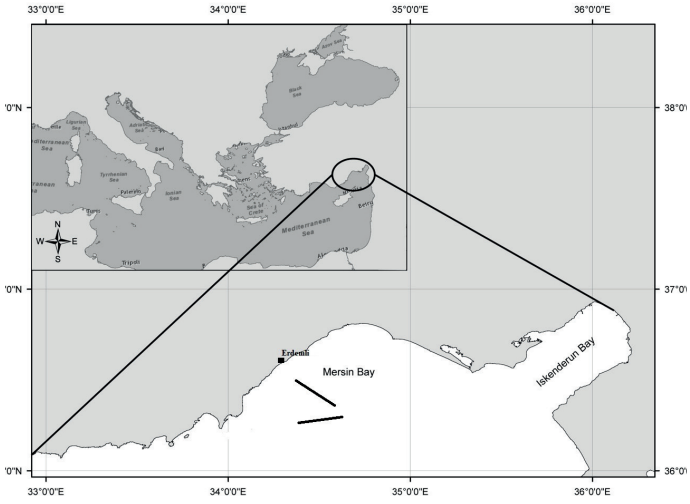

Figure 1. Sampling area.

During the sea surveys, 102 deep sea fish specimens belonging to three family were caught by commercial bottom trawler at a depth of 400 to 595 m off Mersin Bay (Erdemli coast) (Coordinates; 36° 12′ 383 N - 034° 23′ 019″ E; 36° 08′ 926″ N - 034° 42′ 057″ E). Samplings were carried out between June and July 2019. The trawler was equipped with 44 mm stretched mesh size nets at the cod-end. Trawling lasted 4 hours and the trawling speed was 2.7 knots (Figure 1). Captured fish specimens were photographed on board and then preserved in ice boxes for examination in the laboratory.In the laboratory, each fish was measured for total length to the nearest 0.1 cm, weight was measured to the nearest 0.1 g, and the sex was determined by macroscopic observation of the gonads.

Estimation of the length-weight relationship was made by adjustment of an exponential curve to the data (Ricker, 1975): $W=aL^b$. Where; W is body weight (g), L is total length (cm), a is a coefficient related to body form, and b is an exponent indicating isometric growth when equal to 3 (Beverton & Holt, 1996). The parameters a and b were estimated by linear regression on the transformed equation: log (W)= log(a) + b log(L). The *b* value for each species was tested by a *t*-test at the p=0.05 significance level to verify if it was significantly different from 3 (Pauly, 1993). All statistical analyses were performed using SPSS v. 21.0. Species identification was done according to Whitehead et al. (1986). The scientific name for each species was checked against FishBase (Froese & Pauly, 2019).

RESULTS

Lengths (TL) and weights (g) of a total of 102 fish specimens belonging to 3 fish species from 3 families were measured, recorded and analyzed (Figure 2, Figure 3, and Figure 4). The sample size, minimum maximum length as well as the LWRs, the coefficient of determination (r^2), the standard error and confidence interval (CI) of b for each species are presented in Table 1.

Figure 2. *Nettastoma melanura* Rafinesque, 1810 in the North-eastern Mediterranean.

Figure 3. *Lampanyctus crocodilus* (Risso, 1810) in the North-eastern Mediterranean.

Figure 4. *Chauliodus sloani* Bloch & Schneider, 1801 in the North-eastern Mediterranean.

The exponent b often has a value close to three, but varies between two and four (Tesch 1971).In the present study, b values (based on TL) of the *N. melanura* species were negative allometric growth for males, females and sexes combined (b<3). However, b values of *L. crocodilus* and *C. sloani* were positive allometric growth for males, females and sexes combined (b>3), (t-test: p<0.05).

In the present study, 102 specimens had b values within the expected range of 2.5-3.5 (Bílge et al., 2014; Deval et al., 2014) for three deep sea fish species (*N. melanura, C. sloani* and *L. crocodilus*). The calculated allometric coefficient b ranged from a minimum of 2.458 for males of *N.melanura*, to a maximum 3.496 for males of *L. crocodilus*. All regression values were found to be highly significant (p<0.001), with the coefficient of determination (r^2) values being >0.95 for all three fish species (Table 1).

Table 1. Descriptive statistics and length–weight relationships (LWRs) for three deep-sea fish species, North-eastern Mediterranean coast of Turkey.

Family	Species	Sex	N	TL (cm) L_{min}-L_{max} (L_{mean}±SD)	TW (g) W_{min}-W_{max} (W_{mean}±SD)	a	b	SE of b	95% CI of b	r^2	P	Growth Type
Nettasto-matidae	*N. melanura*	F	27	19.50-63.00 (34.57±12.21)	2.89-77.80 (23.05±19.84)	0.0017	2.622	0.111	2.393-2.851	0.957	<0.05	A-
		M	18	21.00-58.80 (42.08±11.01)	4.56-67.56 (34.56±18.71)	0.0032	2.458	0.084	2.280-2.637	0.982	<0.05	A-
		F+M	45	19.50-63.00 (37.61±12.21)	2.89-77.80 (27.66±20.01)	0.0021	2.573	0.072	2.428-2.719	0.967	<0.05	A-
Stomiidae	*C. sloani*	F	16	13.30-23.60 (18.26±3.13)	2.30-15.60 (7.52±4.36)	0.0003	3.474	0.132	3.191-3.757	0.980	<0.05	A+
		M	14	14.20-23.40 (17.71±2.78)	3.43-15.02 (6.71±3.85)	0.0005	3.261	0.172	2.885-3.636	0.967	<0.05	A+
		F+M	30	13.30-23.60 (18.00±2.93)	2.30-15.60 (7.14±4.08)	0.0004	3.383	0.104	3.171-3.195	0.974	<0.05	A+
Myctophi-dae	*L. crocodi-lus*	F	17	12.70-19.50 (16.97±1.81)	11.07-47.96 (29.67±9.93)	0.0020	3.373	0.143	3.069-3.678	0.973	<0.05	A+
		M	10	11.50-19.20 (16.16±2.18)	8.05-43.53 (25.92±11.42)	0.0014	3.496	0.194	3.050-3.944	0.976	<0.05	A+
		F+M	27	11.50-19.50 (16.67±1.95)	8.05-47.96 (28.28±10.46)	0.0017	3.431	0.108	3.028-3.654	0.975	<0.05	A+

N= sample size, *L* = Length [cm], min = Minimum, max = Maximum, *r2* = Coefficient of determination, *a* = Intercept, *b* = Slope, SE of *b* = Standart error of *b*, CI = Confidence Interval, A (+) = Positive allometry, A (–) = Negative allometry

Table 2. Length-weight relationships of three deep-sea fish species from different geographical areas.

Reference	Locality	Country	Species	Sex	N	TL (cm) L_{min}-L_{max}	TW (g) W_{min}-W_{max}	a	b	r^2
Deval et al. (2014)	Antalya Bay, eastern Mediterranean	Turkey	*Nettastoma melanura*	Mixed	75	25.1 - 79.8	5.4 -255.5	0.00020	3.180	0.940
Porcu et al. (2013)	South-eastern Sardinian Sea	Italy	*Nettastoma melanura*	Male	171	30.2 - 66.8	-	0.00200	3.247	0.820
				Female	226	32.5 - 75.3	-	0.00004	3.602	0.860
Bílge et al. (2014)	Southern Aegean Sea	Turkey	*Lampanyctus crocodilus*	Mixed	80	9.4 - 16.2	-	0.00690	3.143	0.967
Merella et al. (1997)	Balearic Islands (western Mediterranean)	Spain	*Lampanyctus crocodilus*	Mixed	25	9.0 - 21.0	-	0.00510	2.980	0.990
Merella et al. (1997)	Balearic Islands (western Mediterranean)	Spain	*Chauliodus sloani*	Mixed	11	15.1 - 30.5	-	0.00090	3.180	0.988

The length-weight relationship for the three deep sea fish species was found as W=0.0021 $L^{2.573}$ (R^2=0.967) for *N. melanura*, W=0.0004 $L^{3.383}$ (R^2=0.974) for *C. sloani* and W=0.0017 $L^{3.431}$ (R^2=0.975) for *L. crocodilus*. Estimation of length-weight relationship of combined sexes for the three fish species are given in Figure 5.

Porcu et al. (2013) reported positive allometric growth (male; b=3.247, female; b=3.602) for *N. melanura* from South-eastern Italy. Similarly, Deval et al. (2014) stated in the Antalya Bay, Turkey positive allometric growth (b=3.180), Bílge et al. (2014) reported positive allometric growth (b=3.143) for *L. crocodilus* in the study conducted from the southern Aegean Sea, Turkey, and Merella et al. (1997) reported in the western Mediterranean negative allometric growth (b=2.980) for *L. crocodilus* andpositive allometric growth (b=3.180) for *C. sloani*.

The previous studies on the presence for length characteristics of the length–weight relationships of the three deep-sea fish species in the other Mediterranean regions are given in Table 2. For all of the studied species presented in this paper, the b val-

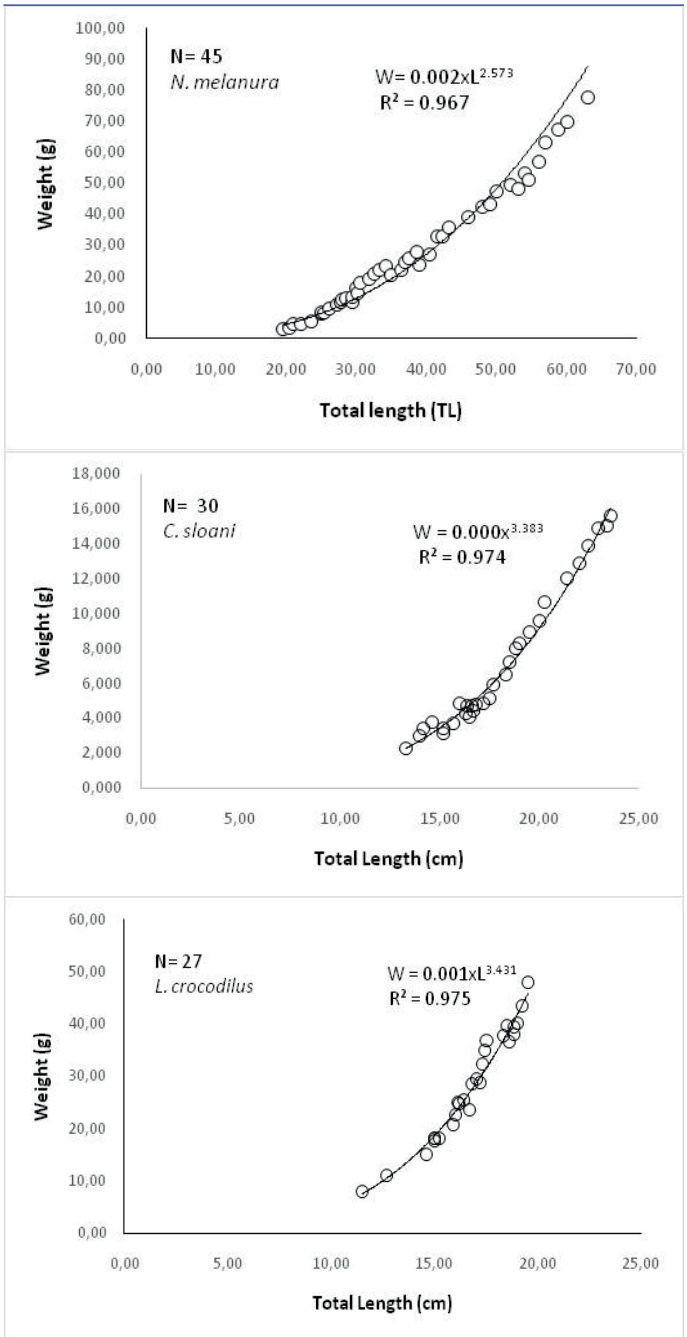

Figure 5. Length-weight relationship of combined sexes for three deep-sea fish species from the North-eastern Mediterranean, Turkey.

ues were generally in agreement with results for fishes of the same family obtained from other geographical regions except for *N. melanura*. These differences for *N. melanura* could be the result of ecological differences between regions or environmental differences (Frost, 1945). At the same time, the differences in the sampling time and sampling methods may also affect the relationships, as the numbers of specimens and length ranges of the species were distinct among localities (Tesch, 1971; Froese, 2006).

Length and weight relationships are used widely in fish exploration and supervision, and LWRs are essential to recognize the ecology and life of fish species (Froese, 2006). However, the length-weight relationship in fishes is affected by a number of factors including season, habitat, gonad, sex, diet and stomach fulness and preservation techniques (Tesch, 1971; Bagenal and Tesch, 1978), all of which were not accounted for in the present study.

CONCLUSION

The present study was conducted to give length and weight data of three fish species. To date, no information regarding the *C. sloani* and *L. crocodilus* fish species for the Eastern Mediterranean is available in Fishbase (Froese & Pauly, 2019).

To the best knowledge of the authors, this study presented the first compherensive reference on length-weight relationships for three deep sea fish species from the Eastern Mediterranean coast of Turkey. The results obtained from this study are useful to researchers and fisheries biologists, because the data were sampled from a fairly deep waters area.

Conflicts of interest: The authors have no conflicts of interest to declare.

Ethics committee approval: This study was conducted in accordance with the ethics committee procedures of animal experiments.

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