RESEARCH ARTICLE

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Growth and feeding features of the four-spotted megrim *Lepidorhombus boscii* (Risso,1810) in the Aegean Sea, Türkiye

Cangidez (*Lepidorhombus boscii* (Risso, 1810))'in Ege Denizi'ndeki büyüme ve beslenme özellikleri

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Abstract: The growth and feeding features of *Lepidorhombus boscii* (Risso, 1810) (four-spotted megrim) obtained from the Aegean Sea between May 2009 and April 2010 were determined. During the study, 1864 specimens were examined, and the female/male ratio was 1:0.41. The total length range and age distribution for all specimens were 6.3-34.8 cm and I-XIV, respectively. The Von-Bertalanffy growth equations were calculated by using the mean total lengths of each age group. The results were as follows: (L∞= 43.24 cm, K=0.088, t0= -2.897) for females, (L∞= 28.53 cm; K= 0,.25; t0= -0.829) for males, and (L∞= 42.9 cm, K=0.097, t0= -2.070) for all specimens. The stomach contents of the *L. boscii* species were also examined; a total of 9 main groups (Amphipoda, Mysidacea, Natantia, Anomura, Brachyura, Stomatopoda, Cephalopoda, Teleostei, Decapod Crustacea) were determined.

Keywords: Aegean Sea, feeding features, growth, Lepidorhombus boscii

Öz: Mayıs 2009-Nisan 2010 tarihleri arasında Ege Denizi'nden temin edilen *Lepidorhombus boscii*'nin (Risso, 1810) (Cangidez) büyüme ve beslenme özellikleri belirlenmiştir. Çalışma süresince, 1864 birey incelenmiş ve dişi erkek oranı 1:0.41 olarak bulunmuştur. Tüm ömekler için total boy aralığı ve yaş dağılımı sırasıyla, 6,3-34,8 cm ve I-XIV 'dir. Von-Bertalanffy büyüme denklemleri, her yaş grubunun ortalama Total Boy uzunlukları kullanılarak hesaplmıştır. Sonuçlar sırasıyla, dişiler için (L∞= 43,24 cm, K=0,088, t0= -2,897), erkekler için (L∞= 28,53 cm; K= 0,225; t0= -0,829) ve tüm bireyler için (L∞= 42,9 cm, K=0,097, t0= -2,070) dir. *L. boscii* türüne ait mide içerikleri incelendiğinde toplam 9 ana besin grubu (Amphipoda, Mysidacea, Natantia, Anomura, Brachyura, Stomatopoda, Cephalopoda, Teleostei, Decapod Crustacea) tespit edilmiştir.

Anahtar kelimeler: Ege Denizi, beslenme özellikleri, büyüme, Lepidorhombus boscii

INTRODUCTION

Lepidorhombus boscii is a flatfish species that is distributed across the Northeast of the Atlantic Ocean and the Mediterranean basin except The Black Sea region and it inhabits sandy-muddy bottoms that are about 50-800 m in depths (Norman, 1934; Froese and Pauly, 2015). They are most likely to be inhabited at depths between 100 and 450 m (Sanchez et al., 1998). They are usually caught with Lepidorhombus whiftagonis Walbaum 1792 (common megrim) which is another specimen in the same genus and both of them become economically important in European fisheries (Crego-Prieto et al., 2012). Especially lengths of 20 cm and above individuals are of commercial interest on the Mediterranean seas (Vassilopoulou, 2000; Ulutürk et al., 2012; Cengiz et al., 2013).

There are many studies on four spotted megrim because of a common component of benthic fauna (Castilho et al., 1993) and also economic value (Crego-Prieto et al., 2012). These studies mainly related to age and growth (Castilho et al., 1993; Santos, 1994; Landa, 1999; Robson et al., 2000; Landa et al., 2002; Teixeira et al., 2010; Landa and Fontela, 2016; Landa and Hernandez, 2020) in the Atlantic and (Bello

and Rizzi, 1987; Vassilopoulou and Ondarias, 1999; Bostanci and Polat, 2008; Cengiz et al., 2013) in the Mediterranean Sea. Despite the importance of benthic fauna, information on the feeding habits of these specimens is limited. These were in the Portuguese waters (Teixeira et al., 2010) and the Mediterranean Sea (Morte et al., 1999; Vassilopoulou, 2006).

The main aim of our study is to determine the growth parameters and feeding habits of the four-spotted megrim in the central Aegean Sea; furthermore, it is thought to contribute to limited studies for the four-spotted megrim in the Aegean Seas.

MATERIALS AND METHODS

This study was carried out between May 2009 to April 2010 and fish specimens were obtained from bottom trawl ships targeting *Parapenaeus longirostris* (Lucas, 1846) from Karaburun and Kusadası in the central Aegean Sea. Three main areas (Karaburun; Sığacık Bay and Kuşadası Bay) have been identified for our sampling (Figure 1).

Fish lengths were measured to the nearest 1 mm and

weights weighed to the nearest 0.01 g. Otolith for age determination was suspended in a 10% solution of NaOH and cleared with pure water then passed through five stages of ethanol series (90%, 70%, 50%, 30%, 10%). Otoliths were read using a black background filled with 50% water and 50% glycerin under reflected light. The stomachs were fixed in 4% formalin after the specimens were dissected.

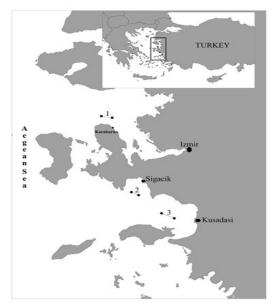


Figure 1. Sampling locations of L. boscii in the Aegean Sea

The form of the exponential equation "W= a \cdot L^b" was used to indicate the relationship between individuals' weight and length parameters (Ricker, 1979). The calculation of using The Von Bertalanffy growth "Lt = L ∞ [1-e-k(t - to)]" equation is used to calculate the growth of *L. boscii* specimens. In the equation: Lt is the fish's total length (cm) at age t, L ∞ is the asymptotic fish length (cm), t is the fish age(year), to(year) is the hypothetical time at which the fish length is zero, k is the growth coefficient (year -1) (Sparre and Venema, 1998). The Gulland and Holt plotting method was used to estimate the parameters of the von Bertalanffy growth function (Avşar, 2005).

The stomach fullness index was estimated by using the equation FI= Fs/(Fs+Es) (Sartor and Ranieri, 1996).

In this formula; FI: Stomach Fullness Index; Fs: Number of the full stomach; Es: Number of Empty Stomachs.

Food items were identified to the lowest taxonomic level possible. Their number was recorded and weights were determined with the nearest 0.0001 g. The diet of L. boscii specimens was determined by four indices as follows: (i) frequency of occurrence (F%), based on the number of stomachs in which a food item was found; (ii) weight percentage (W%), based on weights of each prey item expressed as the percentage of the total weight of stomach contents; (iii) numerical abundance (N%), based on the number of each prey item in all non-empty stomachs; (iv) the index of relative importance IRI=[N%+W%]* F%*100 (Pinkas et al., 1971) and The IRI value was expressed as a percentage [IRI%= (IRI/∑IRI) x 100] (Cortes,1997). Food items were grouped into preference categories using the method recommended by Morato-Gomes et al. (1998) and Sever et al. (2008). The categories were defined as follows:

Main important prey (MIP): IRI≥ 30*(0.15*∑%F),

Secondary prey (SP): $30*(0.15*\Sigma\%F)>IRI>10*(0.05*\Sigma\%F)$,

Occasional prey (OP): IRI≤10*(0.05*∑%F)

RESULTS

A total of 1864 individuals were obtained during the observation. The number of females was 1283(68.83%), the male was 521 (27.95%) and undetermined was 60 (3.22%). The sex ratio for *L. boscii* specimens was 1:0.41. Significant differences were observed between the sexes (χ^2 =160.83, p≤0.05).

Maximum and minimum total lengths of female and male *L. boscii* specimens were 34.8 and 11.1 cm, 26.7 and 6.3 cm respectively. Specimens between 15.0-17.9 cm were abundant in both sexes (Figure 2).

The mean total lengths value was significant differences between female and male individuals (df: 1802, p<0.0001). Length-weight relationships were calculated separately as female, male, and all individuals. The t-test results of b values results showed positive allometric growth for all individuals and female specimens, and isometric growth for male specimens (Table 1).

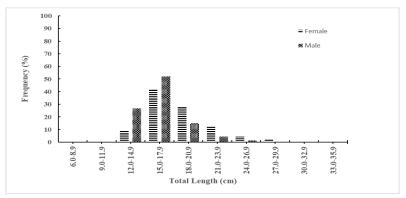


Figure 2. Length distribution of *L. boscii* specimens

The age distribution was observed between I to XIV. Dominant age groups in female, male, and all specimens were II (26.57%), III (26.83%), and II (26.24%) years respectively. (Table 2). Significant differences were observed in age and sex composition between female and male specimens (χ^2 =59.38, p≤0.05). Total length values corresponding to the age group of female and male megrim individuals were evaluated separately and obtained from the sampling results (Table 3). The Von Bertalanffy growth equations were calculated as L ∞ =43.24 cm, k=0.08 year $^{-1}$, t₀=-2.897 year for females, L ∞ =28.53 cm, k=0.11 year $^{-1}$, t₀=-0.829 for males and L ∞ = 42,9 cm, K=0.097, t0= -2.070 for all individuals. A total of 1864 stomach samples were examined,

of which 1313 were found to be empty and 521 were full. Stomach fullness index values were found for females, males, and all individuals 0.31, 0.27, and 0.30 respectively. A total of 9 prey groups were identified from the stomach contents of *L. boscii* specimens. According to the IRI% value to the overall feeding composition, Natantia (IRI%: 70.15) was the first place and Mysidacea (IRI%: 23.42), Anomura (IRI%: 3.09), Teleostei (IRI%: 1.72) and Brachyura (IRI%: 1.30) followed them respectively. According to preference categories, Natantia and Mysidacea specimens were found main important prey (MIP), and Decapod Crustacea, Anomura, and Brachyura specimens were secondary prey (SP). Other groups of diet were found as occasional prey (OP) (Table 4).

Table 1. Length-Weight relationship values of L. boscii individuals (♀: Female Specimens; ♂: Male Specimens; ?: Undetermined Specimens)

Sex	N	LmaxLmin.(cm)	WmaxWmin.(g)	а	W=a*Lbb±%95CI	r	Growth
♀+♂+?	1864	34.8-6.3	393.57-1.24	0.0053	3.13±0.027	0.982	Allometric(+)
2	1283	34.8-11.1	393.57-8.64	0.0053	3.14±0.32	0.982	Allometric(+)
3	521	26.7-6.3	152.35-2.00	0.0071	3.03±0.058	0.975	İsometric

Table 2. Age and sex composition of *L. boscii* specimens

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٨٥٥	2	+3		2		♂	0.7
Age	N	N%	N	N%	N	N%	₽:♂
1	8	1.72	4	1.14	4	3.48	1:1.00
II	122	26.24	93	26.57	29	25.22	1:0.31
III	81	17.42	49	14.00	32	27.83	1:0.65
IV	70	15.05	47	13.43	23	20.00	1:0.49
V	61	13.12	47	13.43	14	12.17	1:0.30
VI	40	8.60	35	10.00	5	4.35	1:0.14
VI	28	6.02	25	7.14	3	2.61	1:0.12
VIII	22	4.73	21	6.00	1	0.87	1:0.05
IV	14	3.01	12	3.43	2	1.74	1:0.17
Χ	7	1.51	5	1.43	2	1.74	1:0.40
XI	2	0.43	2	0.57	-	-	-
XII	4	0.86	4	1.14	-	-	-
XIII	4	0.86	4	1.14	-	-	-
XIV	2	0.43	2	0.57	-	-	-
Total	465	100	350	100	115	100	1:0.33

Table 3. Total length values corresponding to the age group of *L. boscii* specimens

Sex	Age	N	Lmax (cm)	Lmin (cm)	Lmean (cm)
		4	13.00	11.50	12.55
	II	93	16.90	13.20	15.50
	III	49	18.40	17.00	17.61
	IV	47	20.40	18.50	19.38
	V	48	23.90	20.50	21.41
	VI	34	24.40	22.50	23.51
0	VII	25	25.90	24.50	24.99
₽	VIII	21	27.90	26.00	26.71
	IV	12	28.90	28.00	28.34
	Χ	5	29.90	29.20	29.58
	XI	2	30.30	30.20	30.25
	XII	4	31.50	31.00	31.30
	XIII	4	32.70	32.00	32.33
	XIV	2	34.80	33.50	34.15
		4	13.00	6.30	9.63
	II	29	15.60	13.40	14.46
	III	32	17.80	14.20	16.20
	IV	23	19.70	16.20	18.30
8	V	14	22.00	18.10	20.58
0	VI	5	22.60	22.00	22.30
	VII	3	23.50	23.20	23.30
	VIII	1	24.20	24.20	24.20
	IV	2	25.50	25.20	25.35
	Χ	2	26.70	26.30	26.50

Table 4. Dietary groups for *Lepidorhombus boscii*, concerning the frequency of occurrence (F%), weight percentage (W%), (IRI: Percent of the Index of Relative Importance, PC: Preference categories)

Prey Groups	N	N%	F%	W%	IRI%	PC
Amphipoda	14	0.83	2.54	0.01	0.0004	OP
Mysidacea	843	49.82	152.99	10.79	23.42	MIP
Natantia	522	30.85	94.74	52.20	70.15	MIP
Anomura	129	7.62	23.41	9.31	3.09	SP
Brachyura	95	5.61	17.24	5.31	1.30	SP
Stomatopoda	10	0.59	1.81	0.71	0.02	OP
Cephalopoda	12	0.71	2.18	3.95	0.12	OP
Teleostei	47	2.78	8.53	14.24	1.72	OP
Decapod Crustacea	20	1.18	3.63	3.49	0.18	SP

DISCUSSION

Sexes of flatfish specimens show differences in growth and also female individuals reach a larger size than males (Rljnsdorp and Ibelings, 1989; Vassilopoulou and Ondarias, 1999). Differences in growth between male and female individuals have been found in other studies for *L.boscii* specimens (Bello and Rizzi, 1987; Castilho et al., 1993; Vassilopoulou and Ondarias, 1999; Robson et al., 2000; Teixeira et al., 2010; Cengiz et al., 2013; Landa and

Hernandez, 2020). Growth between sexes was determined as statistically different in this study. Growth studies of *L.boscii* specimens have focused on two different areas (Northeast Atlantic and Mediterranean Sea) (Table 5). According to these studies, the Maximum Total length of *L.boscii* specimen was reported in the North Aegean Sea at 48 cm (Sartor et al., 2002).

In this study the growth of megrim, in females was allometric (P<0.005, tcal:8.53, t0.05(1282):1.96), and in males was isometric (p>0.005, tcal:1.00, t0.005(520): 1.96). These growth results have found some studies on length-weight relationships for L. boscii by other authors (Table 6). As a result age determination from L.boscii specimens ranged between I to XIV. Mean lengths of age groups were observed in this study and other *L. boscii* studies were given in Table 7. Differences in age group and mean lengths related to age depend on various factors such as food ability; latitudinal differences, fishing efforts, and temperature (Nash and Geffen, 2005). It was observed that female individuals reached older ages than males. This difference in the growth of L. boscii specimens was found in other studies (Castilho et al., 1993; Santos, 1994; Vassilopoulou and Ondrias, 1999; Robson et al., 2000; Landa et al., 2002; Cengiz et al., 2013; Landa and Fontela, 2016; Landa and Hernandez, 2020).

Table 5. A comparison of L. boscii specimen's maximum and minimum length values

Area	Locality	N	Length	Max. (cm)	Min. (cm)	References
	Portuguese Coasts	578	TL	39.1 (♀)	10.2 (♂+♀)	Castilho et al. (1993)
Atlantic	The West Coast of Ireland	150	TL	41.9 (♀)	24.2 (♂)	Robson et al. (2000)
	Portuguese Coasts	302	TL	34.6	14,9	Teixeira et al. (2010)
	North Aegean Sea	15323	TL	29.5 (♀)	5 (♂)	Vassilopoulou and Ondarias (1999)
	Morroco-Spain-France Coasts	3427	TL	36	3	Sartor et al. (2002)
	Sardinia- Tyrrhenian Sea	6163	TL	38	4	Sartor et al. (2002)
Mediterranean	Adriatic Sea	900	TL	35	7	Sartor et al. (2002)
Sea	Ionian Sea	1628	TL	41	2	Sartor et al. (2002)
	North Aegean Sea	4080	TL	48	2	Sartor et al. (2002)
	Saros Bay North Aegean Sea	788	TL	40.8	10.9	Cengiz et al. (2013)
	Aegean Sea	1864	TL	34.8 (♀)	6.3 (♂)	This study

Table 6. Comparison of the Length-Weight Relationship values between L. boscii's studies

Area	Locality	Sex	N	а	b	r	Growth Types	References
	Portuguese Coasts	9	250 315	2.01x10 ⁻⁶ 4.11x10 ⁻⁶	3.25 3.11	0.98 0.98	Allometric Allometric	Castilho et al. (1993)
Atlantic	Portuguese Coasts	9	306 351	0.0025 0.0045	3.36 3.16	-	-	Santos (1994)
	Ireland Coasts	♀+♂+?	150	0.0062	3.37	0.96	Allometric (+)	Robson et al. (2000)
	South Adriatic Coasts	♀ +♂+? ♀ ♂	237 109 128	0.00333 0.00323 0.00344	3.25 3.26 3.24	0.99 0.99 0.99	- - -	Bello and Rizzi (1987)
Maditananaaa	Saros Bay	♀ +♂+?	521	0.00316	3.29	0.99	-	İşmen et al. (2007)
Mediterranean Sea	Saros Bay	9	553 235	0.0032 0.0069	3.31 3.04	0.99 0.98	Allometric (+) Isometric	Cengiz et al. (2013)
	Aegean Sea (Karaburun-Kusadası)	♀ +♂+? ♀ ♂	1864 1283 521	0.0053 0.0053 0.0071	3.13 3.14 3.03	0.98 0.98 0.97	Allometric (+) Allometric (+) Isometric	This study

Table 7. Lengths of age groups for L. bosoil specimens were stated by other studies (mean or range of TL in cm as given in the related studies)

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Area	Locality	Sex	z	÷	_	=	≡	2	^	IV	IIA	NIII	×	×	×	^ IIX	IX IIIX	XIV Re	References
	Portuguese Coasts	⁶ 0 0+	327		11	13.3	16.4	18.7	22	24.4	25.2 30.2	27.1	31.8 35.1	37.1	- 3 37.2 3	32.5 37.6		Castill	Castilho et al. (1993)
Atlantic	Northern Coasts of Spain	₹0 O+	1895	1 1	5-10	10-15	15-17	17-20	20-22	22-23	23-24	24-25	33-34	35-36	t t			Landa	Landa et al. (2002)
	Cantabrian Sea & Galician waters	\$ +	Ç+♂ 11741	7.4	14.3	18.8	22	24.3	26.6	28.6	30.5	32.4	33.8		1			- Landa	Landa and Fontela (2016)
	Porcupine Bank	\$ + \$	⊋+∂ 1334	4.6	11.9	15.9	19.2	22.4	24.1	27.2	29.4	32.0	34.3	37.3	39.4			- Landa	Landa and Hemandez (2020)
	South Adriatic Coasts	⁵ O O+	82	1 1	9.81	14.13	16.45	18.22	19.6	22.3		1 1						Bello 8	Bello and Rizzi (1987)
Modiferranean	North Aegean Sea	⁶ O O+	923		7.1-16.0	5.1-11.0 7.1-16.0 10.1-17.0 12.1-19.0 15.1-21.0 17.1-22.0 20.1-22.0 5.1-12.0 8.1-15.0 12.1-19.0 15.1-21.0 16.1-23.0 18.1-25.0 20.1-27.0 23.1-26.0 23.1-28.0	12.1-19.0	15.1-21.0	17.1-22.0	20.1-22.0	-23.1-26.0	-23.1-28.0			1 1	т г		Vassik	Vassilopoulou and Ondarias (1999)
Sea	North Aegean Sea (Saros Bay)	₹O O+	140	1 1	12.5	14.3	17.1	19.5	20.8	24 26.8	25 28.9	31.7	29.9	34.6	- 36.4 3	- 37.2 3	38.2	- Cengiz	Cengiz et al. (2013)
	Aegean Sea (Karaburun-Kuşadası)	⁵ 0 O+	115 350	1 1	9.63	14.46	16.2	18.3	20.6	22.3	23.3	24.2	25.35 28.34	26.5	26.5 29.58 30.25 31.3 32.3	31.3 3		- т 34	This Study

When we compare growth parameters between other $L.\ boscii$ studies and our results, there are some differences between Atlantic and Mediterranean studies. $L.\ boscii$'s L^∞ values were determined higher in the studies of the Atlantic than in the Mediterranean. Differences in studies may be attributed to

many factors (sample sizes, geographical differences, aging methodology, water temperature, etc.) (Cengiz et al., 2013; Landa and Hernandez, 2020) and the different methodologies to find age estimation and length frequency analysis (Landa and Fontela, 2016). Obtained results of growth parameters are given in Table 8.

Table 8. Some of the studies on growth parameters of *L. boscii* specimens [The methodology of age estimation: Direct age estimation (DAE), Back-calculation (BC), and Direct age estimation & back-calculation (DB)]

Area	Locality	N	Sex	Methodology	L∞(cm)	k (year-1)	t ₀ (year)	References
	Portuguese Coasts	250 328	97	DAE DAE	44.0 37.5	0.14 0.14	-1.52 -1.93	Castilho et al. (1993)
Atlantic	Portuguese Coasts	227 217	9	DAE DAE	39.77 34.79	0.157 0.198	-1.858 -1.436	Santos (1994)
Additio	The West Coast of Ireland	150	₽+♂	DAE	34.39	0.27	-1.997	Robson et al. (2000)
	Porcupine Bank (ICES div. 7/b/c/k)	1334	₽+♂	DAE BC DB	56.46 43.74 50.09	0.11 0.17 0.13	-0.09 -0.13 -0.30	Landa and Hernandez (2020)
	South Adriatic Coasts	55 82	9 %	DAE DAE	28.5 27.6	0.26 0.21	-0.85 -1.27	Bello and Rizzi (1987)
Mediterranean	North Aegean Sea	1241 923	9	DAE DAE	30.5 25.53	0.179 0.219	-1.103 -1.087	Vassilopoulou and Ondarias (1999)
Sea	North Aegean Sea (Saros Bay)	553 235	9	DAE DAE	49.8 39.1	0.09 0.11	-2.15 -2.59	Cengiz et al. (2013)
	Aegean Sea (Karaburun-Kusadası)	1283 521	9	DAE DAE	43.2 28.5	0.09 0.23	-2.9 -0.83	This study

A total of 1864 four spotted megrim's stomach contents were examined during the study. The number of empty stomachs was 1313 (70%) and the number of full stomachs was 511 (30%). In the cost of Spain in the West Mediterranean Sea, it was determined that 81% of the examined specimens had full stomachs (Morte et al., 1999). The difference between the two studies may be thought that the stomachs of *L boscii* individuals in the study on the coast of Spain were fixed immediately to stop digestive activity thus this may be caused the number of full stomachs to be high. In our sample stomachs could be fixed after coming to the laboratory. For this reason, it is thought that some half-digested or easily digestible foods continue to be digested even after death, causing empty stomachs to count to be high.

Other diet studies of *L.boscii* stated that the food of specimens is composed of living creatures that mostly live buried in the benthic zone (Morte et al., 1999). *L.boscii* like other Scolphthalmid fish species, has a large and muscular esophagus, stomach, and intestine. This advanced nutrition system makes it easy to swallow and digest great food such as shrimp, fish, and crustaceans (De Groot, 1971). The stomach contents of the individuals in our study were obtained when viewed based on IRI value %, shrimp specimens of Natantia were obtained as the first group with a rate of 70.15 %. The obtained results seem to support the above studies.

The study of the feeding regime of four spotted megrim specimens in Portugal waters has been found to mainly Decapoda and Teleostei individuals (Teixeira et al., 2010). In our study, the Mysidacea group of individuals was found an important prey group in the diet of *L. boscii* specimens. This difference is thought to be due to the sampling of more small-sized than those studied in Portugal waters and these small individuals generally preferred the Mysidacea group.

In a study on the Western Mediterranean coast of Spain, are mainly Mysidacea and Natantia groups of individuals in *L. boscii* specimens (Morte et al., 1999). In another study about the feeding regime of four spotted megrim specimens on the North Aegean sea coast of Greece, mainly feeding groups of specimens are Mysidacea and Natantia group of individuals (Vassilopoulou, 2006). The similarities between our results and studies in Spain and Greece are observed.

CONCLUSION

International waters in the rest of Kusadasi – Karaburun (located in the central zone of the Aegean Sea) defined as the study area is an important fishing area for the Aegean region. It is being carried out in this region of intensive trawl fisheries. While Deep-water rose shrimp [Parapenaeus longirostris (Lucas, 1847)] and European hake [Merluccius merluccius (Linnaeus, 1758)] were identified as target species, Fourspotted Megrim (L. boscii) individuals are also considered to be economic in recent years. It is believed that the presented

results improve our knowledge of the feeding features and growth parameters of *L. boscii* specimens in the Aegean sea stocks.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

All relevant data is inside the article

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