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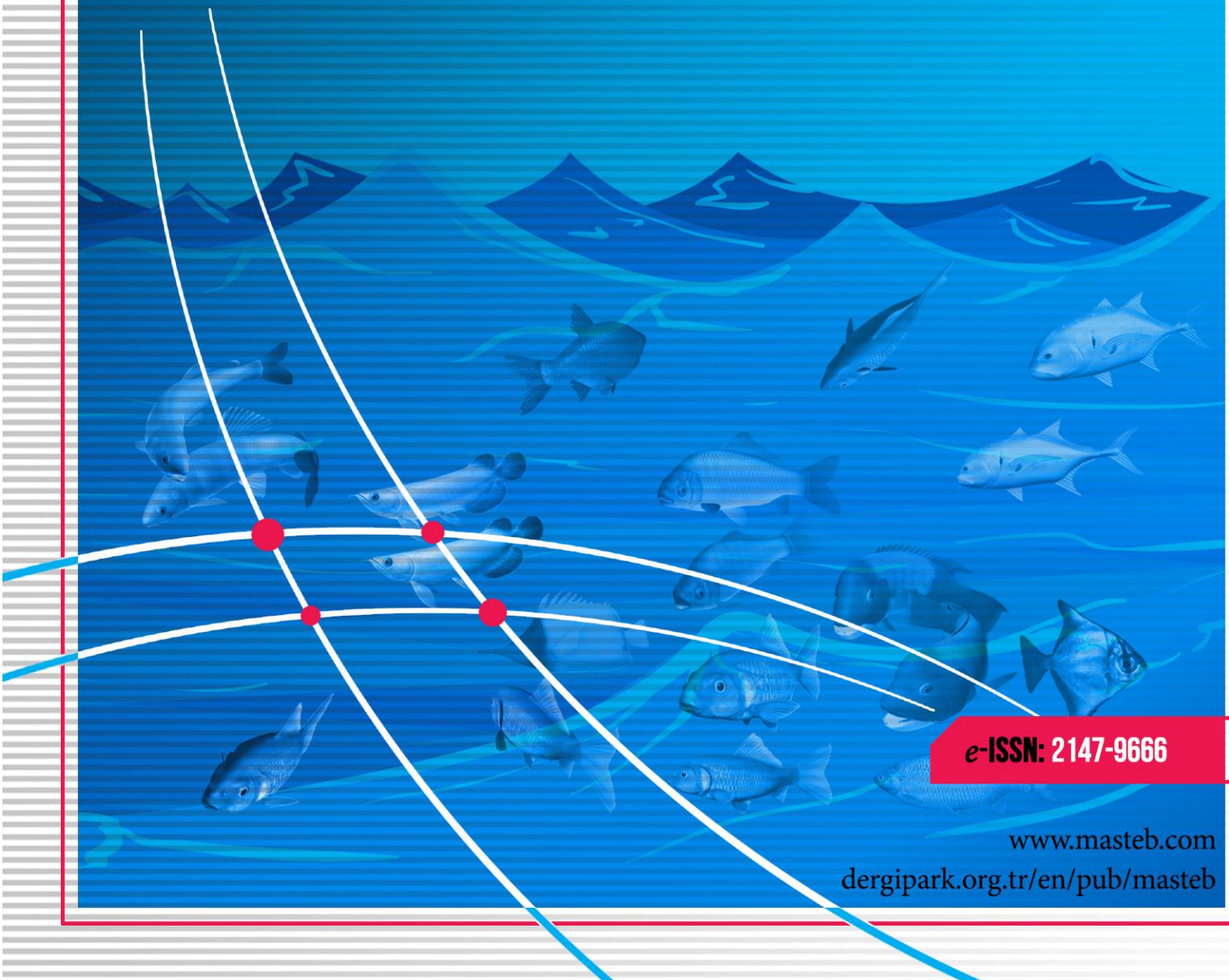

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SHORT COMMUNICATION

The first substantiated record of blunthead puffer *Sphoeroides pachygaster* (Müller and Troschel, 1848), from the coast of northern Cyprus (eastern Mediterranean)

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ABSTRACT

Blunthead puffer *Sphoeroides pachygaster* (Müller and Troschel, 1848) is a circumglobally distributed Tetraodontid found in temperate and tropical waters. It is believed to have colonized the Mediterranean Sea from the Atlantic Ocean. There are relatively few records of this fish in the Mediterranean Sea. On March 2020, a mature female individual was caught incidentally by a commercial fisher in Northern Cyprus. The fish was 520 mm in total length and 1200 grams in total weight. Detailed morphometrics and meristics were reported. This study is the first substantiated record of *S. pachygaster* from Cyprus waters, and the specimen is the largest blunthead puffer reported with a 455 mm standard length and 520 mm total length.

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Introduction

Four genera of Tetraodontidae are found in the Mediterranean, which are *Torquigener, Ephippion, Sphoeroides,* and *Lagocephalus* (Golani, 1987, 1996; Reina-Hervás et al., 2004; Akyol et al., 2005; Corsini et al., 2005; Vacchi et al., 2007; Vella et al., 2017). The blunthead puffer, *Sphoeroides pachygaster* (Müller and Troschel, 1848), inhabits warm and tropical waters circumglobally and is distributed widely in the Atlantic Ocean (Sampaio et al., 2001), generally inhabiting the deep sea and found between depths of 50-480 m (Matasuura and Tyler, 1997). In the Mediterranean Sea, *S. pachygaster* was reported for the first time in 1979 in the waters of the Balearic Islands, Western Mediterranean Basin (Oliver, 1981). The species was first reported for the Eastern Mediterranean Basin of Israel in 1991 (Golani, 1996). Two specimens of *S. pachygaster* were reported from the North Aegean Sea in 2003 (Eryılmaz et al., 2003), and its northernmost record was from Slovenia (Adriatic) in 2012. *S. pachygaster* is broadly distributed throughout the Mediterranean Basin according to the available literature given for this species. But, reports of its existence in some areas are still relatively low (Lipej et al., 2013). The presence of *S. pachygaster* in Cyprus was first reported in 2005 by Katsanevakis et al. (2009).

Like other pufferfish species, *S. pachygaster* is a predatory fish species. They mostly feed on mollusks, crustaceans, and teleosts (Carbonara et al., 2017). The largest individual reported for the species is 466 mm in total length (Rahman et al., 2014). Here we present the first record of *S. pachygaster* from the west coast of Northern Cyprus, maximum length report of the species up to date, and the first detailed morphometrics for this species on the island.

Material and Methods

During a fishing port survey carried out by bycatch observers of the non-governmental organization (NGO) Society for Protection of Turtles on March 11, 2020, a *S. pachygaster* specimen was noted among the threatened species bycatch reported by a participating commercial fisher. As part of a bycatch monitoring program, the fisher was being tracked using a remote GPS data logger (I Got U, GT-.600 recording one location every 5 minutes). The specimen was caught incidentally at a bottom-set trammel net at 250 m deep (recorded on the fishing vessel depth sounder), between four to seven nautical miles from the coast of Yedidalga, Northern Cyprus (Coordinates: 35°12.452 N, 32°49.891 E) (Figure 1).

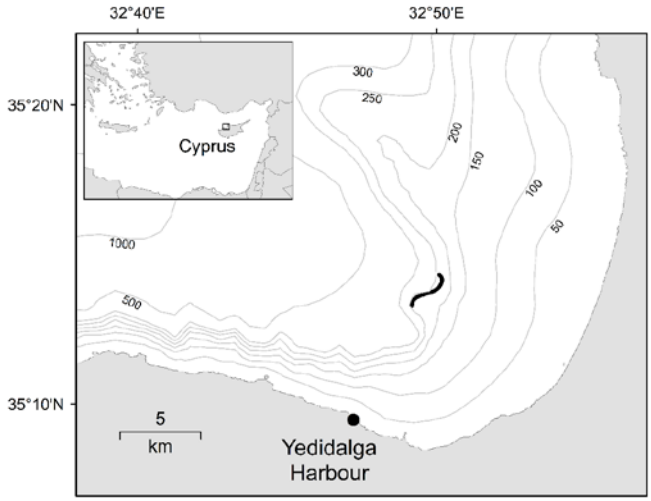

Figure 1. GPS track (dark black line) from an onboard data logger of the set placement in which S. *pachygaster* was caught off the coast of the west coast of Northern Cyprus. Bathymetric contours labeled are in meters

The fisher was targeting European hake (*Merluccius merluccius* (Linnaeus, 1758)) using a trammel net with a 25 mm internal mesh bar length,140 mm outer nets, and a net height of 1.5-2 m. The length of the set was 3000 m. The setting of the net began at 03:34 and ended at 04:32. According to the fisher, the benthos substrate at the set location was muddy. The haul began at 06:02 and ended at 09:42. At the port, the participating fisher informed the observers of an unusual fish among the longnose spur dogs (*Squalus blainville* (Russo, 1827)) and thornback rays (*Raja clavata* (Linnaeus, 1758)) which were being reported routinely as bycatch. The specimen was transferred to the SPOT offices in Nicosia and immediately frozen.

On thawing for examination, species identification was accomplished, according to Cherif et al. (2010). Morphometric measurements were taken, stomach content was analyzed macroscopically, and gender was determined through macroscopic examination of the gonads.

Results

The *S. pachygaster* specimen was found to be a mature female. Its total length was 520 mm, and it weighed 1200 g. The stomach was empty, and part of the intestine had become prolapsed. The specimen (Figure 2) had a smooth skin without any scales or spines. The dorsal fin was short and located in the same vertical line with the anal fin. The pelvic fin was absent. The edges of the caudal fin were spherical, and the tip was flat. The pectoral fin was more prominent than other fins and, the base was darker. The lower part of the body was rough and white, with the potential for inflation of the body, like other puffers. The dorsal of the body was greyish-brown-green, and

Table 1. Comparison of morphometric and meristic characters of *S. pachygaster* with some previous studies

	This study		Cherif et al. (2010)		Hemida et al. (2009)	
Measurements	***Value***	***%TL***	***Value***	***%TL***	***Value***	***%TL***
Morphometrics (mm)						
TL	520	100	101	100	330	100
SL	455	87.5	90	89.1	295	89.4
HL	150	28.8	32.1	31.8	100	30.3
HH	92	17.7	25.3	25	60	18.2
EHD	28	5.4	8.1	8	21	6.4
EVD	18	3.5	4.1	4.1	21	6.4
IOS	65	12.5	17.9	17.7	30	9.1
SNL	79	15.2	11.4	11.3	40	12.1
POL	50	9.6	10.7	10.6	35	10.6
WOP	49	9.4	12.1	12	35	10.6
WGO	31	6.0	8.2	8.1	25	7.6
PDL	335	64.4	61.5	60.9	215	65.2
DFL	42	8.1	9.5	9.4	25	7.6
DFB	18	3.5	6.1	6	11	3.3
AFL	34	6.5	10.2	10.1	30	9.1
AFBL	12	2.3	3.5	3.5	11	3.3
PFL	63	12.1	13.4	13.3	30	9.1
CFL	65	12.5	15.6	15.4	38	11.5
BT	93	17.9	28.9	28.6	90	27.3
BH	82	15.8	33.9	33.6	90	27.3
NGD	7	1.3	3.7	3.7	6	1.8
NLD	4,3	0.8	2.6	2.6	4	1.2
INS	32	6.2	13.6	13.5	30	9.1
Meristics (mm)						
DFSR	8		8		8	
AFSR	8		8		8	
PFSR	16		15		15	
CFSR	13		12		10	
Weights (g)						
TW	1200		47.91		650	
LW	70		2.59			
GW	20		0.16			
Gender	Female		Female		Male	

Note: Abbreviations used: TL (Total length), SL (Standard length), HL (Head length), HH (Head height), EHD (Eye horizontal diameter), EVD (Eye vertical diameter), IOS (Interorbital space), SNL (Snout length), POL (Postorbital length), WOP (Width of pedunculum), WGO (Width of gill opening), PDL (Predorsal length), DFL (Dorsal fin base), AFL (Anal fin length), AFBL (Anal fin base length), PFL (Pectoral fin length), CFL (Caudal fin length), BT (Body thickness), BH (Body height), NGH (Nostril greatest diameter), NLD (Nostril lesser diameter), INS (Internarial space), DFSR (Dorsal fin soft rays), AFSR (Anal fin soft rays), PFSR (Pectoral fin soft rays), CFSR (Caudal fin soft rays), TW (Total weight), LW (Liver weight), GW (Gonad weight).

Table 2. Some capture data from the Mediterranean

Location	Year	Depth (m)	Catch method	Sex	Total Length (mm)	Reference
Strait of Sicily	1990-1994	80-400	Bottom trawl	♀,♂	95-455	Ragonese et al. (1997)
Saros Bay	1999-2001	125-180	Bottom trawl	N/A	167-395	Eryılmaz et al. (2003)
Tyrrhenian Sea	2004	360	Bottom trawl	♂	137	Psomadakis et al. (2008)
Algerian Coast	2008	150	Trawl	♂	330	Hemida et al. (2009)
Cyprus	2009	N/A	N/A	N/A	N/A	Katsanevakis et al. (2009)
Strait of Messina	2012	N/A	N/A	♂	280	Giordano et al. (2012)
Syria	2012	250	Bottom longline	♀	312-466	Rahman et al. (2014)
Adriatic Sea	2012-2015	75-125	Trawl and longline	♀,♂	200-355	Carbonara et al. (2017)
Cyprus	2020	250	Bottom trammel net	♀	520	Present study

there were dark blotches on the body. Its large eyes were oval-shaped, located dorsally in the head, and orientated longitudinally to the body. The head was large, and the snout was round. A pair of teeth in the upper and lower jaw were fused, and beak-shaped.

Figure 2. Photo taken by the observer while the specimen caught

All morphometric measurements and meristics of the specimen are given in Table 1, where they are also compared with those taken in two other chosen studies. Also, some capture data of *S. pachygaster* from the Mediterranean Sea were given in Table 2.

Discussion

The introduction of pufferfish species to the Mediterranean is a well-known issue. *Lagocephalus lagocephalus* (Linnaeus, 1758) is a native pufferfish species for the Mediterranean Sea. *Ephippion guttifer* (Bennett, 1831), *Sphoeroides marmoratus* (Lowe, 1838), *S. pachygaster* (Müller and Troschel, 1848), and S. spengleri (Bloch, 1785) are migrated from the Atlantic Ocean. *Tylerius spinosissimus* (Regan, 1908), *Torquigener flavimaculosus* (Hardy and Randall, 1983), *L. sceleratus* (Gmelin, 1789), *L. spadiceus* (Richardson, 1845), *L. suezensis* (Clark and Gohar, 1953) and *L. guentheri* (Miranda Ribeiro, 1915) are Lessepsian migrants (Bariche et al., 2015; Vella et al., 2017). The global conservation status of *S. pachygaster* is reported as Least Concern in The IUCN Red List of Threatened Species (Shao et al., 2014).

With the ability to inflate their bodies with air or water, they can protect themselves from many predator fishes who ingest by vacuum in the Mediterranean Sea (Brainerd, 1994). Besides, many types of pufferfish cannot be used as a food source for humans, thanks to the tetrodotoxin they contain, and so are not commercially exploited and can be returned to the sea alive. Some species of pufferfish, including *S. pachygaster*, are known to be non-toxic (Tani, 1945; Jeong et al., 1994). A study shows that *S. pachygaster* can accumulate TTX if incubated with it for a long time (Nagashima et al., 2018). Since it is known that TTX is transferred to pufferfish through the food chain (Bane et al., 2014), it would be more accurate to say that *S. pachygaster* can accommodate TTX in favorable conditions. Therefore, consumption can lead to severe poisoning cases. *S. pachygaster* can be distinguished from other Tetraodontidae members by several characteristics. First, it prefers to live in deeper waters than other species living in the Mediterranean Sea. Its body structure is chunkier than other species, and the skin is completely smooth. The snout is rounded. The dorsal of the body is grayish and covered with brownish spots. Fins are relatively shorter than other species of their size (Rahman et al., 2014).

With its 455 mm standard length and 520 mm total length, our specimen is the largest blunthead puffer ever reported globally, but it is not the heaviest. The most massive individual was reported by Rahman et al. (2014) as 1850 gr. The fish's stomach was found to be empty, which may, in part, be due to vomiting and prolapse during capture. In order to reach a more reliable conclusion on the spawning season, more fish samples must be collected, and maturity studies should be conducted. All the morphometric and meristic data agreed with the

previous studies. According to Table 1, anal fin length, anal fin base length, body thickness, and body height percentages are smaller in our study. These differences may be coincidental or related to the age and biological condition of the fish. In order to make healthy comments on this subject, length-weight and age analysis should be done on more samples.

The most common technique to catch *S. pachygaster* is trawling. The catching of fish occurred mostly at depths of more than 100 m. The genders of the captured samples are variable. Juvenile and adult individuals were caught at similar depths together in the same studies (Ragonese et al., 1997; Eryılmaz et al., 2003; Psomadakis et al., 2008; Hemida et al., 2009; Rahman et al., 2014; Carbonara et al., 2017). *S. pachygaster* caught in our research is the first case of catch by using a bottom trammel net.

In Cyprus waters, introduced *Lagocephalus sceleratus* (Gmelin, 1789) and *Torquigener flavimaculosus* (Hardy and Randall, 1983) are the most abundantly observed and caught pufferfish species according to local fishers (Personal observation). Some commercial vessels land more than a ton of *L. sceleratus* annually (Robin Snape, unpublished data). Blunthead puffer is a deep-sea species and generally does not frequent the shallower depths of most commercially targeted species in Cyprus (most commercial fishing in Northern Cyprus occurs on the benthos at <100 m depth, Robin Snape personal observation). This species is somewhat protected from fisheries through its habitat preferences and may also be underrepresented in related research. *S. pachygaster* was recorded for the first time from Cyprus in 2005 (Katsanevakis et al., 2009). For *L. sceleratus* in Cyprus, gonadosomatic index and toxicity studies are present (Rousou et al., 2014; Akbora et al., 2020). There is a gap of information for the toxicity of *S. pachygaster* in Cyprus. Under the Fisheries Retailing Places Regulation (14.10.2005 - R.G. 176 - ANNEX III - A.E. 596 Regulation); "It is forbidden to sell pufferfish (Tetraodontidae, Canthigasteridae), porcupine fish (Diodontidae), and prop/moons (Molidae)" (KKTC Mahkemeleri, 2020). Despite the stated ban in the regulation, many species of pufferfish are consumed unconsciously by many people due to their consumption in the Far Eastern countries. To prevent the risks that may arise in the case of encountering this rare species in the region, the toxicity study should be conducted by conducting targeted sampling of the species. Locals must be informed of the results collected.

Conclusion

In Cyprus, trawling is prohibited, and this reduces the chance of encounters of organisms such as *S. pachygaster*, which are living in the deep seas. The specimen presented in this study is the biggest individual reported up to date, and the first *S. pachygaster*, which was reported to catch by a bottom trammel net.

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Compliance with Ethical Standards

Authors' Contributions

Author HDA designed the study, HDA and RS wrote the first draft of the manuscript, DA and BAÇ contributed to the presentation of the results. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Akbora, H. D., Kunter, İ., Erçetïn, T., Elagöz, A. M. & Çïçek, B. A. (2020). Determination of tetrodotoxin (TTX) levels in various tissues of the silver cheeked puffer fish (*Lagocephalus sceleratus* (Gmelin, 1789)) in Northern Cyprus Sea (Eastern Mediterranean). *Toxicon*, ***175***: 1-6. https://doi.org/10.1016/j.toxicon.2019.12.002

Akyol, O., Ünal, V., Ceyhan, T. & Bilecenoglu, M. (2005). First confirmed record of *Lagocephalus sceleratus* (Gmelin, 1789) in the Mediterranean Sea. *Journal of Fish Biology*, ***66***(4): 1183-1186. https://doi.org/10.1111/j.0022-1112.2005.00667.x

Bane, V., Lehane, M., Dikshit, M., O'Riordan, A. & Furey, A. (2014). Tetrodotoxin: Chemistry, toxicity, source, distribution and detection. *Toxins,* ***6***(2): 693-755. https://doi.org/10.3390/toxins6020693

Bariche, M., Torres, M., Smith, C., Sayar, N., Azzurro, E., Baker, R. & Bernardi, G. (2015). Red Sea fishes in the Mediterranean Sea: A preliminary investigation of a biological invasion using DNA barcoding. *Journal of Biogeography*, ***42***(12): 2363-2373. https://doi.org/10.1111/jbi.12595

Brainerd, E. L. (1994). Pufferfish inflation: functional morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). *Journal of Morphology*, ***220***(3): 243-261. https://doi.org/10.1002/jmor.1052200304

Carbonara, P., Kolitari, J., Đurović, M., Gaudio, P., Ikica, Z., Kroqi, G., Milone, N. & Spedicato, M. T. (2017). The presence of Tetraodontidae species in the Central Mediterranean: An update from the southern Adriatic Sea. *Acta Adriatica: International Journal of Marine Sciences*, ***58***: 325–338. https://doi.org/10.32582/aa.58.2.11

Cherif, M., Amor, M. M. B., Bdioui, M., Salem, S. B., Missaoui, H. & Capapé, C. (2010). Additional records of the blunthead puffer, *Sphoeroides pachygaster* (Osteichthyes: Tetraodontidae) off the Tunisian coast (Central Mediterranean). *Annales: Series Historia Naturalis, **20***: 33-36.

Corsini, M., Margies, P., Kondilatos, G. & Economidis, P. S. (2005). Lessepsian migration of fishes to the Aegean Sea: First record of *Tylerius spinosissimus* (Tetraodontidae) from the Mediterranean, and six more fish records from Rhodes. *Cybium*, ***29***(4): 347-354.

Eryılmaz, L., Özuluğ, M. & Meriç, N. (2003). The smooth pufferfish, *Sphoeroides pachygaster* (müller & troschel, 1848) (Teleostei: Tetraodontidae), new to the northern Aegean Sea. *Zoology in the Middle East*, ***28***: 125-126. https://doi.org/10.1080/09397140.2003.10637962

Giordano, D., Profeta, A., Pirrera, L., Soraci, F., Perdichizzi, F., Greco, S., Perdichizzi, A. & Rinelli, P. (2012). On the occurrence of the blunthead puffer, *Sphoeroides pachygaster* (Osteichthyes: Tetraodontidae), in the Strait of Messina (Central Mediterranean). *Journal of Marine Biology*, ***2012***: 462407. https://doi.org/10.1155/2012/462407

Golani, D. (1996). The marine ichthyofauna of the eastern Levant - History, inventory, and characterization. *Israel Journal of Zoology*, 42(1): 15-55. https://doi.org/10.1080/00212210.1996.10688830

Golani, D. (1987). The Red Sea pufferfish, *Torquigener flavimaculosus* Hardy & Randall 1983, a new Suez Canal migrant to the Eastern Mediterranean (Pisces: Tetraodontidae). *Senckenbergiana Maritima: wissenschaftliche Mitteilungen der Senckenbergischen naturforschenden Gesellschaft*, ***5-6***: 339-343.

Hemida, F., Ben Amor, M. M. & Capapé, C. (2009). First confirmed record of the blunthead puffer, *Sphoeroides pachygaster* (Osteichthyes: Tetraodontidae) off the Algerian coast (south-western Mediterranean). *Pan-American Journal of Aquatic Sciences*, ***4***(2): 188-192.

Jeong, D. Y., Kim, D. S., Lee, M. J., Kim, S. R., Byun, D. S., Kim, H. D. & Park, Y. H. (1994). Toxicity of several puffers collected at a fish market of Pusan, Korea. *Korean Journal of Fisheries and Aquatic Sciences*, ***27***(6): 682-689.

Katsanevakis, S., Tsiamis, K., Ioannou, G., Michailidis, N. & Zenetos, A. (2009). Inventory of alien marine species of Cyprus (2009). *Mediterranean Marine Science, **10***(2): 109-133. https://doi.org/10.12681/mms.113

KKTC Mahkemeleri. (2020). Tüzükler. Retrieved on May 17, 2020 from https://www.mahkemeler.net/cgi-bin/showtuzuk2.aspx

Lipej, L., Mavrič, B. & Paliska, D. (2013). New northernmost record of the blunthead pufferfish, *Sphoeroides pachygaster* (Osteichthyes: Tetraodontidae) in the Mediterranean Sea. *Annales: Series Historia Naturalis, **23***: 103-114.

Matsuura, K. & Tyler, J. C. (1997). Tetraodontiform fishes, mostly from deep waters, of New Caledonia. *Résultats des campagnes MUSORSTOM*, ***17***: 173-208.

Nagashima, Y., Ohta, A., Yin, X., Ishizaki, S., Matsumoto, T., Doi, H. & Ishibashi, T. (2018). Difference in uptake of tetrodotoxin and saxitoxins into liver tissue slices among pufferfish, boxfish and porcupinefish. *Marine Drugs*, ***16***(1): 17. https://doi.org/10.3390/md16010017

Oliver, P. (1981). Sobre la aparicio´n de algunos peces raros en las Islas Baleares. *Boletín Instituto Español de Oceanografía, **6***: 59-64.

Psomadakis, P. N., Ceddia, P. & Vacchi, M. (2008). Additional record of *Sphoeroides pachygaster* (Pisces: Tetraodontidae) in the Tyrrhenian Sea and notes on the distribution of the species in the Mediterranean. *Marine Biodiversity Records,* 1: e18. https://doi.org/10.1017/s1755267206001862

Ragonese, S., Jereb, P. & Morara, U. (1997). Morphometric relationships of *Sphoeroides pachygaster* (Pisces - Tetraodontidae) of the strait of Sicily (Mediterranean Sea). *Cahiers de Biologie Marine*, ***38***(4): 283-290.

Rahman, W. A., Galiya, M. & Ali, A. K. (2014). First record of the blunthead puffer *Sphoeroides pachygaster* (Osteichthyes: Tetraodontidae) in Syrian marine waters (eastern Mediterranean). *Marine Biodiversity Records*, ***7***: e31. https://doi.org/10.1017/S1755267214000244

Reina-Hervás, J. A., García Raso, J. E. & Manjón-Cabeza, M. E. (2004). First record of *Sphoeroides spengleri* (Osteichthyes: Tetraodontidae) in the Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom*, ***84***(5): 1089-1090. https://doi.org/10.1017/S0025315404010495h

Rousou, M., Ganias, K., Kletou, D., Loucaides, A. & Tsinganis, M. (2014). Maturity of the pufferfish *Lagocephalus sceleratus* in the southeastern Mediterranean Sea. *Sexuality and Early Development in Aquatic Organisms*, ***1***: 35-44. https://doi.org/10.3354/sedao00005

Sampaio, C. L. S., Lopes, P. R. D. & Olavo, G. (2001). Registros de *Lagocephalus lagocephalus* (Linnaeus, 1758) e *Sphoeroides pachygaster* (Müller & Troschel, 1848) (Actinopterygii: Tetraodontidae) para o litoral da Bahia, nordeste do Brasil. *Interciencia*, ***26***: 157-160.

Shao, K., Liu, M., Jing, L., Matsuura, K., Hardy, G. & Leis, J. L. (2014). *Sphoeroides pachygaster*. The IUCN Red List of Threatened Species 2014: e. T190193A1943625. https://doi.org/10.2305/IUCN.UK.2014-3.RLTS.T190193A1943625.en Downloaded on 09 April 2020.

Tani, T. (1945). Nihonsan fugu no chudokugakutekikenkyu (Toxicological studies on Japanese puffer). *Tokyo, Teikokutosho*, ***1945:*** 15-27.

Vacchi, M., Bussotti, S., Miglietta, A. M. & Guidetti, P. (2007). Presence of the Guinean puffer *Sphoeroides marmoratus* (Lowe, 1838) in the Mediterranean Sea. *Journal of Fish Biology*, ***71***(4): 1215-1219. https://doi.org/10.1111/j.1095-8649.2007.01578.x

Vella, A., Vella, N., Karakulak, F. S. & Oray, I. (2017). DNA barcoding of Tetraodontidae species from the Mediterranean Sea: Filling knowledge gaps for improved taxonomic accuracy. *Genetics of Aquatic Organisms,* ***1***: 61-69. https://doi.org/10.4194/2459-1831-v1_2_05

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RESEARCH ARTICLE

Morphologic characteristics and length-weight relationships of *Sciaena umbra* (Linnaeus, 1758) in the Black Sea coast

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ABSTRACT

In this study, the morphological characteristics and length-weight relationships of *Sciaena umbra* (Linnaeus, 1758) belonging to the Sciaenidae family, which is represented by five species in the Mediterranean basin and two species in the Black Sea, were investigated. Sampling was carried out in the Black Sea Region (Samsun, Ordu, Giresun, Trabzon) between March 2019 and February 2020. A total 54 of individuals were sampled and 15 different metric measurements were performed in each sample to determine their morphological characteristics. The mean total length and weight were estimated as 357.8 mm (117-580) and 845.3 g (16.4-2485.1), respectively. Total length was compared with morphometric characters and the lowest ratio was found with eye diameter (4.3%) and the highest ratio was with anal distance (59.9%). In the relation between the total length and morphological characters of the highest and the lowest correlation were observed in dorsal distance with r^2=0.993 and the anal height with r^2=0.938. A strong correlation (r^2 = 0.993) was found between the total length and weight relationship and the growth was positive allometric b> 3. This paper reports the first documented of morphometric characteristics of the species. It is considered to contribute to fisheries biology and international scientific literature.

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Introduction

The brown meagre, *Sciaena umbra* Linnaeus, 1758, is one of the five species of the Sciaenidae (croakers or drums) family present in the Mediterranean Sea (Fischer et al., 1987). It is a demersal species with a wide distribution from the East Atlantic Ocean to the Mediterranean, Aegean, Black Sea and Azov Sea (Artüz, 2006; La Mesa et al., 2008; Chao, 2015). This species, which mostly lives on rocky and hard substrata, can grow up to a maximum length of 70 cm, but they are mostly found around

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30 cm (Bauchot, 1987). The brown meagre is distributed in all the coasts of Turkey. This species is social and lives in small groups (20-150 individuals) (Artüz, 2006). The brown meagre is a sedentary and gregarious species living in shelters on rocky bottoms close to caves or large crevices in which it can shelter, or hidden within Posidonia and Zostera beds (Harmelin, 1991; Keskin, 2007). It is a nocturnal fish but it can sometimes to be found during the day (Frimodt, 1995). The brown meagre occurs in shallow coastal waters but especially when the water temperature down they prefer deeper waters and it may be found 200 m depth (Chauvet, 1991; Artüz, 2006). In the North Mediterranean Region, it has been reported that the species stocks have decreased significantly due to factors such as its life history, behavioral characteristics, habitat degradation, and pressures of small-scale professional and amateur fishing (Harmelin, 1991). In addition, spearfishing had a negative impact on its stocks (Harmelin-Vivien et al., 2015). There are 289 different species belonging to the Sciaenidae family (Chao, 1986; Chao, 2015; Parenti, 2020). The family is represented by two species (*Sciaena umbra* and *Umbrina cirrosa*) in the Black Sea (Fischer et al., 1987; Chao, 2015). There are some studies on the growth, reproduction and feeding habits of the species (Chakroun and Ktari, 1981; Fabi et al., 1998; Froglia and Gramitto, 1998; Chakroun-Marzouk and Ktari, 2003; Fabi et al., 2006; Derbal and Kara, 2007; Engin and Seyhan, 2009). However, there is no detailed study on the morphometric character of the species. Identification of morphometric characters is very important for fish fauna studies in marine ecosystem and determination of intra-species variations (Çoban et al., 2013). In addition, length-weight relationships allow morphological comparisons between different fish species or fish populations from different habitats and different regions (Gonçalves et al., 1997; Oscoz et al., 2005; Gül et al., 2017).The aim of this study was to provide data on the length and weight and morphometric characters of *S. umbra* species in the Black Sea.

Material and Methods

A total of 54 individuals were collected on a monthly and transported to the laboratory then measurements were made during the day. Fifteen metric measurements from *S. umbra* were performed. These measurements were 1. Total length (TL), 2. Standard length (SL), 3. Head length (HL), 4. Post-orbital distance (POD), 5. Eye diameter (ED), 6. Pre-dorsal distance (PDD), 7. Length of D1 fin basis (D1L), 8. Length of D2 fin basis (D2L), 9. Pre-anal distance (PAD), 10. Length of anal fin basis (AL), 11. Depth of anal fin (DAF), 12. Max. body depth (MBD), 13. Caudal peduncle minimal depth (CPMD), 14. Pectoral length (PecL), 15. Pelvic length (PelL), respectively (Figure 1).

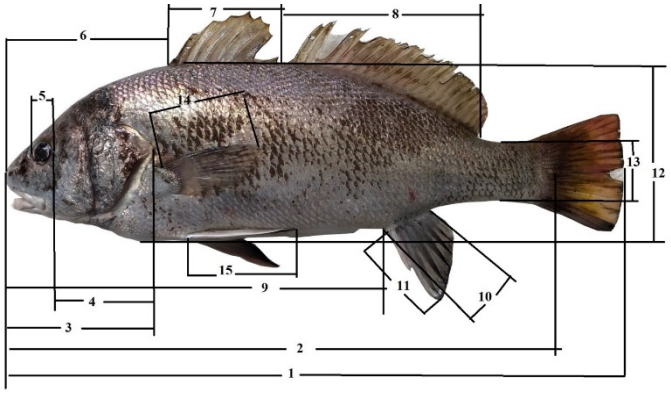

Figure 1. Overview of the morphometric measurements of *Sciaena umbra* (1:Total length (TL), 2: Standard length (SL), 3: Head length (HL), 4: Post-orbital distance (POD), 5: Eye diameter (ED), 6: Pre-dorsal distance (PDD), 7: Length of D1 fin basis (D1L), 8: Length of D2 fin basis (D2L), 9: Pre-anal distance (PAD), 10: Length of anal fin basis (AL), 11: Depth of anal fin (DAF), 12: Max. body depth (MBD), 13: Caudal peduncle minimal depth (CPMD), 14: Pectoral length (PecL), 15: Pelvic length (PelL)

All individuals were measured for total length (TL, mm) to the nearest 0.1 and weighted (W, g) to the nearest 0.01. Digital compass with 0.1 cm sensitivity was used for morphometric measurements. Lengths that cannot be measured with calipers are used with a ruler. Thirteen morphometric characters were evaluated as TL%. Regression analysis of differences body parts against TL of the fish were drawn by least square method. Dependent and independent variables, TL and morphometric measurements were transformed using log 10.

Length-weight relationship was estimated using the equation $W = aL^b$ (W: Weight (g), L: total length (cm)), where "a" is the coefficient and "b" is an exponent indicating isometric growth when equal to 3. The "b" value was tested by student's t-test to verify if it was significantly different from isometric growth (Ricker, 1975; Pauly, 1984).

Results

Length and Weight Relationships

A total of 54 different size of *S. umbra* (36 female, 18 male) were sampled with the smallest individuals 117 mm and the largest 580 mm. Length and weight relationships of *S. umbra* was shown in Figure 2.

A strong correlation relationship between length and weight ($r^2 = 0.993$) was calculated. The value of "b=3.190" is different than 3 ($p > 0.05$). It was determined that growth was positive

allometric b> 3. The length-weight relationship parameters for *Sciaena umbra* were given regardless of gender (Table 1).

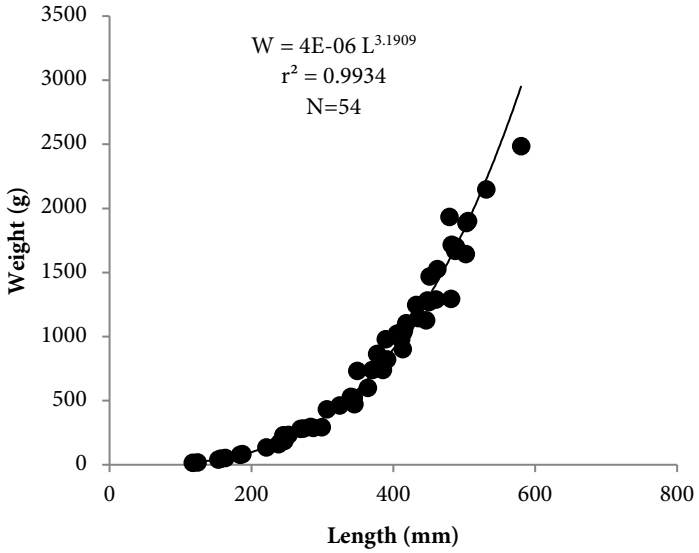

Figure 2. Total length and weight relationships of *Sciaena umbra*

Table 1. The length-weight relationship parameters for *Sciaena umbra* in the Southern Black Sea region

N	a	b	95% CI (+SD)	r^2	Pauly t-test	P
54	0.000004	3.190	3.118-3.263 (±0.035)	0.993	5.06	> 0.05

Note: N: Number of individuals; a: Intercept; b: Slope; CI: Confidence interval; SD: Standard deviation; r^2: Determination coefficient; P: Probability of the t-test (H_0: b = 3).

Morphologic characteristics

S. umbra has a double dorsal fin. The second dorsal fin is longer than the first and located very close to each other. In addition, juvenile individuals have high first dorsal fin. As the individuals grow, the first dorsal and second dorsal highs are almost similar. The pectoral fin position is ahead of the position of the first dorsal and pelvic fin and the length of the pectoral fin does not extend until the end of the pelvic fin. Even though its appearance can change in different habitats, in generally its dorsal part is dark brownish and purplish in color and the lower part of the line lateral has a lighter bronze metallic color. Dorsal fins are bronze metallic light brown, the first rays of the pelvic fin are white, while the other parts are dark black like the anal fin. Also, the anal fin has a white and very thick bony structure. It is surrounded by a black band at the ends of the caudal and dorsal fins and the caudal fin has a single lobed structure. *S. umbra* has a single continuous lateral line extending to hind margin of caudal fin. Scales ctenoid (edge comb-like) cover entire body, except tip of snout. The head is covered with cycloid scales. The head length is about 25.9% of the total length (Table 3). The eye size is relatively larger than the head. Even though some species of the Sciaenidae has barbels, this species has not. Swim bladder is located between the viscera and the backbone and the organ is a carrot-shaped form (Figure 3). The inflated swim bladder is 15 cm long and has a diameter of 5 cm for a fish with a length of 44.3 cm.

S. umbra has 3-4 rows of villiform teeth in both jaws and it also has dense pharynx teeth. On the first gill arch has 14-15 short, blunt shape gill raker (Figure 4).

Six meristic characters were examined. The lists of meristic characters used for analysis of *S. umbra* are presented in Table 2. The first dorsal fin has 10 spine rays and the second dorsal fin has one spine ray and 23 soft rays. The anal fin has two spine rays and 7 soft rays. The second spine of ray is almost 7 times the length of the other spine.

Table 2. Meristic features of *Sciaena umbra*

Meristic features	
Dorsal fin	D1 X, D2 I 23
Pelvic fin	I, 5
Anal fin	II, 7
Pectoral fin	12
Caudal fin	16
Gill rakers	14-15
Linea lateral	72-76

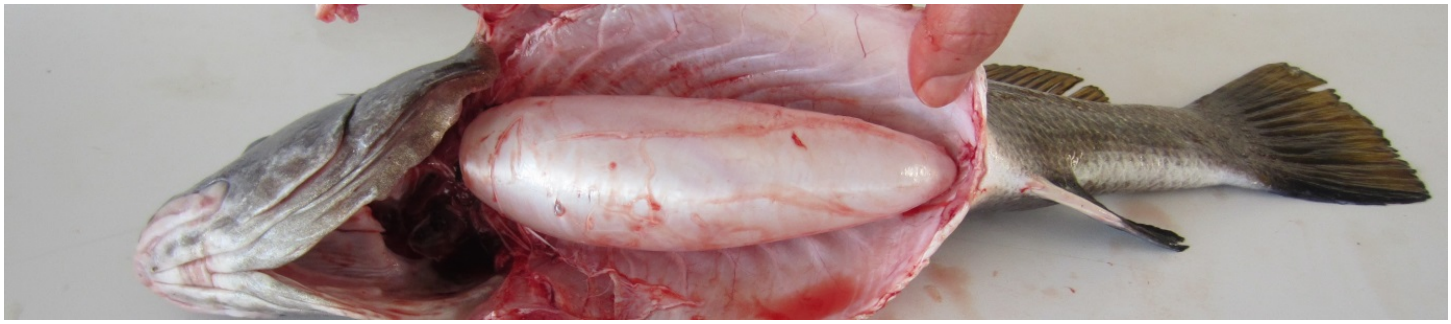

Figure 3. Swim bladder of *Sciaena umbra*

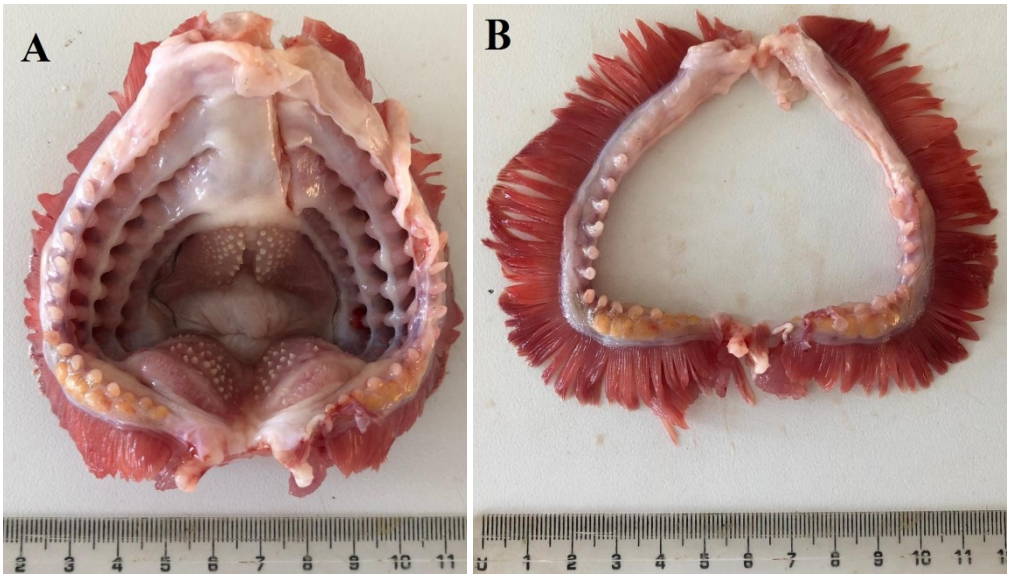

Figure 4. Pharynx teeth (A) and the first gill arch (B) of *Sciaena umbra*

Table 3. Some metric properties of *Sciaena umbra*

Characters	Mean	±SE	Min.	Max.	%TL
Total length (mm)	357.8	116.42	117.0	580.0	---
Standard length (mm)	306.1	104.67	91.5	490.0	---
Head length (mm)	92.8	33.60	29.5	152.1	25.9
Post-orbital distance (mm)	52.9	21.43	12.7	88.7	14.8
Eye diameter (mm)	15.3	4.90	6.51	25.1	4.3
Pre-dorsal distance (mm)	108.6	39.38	33.6	181.1	30.4
Length of D1 fin basis (mm)	65.7	22.47	20.0	112.9	18.4
Length of D2 fin basis (mm)	110.8	36.65	35.1	178.0	31.0
Pre-anal distance (mm)	214.3	75.64	63.5	340.0	59.9
Length of anal fin basis (mm)	27.5	8.50	10.8	39.6	7.7
Depth of anal fin (mm)	53.3	12.40	23.0	76.4	14.9
Max. body depth (mm)	100.5	35.42	28.5	156.1	28.1
Caudal peduncle minimal depth (mm)	29.2	10.80	7.2	50.4	8.2
Pectoral length (mm)	59.2	18.91	20.8	88.6	15.5
Pelvic length (mm)	61.2	15.71	26.7	88.5	17.1
Total weight (g)	845.3	646.03	16.4	2485.1	---

Morphometric characters

The mean total length and weight of the individuals sampled was 357.8 mm (117-580) and 845.3 g (16.4-2485.1), respectively. The mean, standard errors, minimum and maximum values of the morphometric properties of all samples are given in Table 3. In addition, the morphometric properties of the *S. umbra* were proportional to the total length and the smallest ratio was eye size (4.3%) and the highest ratio was the pre-anal distance (59.9%). The maximum body depth of the species is 28.1% of the total length.

The relationship between the morphometric characteristics and total length were analyzed with regression equations. Correlation coefficients of morphometric lengths-total length relationships were given in Table 4. The closest relationship was found between total length (TL) and pre-dorsal distance (PDD)

according to linear regression values (r^2=0.993) and the weakest relationship with depth of anal fin (DAF) (r^2=0.938).

Table 4. Linear regression statistics of various morphometric measurements of *Sciaena umbra* against total length

Regression Formula		r^2
HL	= 0.2865TL – 9.6954	0.985
POD	= 0.1822TL – 12.254	0.979
ED	= 0.0412TL + 05998	0.957
PDD	= 0.3371TL – 11.952	0.993
D1L	= 0.1897TL – 2.1849	0.966
D2L	= 0.3122TL – 0.9396	0.983
PAD	= 0.6454TL – 16.616	0.986
AL	= 0.0712TL – 2.0365	0.950
DAF	= 0.1031TL – 16.434	0.938
MBD	= 0.2991TL – 6.5069	0.969
CPMD	= 0.0912TL – 3.3690	0.965
PecL	= 0.1597TL + 2.0764	0.966
PelL	= 0.1315TL + 14.1080	0.950

Note: 1. Total length (TL), 2. Head length (HL), 3. Post-orbital distance (POD), 4. Eye diameter (ED), 5. Pre-dorsal distance (PDD), 6. Length of D1 fin basis (D1L), 7. Length of D2 fin basis (D2L), 8. Pre-anal distance (PAD), 9. Length of anal fin basis (AL), 10. Depth of anal fin (DAF), 11. Max. body depth (MBD), 12. Caudal peduncle minimal depth (CPMD), 14. Pectoral length (PecL), 15. Pelvic length (PelL).

Discussion

In the study, a total of 54 individuals were sampled (36 females and 18 males). Length of all individuals ranged from 117-580 mm with 357.8 mm average. Karakulak et al. (2006) reported the maximum length as 29.8 cm, Karachle and Stergiou (2008) as 16 cm, Bilge et al. (2014) as 40.4 cm for *S. umbra* in the Aegean Sea. Cengiz et al. (2019) declared that the largest individual was 41.7 cm for the Aegean Sea. Engin (2003) determined the maximum length as 72 cm for Black Sea. Considering these results, it can be said the population in the Black Sea has larger individuals than the Aegean Sea population. The "a" and "b" coefficients obtained in the relationship between length and weight may differ depending on reasons such as environmental factors, nutrient abundance, reproductive activities (Mommsen, 1998).

In this study, "b" value was calculated as 3.1909 and it was determined that growth was positive allometric (b>3). Few studies on the species reported that the growth were negative allometry (Karachle and Stergiou, 2008; Maci et al., 2009; Crec'hriou et al., 2013), while most of study reported to be positive allometric growth (Morey et al., 2003; Karakulak et al., 2006; La Mesa et al., 2008; Engin and Seyhan, 2009; Grau et al., 2009; Bilge et al., 2014; Chater et al., 2018). The "b" value may be different from one population to another of the same species. The fluctuating can be assigned to factors such as food availability, feeding rates, whether sampling was done during the spawning season, differences in the number of specimen sampled, the period of sampling (Bagenal and Tesch, 1978; Moutopoulos and Stergiou, 2002; Mahé et al., 2018). Karachle and Stergiou (2008) and Maci et al. (2009) were used very small individuals in their study. Therefore they maybe have estimated the "b" value less than 3. Crec'hriou et al. (2013) reported "b" value as 2.91. It can be said to be use few individuals (n: 16). It is determined that the species has a highly developed swim bladder. Similarly, Picciulin et al. (2016) stated that the swim bladder of the species has a highly developed (Figure 3), it can make sounds using the muscles in the lower parts and they can establish social relationships with other individuals around them. There may be some changes in the morphometric characters of the fish after adaptation of a fish species to different environmental conditions (Blackith and Albrecht, 1959; Avşar, 1995). Morphometric measurements are used to determine similarities or differences between one stock and another. In addition, it is widely use taxonomic categories for fisheries biology area (Dwivedi and Dubey, 2013). Although the *S. umbra* species is distributed to the East Atlantic Ocean, the Mediterranean, Aegean, Marmara, Black Sea and the Sea of Azov (Artüz, 2006; Chao, 2015), there are very few biological studies (Engin, 2003, Engin and Seyhan, 2009) on the species, but no data are available about morphometric characters. Recent recreational fishing activity particularly from spearfishing had a negative impact on its stocks on the Mediterranean Sea and Black Sea (Harmelin-Vivien et al., 2015). On the one hand, in recent years a large part of the coastal area of the Southern Black Sea has been filled up for the highway and airport construction as well as land acquisitions. It is thought that this development had a positive impact on *S. umbra* species in terms of population increase. Lately, a noticeable increase has been observed on such species (Aydın and Sözer, 2016). Since the habitat structure of the Black Sea is limited rocky areas, does not allow the shelter for small individuals. It is thought that filled coastal areas provide suitable habitats for these species' juveniles.

Conclusion

The scientists rarely provided samples for research this species because of high economic value, living in limited areas and fishing requires special skills. Consequently, there is few study on the species. Thus, this is the first documented of morphometric characteristics of the species. This paper is considered to contribute to fisheries biology and international scientific literature.

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Compliance with Ethical Standards

Authors' Contributions

Author MA designed the study, BB wrote the first draft of the manuscript, and both authors conducted field work and lab work together. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was conducted in accordance with ethics committee procedures of animal experiments.

References

Artüz, M. L. (2006). Abundance and growth observations of *Sciaena umbra* Linnaeus, 1758 in Sea of Marmara. *Hydrobiologica*, ***1a***: 124-128.

Avşar, D. (1995). Utilization of morphometric and meristic characters for the differentiation of fish stocks. Proceedings of the II. National Ecology Congress, Ankara, Turkey. pp. 203-209.

Aydın, M. & Sözer, A. (2016). Presence of the gilthead seabream in the Black Sea. *Turkish Journal of Maritime and Marine Sciences*, ***2***(2): 49-55.

Bagenal, T. B. & Tesch, F. W. (1978). Age and growth (pp. 101-136). In: Bagenal T. (ed.), Methods for Assessment of Fish Production in Fresh Waters. Oxford, UK: *Blackwell Scientific Publications*.

Bauchot, M. L. (1987). Poissons osseux. (pp. 891-1421). In Fischer, W., Bauchot M. L. & Schneider, M. (eds.) *Fiches FAO d'identification pour les besoins de la pêche. (rev. 1). Méditerranée et mer Noire*. Zone de pêche 37. Vol. II. Commission des Communautés Européennes and FAO, Rome, Italy.

Bilge, G., Yapıcı, S., Filiz, H. & Cerim, H. (2014). Weight-length relations for 103 fish species from the Southern Aegean Sea, Turkey. *Acta Ichthyologica et Piscatoria*, ***44***(3): 263-269.

Blackith, R. J. & Albrecht, F. O. (1959). Morphometric differences between the eye-stripe polymorphy of the red locust. *Scientific Journal of the Royal College of Science*, ***27***: 13-27.

Cengiz, Ö, Kızılkaya, B. & Paruğ, Ş. Ş. (2019). Maximum size record of brown meagre (*Sciaena umbra* Linnaeus, 1758) for Aegean Sea. *KSU Journal of Agriculture and Nature,* ***22***(4): 659-663. https://doi.org/10.18016/ksutarimdoga.vi.515704

Chakroun, N. & Ktari, M. H. (1981). Régime alimentaire des Sciaenidae (Poissons Téléostéens) du Golfe de Tunis. *Bull. Inst. Nat. Sci. Tech. Océanogr. Pêche Salammbô,* ***8***: 69-80.

Chakroun-Marzouk, N. & Ktari M. H. (2003). The brown meagre from Tunisian coasts, *Sciaena umbra* (Sciaenidae): sexual cycle, age and growth. *Cybium*, 27(3): 211-225.

Chao, L. (2015). *Sciaena umbra*. The IUCN Red List of Threatened Species 2015: e.T198707A83232286. Downloaded on 19 March 2020.

Chao, L. N. (1986). Sciaenidae (pp. 865-874). In Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E. (eds.) *Fishes of the north-eastern Atlantic and the Mediterranean*. Volume 2. Unesco, Paris, France.

Chater, I., Romdhani-Dhahri, A., Dufour, J. L., Mahé, K. & Chakroun-Marzouk, N. (2018). Age, growth and mortality of *Sciaena umbra* (Sciaenidae) in the Gulf of Tunis. *Scientia Marina*, ***82***(1): 17-25. https://doi.org/10.3989/scimar.04679.21A

Chauvet, C. (1991). Le corb ou brown meagre (*Sciaena umbra* - Linnaeus, 1758) quelques éléments de sa biologie. p. 229-235. In C.F. Boudouresque, M. Avon and V. Gravez (eds.) Les espèces marines à protéger en Méditerranée. GIS Posidonie publ. France.

Çoban, M. Z., Gündüz, F., Yüksel, F., Demirol, F., Yıldırım, T. & Kurtoğlu, M. (2013). Fish fauna of Uzunçayır Dam Lake (Tunceli). *Yunus Araştırma Bülteni*, ***2***: 35-44.

Crec'hriou, R., Neveu, R. & Lenfant, P. (2013). Length–weight relationship of main commercial fishes from the French Catalan coast. *Journal of Applied Ichthyology*, ***28***(5): 861-862. https://doi.org/10.1111/j.1439-0426.2012.02030.x

Derbal, F. & Kara, M. H. (2007). Diet of the brown meagre *Sciaena umbra* (Sciaenidae), from the eastern coast of Algeria. *Cybium*, ***31***(2): 199–207.

Dwivedi, A. K. & Dubey, V. K. (2013). Advancements in morphometric differentiation: a review on stock identification among fish populations. *Reviews in Fish Biology and Fisheries*, ***23***: 23-39. https://doi.org/10.1007/s11160-012-9279-1

Engin, S. (2003). Some bio-ecological characteristics of Brown meagre (*Sciaena umbra*) in the Eastern Black Sea coastal ecosystem. Master Thesis. Karadeniz Technical University, Trabzon, Turkey.

Engin, S. & Seyhan, K. (2009). Age, growth, sexual maturity and feeding ecology of *Sciaena umbra* in the South Eastern Black Sea Marine Ecosystem, Turkey. *Journal of Applied Ichthyology*, ***25***(1): 96-99. https://doi.org/10.1111/j.1439-0426.2008.01173.x

Fabi, G. Manoukian, S. & Spagnolo, A. (2006). Feeding behavior of three common fishes at an artificial reef in the northern Adriatic Sea. *Bulletin of Marine Science,* ***78***(1): 39–56.

Fabi, G., Panfili, M. & Spagnolo, A. (1998). Note on feeding of *Sciaena umbra* L. (Osteichthyes: Sciaenidae) in the central Adriatic Sea. *Rapport Commission International Mer Méditerranée*, ***35***: 426-427.

Fischer, W, Bauchot, M. L. & Schneider, M. (1987). *Fiches FAO d'identification pour les besoins de la pêche révision 1. Méditerranée et mer Noire.* Zone de pêche 37, vol. 2: Vertébrés, Rome, FAO, pp. 761-1530.

Frimodt, C. (1995). *Multilingual illustrated guide to the world's commercial warm water fish*. Fishing News Books, Osney Mead, Oxford, England. 215 p.

Froglia, C. & Gramitto, M. E. (1998). Osservazioni sull'alimentazione di *Sciaena umbra* ed *Umbrina cirrosa* (Pisces, Sciaenidae) in prossimità di barriere artificiali in Adriatico. *Biologia Marina Mediterranea*, ***5***: 100-108.

Gonçalves, J. M. S., Bentes, L., Lino, P. G., Ribeiro, J., Canario, A. V. M. & Erzini, K. (1997). Weight-length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. *Fisheries Research,* ***30***(3): 253-256. https://doi.org/10.1016/S0165-7836(96)00569-3

Grau, A., Linde, M. & Grau, A. M. (2009). Reproductive biology of the vulnerable species *Sciaena umbra* Linnaeus, 1758 (Pisces: Sciaenidae). *Scientia Marina*, ***73***(1): 67-81. https://doi.org/10.3989/scimar.2009.73n1067

Gül, G., Yılmaz, M., Saylar, Ö., Benzer, S. & Gül, A. (2017). Morphometric and meristic characteristics and length-weight relations of *Cyprinus carpio* in the Mogan Lake Population. *SDÜ Eğirdir Su Ürünleri Fakültesi Dergisi*, ***13***(2): 163-172. https://doi.org/10.22392/egirdir.292000

Harmelin, J. G. (1991). *Statut du Corb (Sciaena umbra) en Méditerranée* (pp. 219-227). In: Boudouresque, C. F., Avon, M., Gravez, V. (eds.), Les espèces marines à protéger en Méditerranée. GIS Posidonie publications, France.

Harmelin-Vivien, M., Cottalorda, J. M., Dominici, J. M., Harmelin, J. G., Le Diréach, L. & Ruitto, S. (2015). Effects of reserve protection level on the vulnerable fish species *Sciaena umbra* and implications for fishing management and policy. *Global Ecology Conservation*, ***3***: 279-287. https://doi.org/10.1016/j.gecco.2014.12.005

Karachle, P. K. & Stergiou K. I. (2008). Length-length and length–weight relationships of several fish species from the North Aegean Sea (Greece). *Journal of Biological Research-Thessaloniki,* ***10***: 149-157.

Karakulak, F. S., Erk, H. & Bilgin, B. (2006). Length-weight relationships for 47 coastal fish species from the Northern Aegean Sea, Turkey. *Journal of Applied Ichthyology*, ***22***(4): 274-278. https://doi.org/10.1111/j.1439-0426.2006.00736.x

Keskin, C. (2007). Temporal variation of fish assemblages in different shallow-water habitats in Erdek Bay, Marmara Sea, Turkey. *Journal of the Black Sea / Mediterranean Environment*, ***13***: 215-234.

La Mesa, M., Colella, S., Giannetti, G. & Arneri, E. (2008). Age and growth of brown meagre *Sciaena umbra* (Sciaenidae) in the Adriatic Sea. *Aquatic Living Resources,* ***21***(2): 153-161. https://doi.org/10.1051/alr:2008029

Maci, S., Longo, E. & Basset, A. (2009). Length-weight relationships for 24 selected fish species from a non-tidal lagoon of the southern Adriatic Sea (Italy). *Transitional Waters Bulletin*, ***3***(3): 1-9. https://doi.org/10.1285/i1825229Xv3n3p1

Mahé, K., Bellamy, E., Delpech, J. P., Lazard, C., Salaun, M., Vérin, Y., Coppin, F. & Travers-Trolet, M. (2018). Evidence of a relationship between weight and total length of marine fish in the North-eastern Atlantic Ocean: physiological, spatial and temporal variations. *Journal of the Marine Biological Association of the United Kingdom*, ***98***(3): 617-625. https://doi.org/10.1017/S0025315416001752

Mommsen, T. P. (1998). *Growth and metabolism* (pp. 65–97). In: Evans, D. H. (Ed.), The Physiology of Fishes. New York, USA: CRC Press.

Morey, G., Moranta, J., Massutí, E., Grau, A., Linde, M., Riera, F. & Morales-Nin, B. (2003). Weight-length relationships of littoral to lower slope fishes from the western Mediterranean. *Fisheries Research,* ***62***(1): 89-96. https://doi.org/10.1016/S0165-7836(02)00250-3

Moutopoulos, D. K. & Stergiou, K. I. (2002). Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). *Journal of Applied Ichthyology,* ***18***(3): 200-203. https://doi.org/10.1046/j.1439-0426.2002.00281.x

Oscoz, J., Campos, F. & Escala, M. C. (2005). Weight-length relationships of some fish species of the Iberian Peninsula. *Journal of Applied Ichthyology*, ***21***(1): 73-74. https://doi.org/10.1111/j.1439-0426.2004.00587.x

Parenti, P. (2020). An annotated checklist of fishes of the family Sciaenidae. *Journal of Animal Diversity*, ***2***(1): 1-92. https://doi.org/10.29252/JAD.2020.2.1.1

Pauly, D. (1984). *Fish population dynamics in tropical waters: a manual for use with programmable calculators*. ICLARM Studies and Reviews 8, International Center for Living Aquatic Resource Management, Philippines, Manila, 325p.

Picciulin, M., Bolgan, M., Corò, A. B., Calcagno, G. & Malavasi, S. (2016). Sound production by the Shi drum *Umbrina cirrosa* and comparison with the brown meagre *Sciaena umbra*: a passive acoustic monitoring perspective. *Journal of Fish Biology*, ***88***(4): 1655–1660. https://doi.org/10.1111/jfb.12926

Ricker, W. E. (1975). Computation and interpretation of biology statistics of fish populations, *Bulletin of the Fisheries Research Board of Canada*, ***191***: 382.

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RESEARCH ARTICLE

Determination of shellfish consumption preferences and habits in Erzurum province

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ABSTRACT

The purpose of this research is to determine the shellfish consumption preferences and habits between November 2019 and February 2020 in Erzurum province. A survey consisting of 15 questions was asked face to face with 122 people randomly selected to obtain some results relevant to participant's average monthly income, educational background, their occupational status. The relationships between the demographic characteristics of the participants and the consumption habits of shellfish were analyzed with the Chi-square test. When demographic data were examined, 46.72% of the participants were female, 53.28% were male. The highest age group with a rate of 41,80% is 21-30 years old, the lowest being 61-70 years old with a rate of 4,92% has been identified as the group. When the education levels of the participants were analyzed, it was determined that the highest rate (60.65%) was belonged to university graduates. According to the result, 83.3% of the participants stated that they had not information about the nutritional value of shellfish. Also, it was determined that 66.2% of the sharers preferred mussels compared to crustaceans. This study in Erzurum, where the consumption of shellfish is very low compared to the seashore cities, is also an important data source in terms of providing ideas for different researches and aquaculture systems.

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Introduction

Since aquaculture is rich in protein, vitamins and minerals, it is among the foods that have an important place in human nutrition and are recommended for health. Aquaculture in terms of consumption; includes molluscs, crustaceans and all kinds of fish and marine mammals. (Baysal, 2004; Oğuzhan et al., 2006).

Shellfish have been used as a food source of people since ancient times (Başçınar, 2007). Due to its animal origin, it is among the foods with high biological value, especially recommended for the nutrition of children. They contain

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mainly protein, iodine, phosphorus, zinc, vitamin E, niacin, vitamin B12, unsaturated fatty acids, omega-3, omega-6, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and many bioactive beneficial components. Because they contain these extremely valuable and useful ingredients, they are classified as more valuable than many animal foods and are in the category of "functional foods", also referred to as nutraceuticals in recent years (Tokuşoğlu, 2016).

Shrimp, crab, lobster, mussel and oyster are the main shellfish products. World fish production (fish, crustaceans and molluscs) is 172.7 million tons in 2017. In 2017, 53.4 million tons of world aquaculture production was fish (66.6%), 17.4 million tons of molluscs (21.7%), 8.4 million tons of crustaceans (10.5%) and 0.9 million. tonnes consisted of other aquatic animal species (1.1%). The production of crustaceans and molluscs is also important in marine fish, which is mostly composed of marine fish. In our country, 21.8% of the total marine products caught production in 2018 was made up of other aquaculture group other than fish. The species with the highest production in this group are striped venus and sea snails, both of which are caught in the Black Sea (Anonymous, 2019).

In this study, it was aimed to determine the consumption preferences and habits of shellfish products by conducting a survey in the form of question and answer to consumers in Erzurum.

Based on this survey, an important place in the world aquaculture production units and shellfish which is high compared to the return of fish and molluscs in Turkey is thought to be done research to increase aquaculture and consumption.

Material and Methods

This study was conducted between November 2019 and February 2020 in order to determine the consumption preferences and habits of shellfish consumption in Erzurum. Erzurum is a province that covers 25,066 km² area in the Eastern Anatolia Region of Turkey. It is bordered by the provinces of Kars and Ağrı to the east, Muş and Bingöl to the south, Erzincan and Bayburt to the west, Rize and Artvin to the north and Ardahan to the northeast. Continental climate rules in the province with long and harsh winters, and short and mild summers. Agricultural activities are carried out in the province. Most of the grain types are cultivated of wheat, barley and rye. Among the industrial plants, mostly sugar beet, potato and sunflower are cultivated. Apple, pear, walnut, apricot, plum, cherry, cherry and cranberry are grown. Livestock in in the province produced. Food industries include beekeeping and trout farming (Anonymous, 2020).

The material of the study consists of data obtained from a survey with a total of 122 individuals (57 women and 65 men). The study was achieved by asking total 15 questions to participants in survey. 10 questions were asked on the seafood and shellfish consumption habits, 5 questions asked to measure demographic characteristics to better understand the underlying factors about consuming habits. The data obtained from the survey were analyzed with the Chi-square test using the SPSS package program and statistical significance level was accepted as $p < 0.05$ (Sümbüloğlu and Sümbüloğlu, 2019).

Results and Discussion

Socio-Demographic Features of Consumers Participating in the Survey

46.72% of the consumers participating in the survey are women and 53.28% are men. While the lowest age group of the participants were 61-70 age with a rate of 4.92%, the highest was determined as 21-30 age with 41.80%. 60.65% of the participants of survey were graduated from a university, 4.92% of the participants were graduated from a primary school, 13.11% of the participants were graduated from a high school, and 21.32% of the participants were graduated from postgraduate. 15.57% of the participants are official, 7.38% are self-employed, 41.80% are students, 13.94% are retired, 8.20% are housewives, and 13.11% are from other occupational groups. They were found to be considering the income levels, 32.79% of 1000 TRY and below, 14.76% of 1001-2000 TRY, 26.23% of 2001-3000 TRY, 11.47% of 3001-4000 TRY, and 14.7% of 4001 TRY and above (Table 1).

Saka and Bulut (2020) reported that 57.6% of the participants were male and 42.4% were female. When the ages of the participants were compared, 30.8% were in the 19-29 age group while 27.8% were in the 30-49 age group. When the educational status was examined, it was determined that 33.4% graduated from high school or equivalent schools and 19.1% graduated from undergraduate programs. When the professions of the participants were researched, 27% were students, 20% were self-employed, 18.6% were workers, 13.6% were homemakers, 13.3% were public officers, and 7% were retired.

In the study in Çan district of Çanakkale, the income levels were determined 18.3% of 2000 TRY and below, 19.5% of 2000-3500 TRY, 41.2% of 3500-5000 TRY, and 21% of 5000 TRY and above (Selvi et al., 2019).

Shellfish Products Preference and Consumption Habits of Consumers Participating in the Survey

Depending on the age, the analysis of the answers given by the participants to the question "*How often do you consume seafood?*" is shown in Table 2.

As a result of the Chi-square independence test for the answers given by the participants to the question "*How often do you consume seafood?*" a significant difference was found between the answers given depending on the age ($p<0.05$). While 66.7% of participants between the ages of 51-60 say they consume every fifteen days, this rate is 9.8% for participants between the ages of 21-30. They responded "*How often do you consume seafood?*" 27.5% of the respondents to the question once a week, 35.83% once a month, 5.83% twice a week, 12.5% twice a month and 18.34% once a year in a study in Palu district of Elazig province (Karaton Kuzgun and Demirbağ, 2018).
In another study in Antalya province, the frequency of seafood consumption was determined to be 43.67% once every two weeks and 26.81% once a week (Arslan and Izci, 2016). The answers given to this question in the studies conducted in different regions are thought to cause from geographical conditions, table culture and habits.

Depending on gender, the analysis of the answers given by the participants to the question "*Which of the crustaceans and molluscs do you prefer more?*" is given in Table 3.

As a result of the Chi-square independence test for the answers given by the participants to the question "*Which of the crustacean and molluscs would you prefer more?*", a significant difference was found between the answers given depending on

Table 1. Gender, age, educational status, income level, and occupational distributions of the participants

Gender	**N**	**%**
Male	65	53.3
Female	57	46.7
Age		
< 21	14	11.5
21-30	51	41.8
31-40	22	18.1
41-50	11	9.0
51-60	18	14.7
61-70	6	4.9
Educational Status		
Primary school	6	4.9
High school	16	13.1
University	74	60.7
Postgraduate	26	21.3
Income Level		
< 1000 TRY	40	32.8
1001-2000 TRY	18	14.7
2001-3000 TRY	32	26.3
3001-4000 TRY	14	11.5
> 4000 TRY	18	14.7
Occupational		
Official	19	15.6
Free	9	7.4
Student	51	41.8
Retire	17	13.9
Housewife	10	8.2
Other	16	13.1

Table 2. Shellfish consuming frequency according to the age group

Age		**How often do you consume?**				**Total**
		Once a week	**Fortnightly**	**Once a month**	**Once a year**	
< 21	Person	2	2	8	2	14
	%	14.3	14.3	57.1	14.3	100
21-30	Person	10	5	24	12	51
	%	19.6	9.8	47.1	23.5	100
31-40	Person	8	6	6	2	22
	%	36.4	27.3	27.3	9.1	100.0
41-50	Person	4	4	3	0	11
	%	36.4	36.4	27.3	0	100
51-60	Person	3	12	2	1	18
	%	16.7	66.7	11.1	5.6	100
61-70	Person	3	0	0	3	6
	%	50	0	0	50	100
Total	Person	30	29	43	20	122
	%	24.6	23.8	35.2	16.4	100

Note: Pearson Chi-square: 45,110 $p<0.05$

Table 3. Consuming preferences according to gender

Gender		Which of the crustaceans and molluscs would you prefer?					Total
		Shrimp	Crab	Lobster	Mussel	None	
Male	Person	10	0	1	43	11	65
	%	15.4	0	1.5	66.2	16.9	100
Female	Person	10	6	0	28	13	57
	%	17.5	10.5	0	49.1	22.8	100
Total	Person	20	6	1	71	24	122
	%	16.4	4.9	0.8	58.2	19.7	100

Note: Pearson Chi-square: 9,853 p<0.05

Table 4. Factors affecting the preference to buy shellfish

Gender		What do you pay attention to when buying shellfish?					Total
		Cleaning of the place taken	Freshness	Price	Species	Other	
Male	Person	9	22	16	10	8	65
	%	13.8	33.8	24.6	15.4	12.3	100
Female	Person	17	28	3	4	5	57
	%	29.8	49.1	5.3	7	8.8	100
Total	Person	26	50	19	14	13	122
	%	21.3	41	15.6	11.5	10.7	100

Note: Pearson Chi-square: 14,879 p<0.05

Table 5. Consuming preferences of shellfish species according to the age group

Age		Which of the crustaceans and molluscs would you prefer more?					Total
		Shrimp	Crab	Lobster	Mussel	None	
< 21	Person	0	2	0	10	2	14
	%	0	14.3	0	71.4	14.3	100
21-30	Person	5	0	1	41	4	51
	%	9.8	0	2	80.4	7.8	100
31-40	Person	9	2	0	8	3	22
	%	40.9	9.1	0	36.4	13.6	100
41-50	Person	5	0	0	4	2	11
	%	45.5	0	0	36.4	18.2	100
51-60	Person	1	2	0	5	10	18
	%	5.6	11.1	0	27.8	55.6	100
61-70	Person	0	0	0	3	3	6
	%	0	0	0	50	50.0	100
Total	Person	20	6	1	71	24	122
	%	16.4	4.9	0.8	58.2	19.7	100

Note: Pearson Chi-square: 58,282 p<0.05

gender (p<0.05). While 66.2% of men prefer the most mussel response, this rate is 49.1% for women. It was found that they did not prefer other crustaceans and molluscs. This situation is caused the fact that the consumption of shellfish is not common in our country and that there is more mussel consumption in the settlements that have a coast. In the study in Ardahan province, the most consumed seafood was found to be mussels with 11.92% (Kılıç et al., 2019). They found that consumers in Ankara province consumed mussels with 47.50%, followed by squid and shrimp respectively (Yavuz et al., 2015). In another study in the province of Burdur has been reported mussels with 71.40%, shrimp with 39.30%, squid with 25%, octopus with 7.10%, lobster with 3.60% and crab with 3.60% (Orhan and Yüksel, 2010).

Depending on gender, the analysis of the answers given by the participants to the question "*What do you pay attention to when buying shellfish?*" is given in Table 4.

As a result of the Chi-square independence test for the answers given by the participants to the question "*What do you*

pay attention to when buying shellfish?", a significant difference was found between the answers given depending on gender ($p<0.05$). While 49.1% of women respond to freshness, this rate is 33.8% for men. 82% of individuals in the province of Denizli reported that they pay attention to freshness while buying fish (Telli, 2018). In the central district of Kahramanmaraş province, 73% of the participants were found to pay attention to freshness while purchasing fish (Beyazbayrak, 2014). They emphasized that the people who buy fish in Rize province attach great importance to keeping the fish fresh (Temel, 2014). The survey results in different regions are similar to our results. Aydın and Bashimov (2020) reported that the freshness of the fish is very effective while buying fish consumers in urban areas of Mary city in Turkmenistan.

Depending on the age, the analysis of the answers given by the participants to the question "*Which of the crustaceans and molluscs would you prefer more?*" is given in Table 5.

In terms of the answers given to "*Which of the crustaceans and molluscs do you prefer more?*", it was observed that there was a significant difference between the ages in accordance to the Chi-square independence test ($p<0.05$). While 80.4% of the participants between the ages of 21-30 say they consume more mussels, this is 27.8% for the age group of 51-60.

Depending on the education level, the analysis of the answers given by the participants to the question "*Do you have information about shellfish?*" is given in Table 6.

In terms of the answers given to "*Do you have information about shellfish?*", it has been revealed that there is a significant difference between the educational status as a result of the Chi-square independence test ($p<0.05$). While 87.5% of the secondary education graduates give no answer, this rate is 30.8% for graduate graduates. This situation thought to be due Erzurum province does not have a seaside, the consumption of shellfish as human food is not widespread enough and due to the table culture. Kılıç et al. (2019) were reported that 78.48% of consumers in Ardahan province had knowledge about fish meat, while 19.21% did not.

Table 6. Knowledge level of consumers on the shellfish according to the education level

Educational Status		Do you have information about shellfish?		Total
		Yes	No	
Primary school	Person	1	5	6
	%	16.7	83.3	100
High school	Person	2	14	16
	%	12.5	87.5	100
University	Person	32	42	74
	%	43.2	56.8	100
Postgraduate	Person	18	8	26
	%	69.2	30.8	100
Total	Person	53	69	122
	%	43.4	56.6	100

Note: Pearson Chi-square: 15,858 $p<0.05$

The analysis of the answers given by the participants to the question "*How often do you consume seafood*?" depending on their income levels is given in Table 7.

As a result of the Chi-square independence test in terms of the answers given to the question "*How often do you consume?*", it was revealed that there is a significant difference in terms of income levels ($p<0.05$). While 42.9% of individuals with 3001-4000 TRY income level answer every fifteen days, this rate is 10% for individuals whose income level is below 1000 TRY. In study in Erzurum province, the frequency of fish consumption

Table 7. Shellfish consuming frequency according to the income level

Income level (TRY)		How often do you consume?				Total
		Once a week	Fortnightly	Once a month	Once a year	
<1000	Person	5	4	21	10	40
	%	12.5	10	52.5	25	100
1001-2000	Person	3	5	7	3	18
	%	16.7	27.8	38.9	16.7	100
2001-3000	Person	9	11	7	5	32
	%	28.1	34.4	21.9	15.6	100
3001-4000	Person	4	6	4	0	14
	%	28.6	42.9	28.6	0	100
>4001	Person	9	3	4	2	18
	%	50	16.7	22.2	11.1	100
Total	Person	30	29	43	20	122
	%	24.6	23.8	35.2	16.4	100

Note: Pearson Chi-square: 25,704 $p<0.05$

Table 8. Consuming preferences of shellfish species according to the occupational group

Occupational		Which of the crustacean and mollusc do you prefer?					Total
		Shrimp	Crab	Lobster	Mussel	None	
Official	Person	7	0	0	10	2	19
	%	36.8	0	0	52.6	10.5	100
Freelancer	Person	2	0	0	4	3	9
	%	22.2	0	0	44.4	33.3	100
Student	Person	5	2	1	39	4	51
	%	9.8	3.9	2	76.5	7.8	100
Retired	Person	2	2	0	7	6	17
	%	11.8	11.8	0	41.2	35.3	100
Housewife	Person	1	1	0	2	6	10
	%	10	10	0	20	60	100
Other	Person	3	1	0	9	3	16
	%	18.8	6.3	0	56.3	18.8	100
Total	Person	20	6	1	71	24	122
	%	16.4	4.9	0.8	58.2	19.7	100

Note: Pearson Chi-square: 34,341 $p<0.05$

was determined to be 26.7% of those with an income of 3501 TRY and above consume fish once a week, this rate is 12.5% for those whose income is 2000 TRY and less (Karakulak et al., 2019).In another study Erzincan province, the rate of those who consume fish fortnightly is 50%, the rate of those who consume fish once a month is 40%, and the rate of those who consume fish once a week is 10% (Karakaya, 2020). Quasim et al. (2020) stated that 38.8% eat fish once a month, 32% two to three times a month, 20.6% four times a month, and 8.03% more than four times a month.

Depending on the profession groups, the analysis of the answers given by the participants to the question "*Which of the crustacean and molluscs do you prefer?*" is given in Table 8.

As a result of the Chi-square independence test conducted to determine whether there is a difference between the professional groups in terms of the answers given to the question "*Which of the crustacean and molluscs do you prefer more?*", it was revealed that there was a significant difference ($p<0.05$). It was determined that the participants consumed the most mussels with a rate of 76.5%, while this rate was 20% for housewives in Table 8.

Conclusion

In conclusion, consumption of shellfish, especially rich in nutrition, is very low in our country compared to other seafood products. According to Turkey Statistical Institute (TurkStat, 2018) fisheries data; 628631 tons of seafood was put into consumption. 35.3% marine fish, 9.9% other seafood, 4.8% inland fisheries and 50% aquaculture products. While the average fish consumption per capita was 5.49 kilograms in 2017, it increased by 11.8 percent to 6.14 kilograms in 2018. There are many reasons for this. In our study, it was seen that the lack of information about the nutritional content of shellfish is an important factor. The fact that most of the participants, whose education level is at the high school level, stated that they do not have information about shellfish products supports this situation. It is thought to be of benefit to increase shellfish training activities in the region. It is another result of our study in the responses that the consumption will increase if the prices of shellfish are reduced. The low use of shellfish in food culture in our country affects consumption significantly. These and similar studies are encouraging factors in the introducing and consumption of shellfish. This study in Erzurum, where the consumption of shellfish is very low compared to the seashore cities, is also an important data source in terms of providing ideas for different researches and aquaculture systems.

Compliance with Ethical Standards

Authors' Contributions

POY designed the study. GA performed statistical analysis. Both authors read and approved the final version of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Anonymous. (2019). Su ürünleri sektör politika belgesi 2019-2023.

Anonymous, (2020). Erzurum ili. Retrieved on August 20, 2020 from https://tr.wikipedia.org/wiki/Erzurum

Arslan, M. & İzci, L. (2016) .Antalya İli Su Ürünleri Tüketim Alışkanlıklarının Belirlenmesi. *Eğirdir Su Ürünleri Fakültesi Dergisi*, ***12***(1): 75-85. https://doi.org/10.22392/egirdir.246325

Aydın, A. & Bashimov, G. (2020). Determination of fish consumption habits of consumers: Case study of Mary city, Turkmenistan. *Marine Science and Technology Bulletin*, ***9***(2): 118-124. https://doi.org/10.33714/masteb.685436

Başçınar, N. (2007). Ülkemizdeki kabuklu ve yumuşakça su ürünleri üretimi ve ihracatı. *Yunus Araştırma Bülteni*, ***7***(2): 14-17.

Baysal, A. (2004). Beslenme. 10. Ed., Ankara, Turkey: Hatipoğlu Yayınevi, 566p.

Beyazbayrak, Z. (2014). Kahramanmaraş ili Merkez ilçede balık tüketim alışkanlıkları. Master Thesis. Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey.

Karakaya, E. (2020). Erzincan ili balık tüketim alışkanlıklarının belirlenmesi. Menba *Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi*, ***6***(1): 18-29.

Karakulak, Y., Arslan, G. & Yanık, T. (2019). Erzurum ili Merkez ilçelerinin su ürünleri tüketim davranışları üzerine araştırmalar. *Acta Aquatica Turcica*, ***16***(2): 290-300. https://doi.org/10.22392/actaquatr.669336

Karaton Kuzgun, N. & Demirbağ, A. (2018). Palu ilçesi balık tüketim alışkanlıklarının belirlenmesi. *Uluslararası Palu Sempozyumu Bildiriler Kitabı,* Elazığ, Turkey. pp. 205-212.

Kılıç, E., Soylu, M. & Uzmanoğlu, M. S. (2019). Ardahan ili su ürünleri tüketim alışkanlıklarının belirlenmesi. *Turkish Journal of Agriculture - Food Science and Technology,* ***7*****(7):** 1028-1039. https://doi.org/10.24925/turjaf.v7i7.1028-1039.2536

Oğuzhan, P., Angiş, S., Haliloğlu, H. İ. & Atamanalp, M. (2006). Gökkuşağı alabalığı (*Oncorhynchus mykiss)* filetolarında sıcak tütsüleme sonrası kimyasal kompozisyon değişimleri. *Ege Üniversitesi Su Ürünleri Dergisi*, ***23***(1/3): 465-466.

Orhan, H. & Yüksel, O. (2010). Burdur İli su ürünleri tüketimi anket uygulaması. *Süleyman Demirel Üniversitesi, Ziraat Fakültesi Dergisi,* ***5***(1): 1-7.

Qasim, M., Qasim, S. & Nazir, N. (2020). Factors affecting fish consumption of traditional subsistence fishers in Khyber Pakhtunkhwa, Pakistan. *Marine Science and Technology Bulletin*, ***9***(2): 178-187. https://doi.org/10.33714/masteb.744894

Saka, F. & Bulut, M. (2020). Determination of fish consumption in Çanakkale. *Marine Science and Technology Bulletin,* ***9***(1): 7-14. https://doi.org/10.33714/masteb.658093

Selvi, K., Kandemir, G. & Özdikmenli Tepeli, S. (2019). Determination of factors affecting on the fish consumption habit in rural areas. *COMU Journal of Marine Science and Fisheries,* ***2***(2): 132-141.

Sümbüloğlu, V. & Sümbüloğlu, K. (2019). Bioistatistik. 13. Ed. Ankara, Turkey: Hatiboğlu Publishing. 299 p.

Telli, Ö. (2018). Denizli ili su ürünleri tüketim alışkanlıkları üzerine bir anket çalışması. Master Thesis. Süleyman Demirel University, Isparta, Turkey.

Temel, T. (2014). Rize İlinde hanelerin balık tüketimi üzerine etkili olan faktörlerin belirlenmesi. Master Thesis. Atatürk University,Erzurum, Turkey.

TurkStat. (2018). Fishery Statistics. Turkish Statistical Institute. Retrieved on August 20, 2020, from http://www.tuik.gov.tr

Tokuşoğlu, Ö. (2016). Kabuklu su ürünleri etleri ve kalite: Midye, istiridye, karides ve yengeç. Retrieved on February 14, 2020 from http://www.gida2000.com/kabuklu-su-urunleri-etleri-ve-kalite-midye-istiridye-karides-ve-yengec.html

Yavuz, G. G., Ataseven, Z. Y., Gül, U., Gülaç, Z. N. (2015). Su ürünleri tüketiminde tüketici tercihlerini etkileyen faktörler: Ankara ili örneği. *Yunus Araştırma Bülteni,* ***1***(5): 73-82.

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SHORT COMMUNICATION

Maximum size of *Stephanolepis diaspros* (Tetraodontiformes: Monacanthidae)

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ABSTRACT

Reticulated leatherjacket, *Stephanolepis diaspros* Fraser-Brunner, 1940 is one of the very first Lessepsian settlers in the Mediterranean. On 22 June 2020 a specimen of *Stephanolepis diaspros* (catalogue number: ESFM-PIS/2020-02) with 305 mm in total length (TL) was captured by an angler off Yassıcaada, Urla, Izmir Bay on sandy bottom at a depth of 18 m. The present ichthyologic record demonstrates maximum size of *S. diaspros* species and even, this large size is unique in the Mediterranean and the world seas until a new one is reported.

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Introduction

Reticulated leatherjacket, *Stephanolepis diaspros* Fraser-Brunner, 1940 is one of the very first Lessepsian settlers in the Mediterranean (Tortonese, 1986; Froese and Pauly, 2019), and lives inshore in sandy and rocky habitats with vegetation to a depth of 20 m (Golani et al. 2006; Froese and Pauly, 2019). Common sizes of *S. diaspros* are 7-15 cm, with the maximum as 20 cm (Golani et al. 2006) and 25 cm TL (Froese and Pauly, 2019).

Stephanolepis diaspros is especially well established in the eastern Mediterranean; however, it is still rare in the Aegean Sea. It has been reported from the Aegean Sea in 1943 for the first time (Tortonese, 1947) and it occasionally occurs in the region, reaching as far as to the Sea of Marmara (Bilecenoğlu and Yokeş, 2013). Also, it reached to the Adriatic Sea (Dulčić and Pallaoro, 2003), Gulf of Palermo, Sicily (Catalano and Zava, 1993), Tunisia (Ben Amor and Capapé, 2008; Zouari-Ktari et al. 2008) and Maltese waters (Deidun et al. 2015).

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This paper reports a new maximum size of *S. diaspros* and even, its large size is unique in the Mediterranean and the world seas for the time being.

Material and Methods

On 22 June 2020, a specimen of *S. diaspros* (Figure 1) with 305 mm in total length (TL) was captured by an angler off Yassıcaada, Urla, Izmir Bay (38°24.271 N - 26°47.589 E), on sandy bottom at a depth of 18 m (Figure 2). The bait was European razor clam. The specimen was measured to the nearest millimetre, fixed in 6% formaldehyde solution and deposited in the Ichthyological Collection of Fisheries Faculty, Ege University with the catalogue number: ESFM-PIS/2020-02.

Figure 1. The specimen of *Stephanolepis diaspros* with 305 mm TL (ref. ESFM-PIS/2020-02), captured off Yassıcaada, Urla in the Bay of İzmir (Photo: O. Akyol)

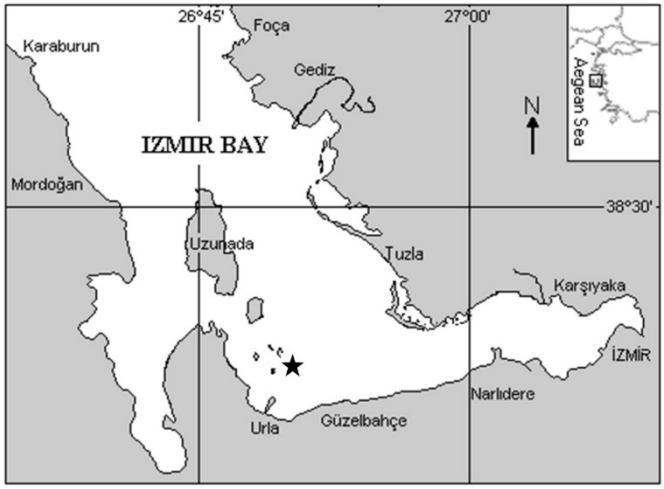

Figure 2. Capture site (black star) of *Stephanolepis diaspros*

Results and Discussion

Description, measurements and percentage in total length (Table 1) of *S. diaspros* are in total accordance with those in Tortonese (1986), Golani et al. (2006), Froese and Pauly (2019).

Table 1. Morphometric measurements as percentage of total length (TL%) and meristic counts recorded in *Stephanolepis diaspros*, captured from İzmir Bay, Aegean Sea

Measurements	Size (mm)	Proportion (TL%)
Total length (TL)	305	-
Standard length (SL)	260	85.2
Pre-second dorsal fin length	138	45.2
Pre-anal fin length	152	49.8
Pre-pectoral fin length	74	24.3
Maximum body depth	122	40.0
Head length	73	23.9
Eye diameter	13	4.3
Preorbitary length	51	16.7
Meristic counts		
First dorsal fin rays	I	
Second dorsal fin rays	30	
Anal fin rays	30	
Pectoral fin rays	13	
Weight (g)	464	

Gücü et al. (1994) firstly reported *S. diaspros* from Mersin and Iskenderun bays, the north-eastern Mediterranean coast of Turkey during 1983, 1984 and 1989 intermittent trawl surveys. This might be the oldest catch record of *S. diaspros* in the Turkish waters and the other previous records of *S. diaspros* are shown in Table 2.

As seen, the size reported in this study might be the maximum for the Mediterranean. Recently *S. diaspros* with 100-300 mm in length was reported by Servonnat and Drakulic (2015) from Lipsi Island, Greece. Even though, it had 300 mm maximum length that close to our finding, this was observed during underwater visual census. Such a report on maximum length needs further approval since it tends to produce less accurate estimates of fish length due to optical characteristics of water (*see*, Harvey et al. 2001a, b; 2002).

Conclusion

In conclusion, maximum size is one of the important parameters used in life history studies and fishery science (Borges, 2001). In addition, the present ichthyologic record demonstrates maximum size of *S. diaspros* species and even, this large size is unique in the Mediterranean and the world seas until a new one is reported.

Table 2. Previous records of *Stephanolepis diaspros* in various seas between 1983 and 2020

Date	n	TL range (mm)	Depth (m)	Area	References
1983/84-89	8	77-206	<100	Anamur-İskenderun	Gücü et al. (1994)
1991-1994	3	100-145	40	Karataş, İskenderun	Torcu and Mater (2000)
1994-1996	2	161-72	40	Karataş, İskenderun	Başusta and Erdem (2000)
1997-1998	207	71-130	10-80	Mersin-İskenderun	Taşkavak and Bilecenoğlu (2001)
2001-2003	52	73-142	5-100	NE Mediterranean	Sangun et al. (2007)
?	3	98-212	10-20	Yeşilova Bay	Oz et al. (2007)
May2003-Dec.2005	1124	37-237	0-40	Gulf of Gabes, Tunisia	Zouari-Ktari et al. (2008)
2004-2007	550	70-261	?	Gulf of Suez, Red Sea	El-Ganainy and Sabra (2008)
2007-2008	56	80-135	12-120	İskenderun Bay	Erguden et al. (2009)
2008-2009	16	80-190*	5-35	S. Aegean Sea	Corsini-Foka et al. (2010)
2009-2010	7	96-139	?	Antalya Bay	Türker et al. (2020)
2010-2011	158	80-202	31-110	İskenderun Bay	Yemişken et al. (2014)
28-29 Nov.2012	2	ca.200	10	Sea of Marmara	Bilecenoğlu and Yokeş (2013)
27 Sep.2013	1	130	20	Piran, Slovenia	Lipej et al. (2014)
10 Dec.2013	1	200	34	Lampedusa Island	Deidun et al. (2015)
20 July 2014	1	170	20	Egadi Island, Sicily	Balistreri and Parasporo (2015)
July-Aug.2014	17	100-300**	1-6	Lipsi Island, Greece	Servonnat and Drakulic (2015)
27 Oct.2014	1	177	6	Urla, İzmir Bay	Akyol and Özgül (2015)
15-19 Sep.2014	3	115-239	6-25	Pemera-Limassol, Cyprus	Iglésias and Frotté (2015)
10 Jan.2018	1	257	1	Türkbükü, Güllük Bay	Akyol et al. (2018)
22 June 2020	1	305	18	Yassıcaada, Aegean Sea	This study

Note: *SL; **estimated by underwater observation

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Compliance with Ethical Standards

Authors' Contributions

Author GM: Conceptualization, investigation, editing; Author OA: Methodology, writing – original draft, writing – review & editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Akyol, O. & Özgül, A. (2015). Record of reticulated leatherjacket *Stephanolepis diaspros* Fraser-Brunner, 1940 (Tetraodontiformes: Monacanthidae) from Izmir Bay, Turkey. *Journal of Black Sea/Mediterranean Environment,* ***21***(3): 316-322.

Akyol, O., Ceyhan, T., Özgül, A. & Ertosluk, O. (2018). Maximum size of reticulated leatherjacket, *Stephanolepis diaspros* Fraser-Brunner, 1940 (Tetraodontiformes: Monacanthidae), for the Turkish Seas. *Journal of Black Sea/Mediterranean Environment*, ***24*****:** 149-156.

Balistreri, P. & Parasporo, M. (2015). First record of *Stephanolepis diaspros* (Tetraodontiformes, Monacanthidae) from the Egadi Islands Marine Protected Area (western Sicily). In: Tsiamis *et al.* New Mediterranean Biodiversity Records (July 2015). *Mediterranean Marine Science*, ***16***: 472-488. https://doi.org/10.12681/mms.1440

Başusta, N. & Erdem, Ü. (2000). A study on the pelagic and demersal fishes of Iskenderun Bay. *Turkish Journal of Zoology*. ***24***(Suppl.): 1-19.

Ben Amor, M. M. & Capapé, C. (2008). Occurrence of a filefish closely related to *Stephanolepis diaspros* (Osteichthyes: Monacanthidae) off northern Tunisian coast (South-western Mediterranean). *Cahiers de Biologie Marine,* ***49*****:** 323-328.

Bilecenoğlu, M. & Yokeş, B. (2013). New Lessepsian fish records from the Aegean and Marmara Sea. In: Bilecenoğlu *et al.,* New Mediterranean marine biodiversity records (December 2013). *Mediterranean Marine Science,* ***14*****:** 463-480.

Borges, L. (2001). A new maximum length for the snipefish, *Macroramphosus scolopax*. *Cybium*, ***25***(2): 191-192.

Catalano, E. & Zava, B. (1993). The presence of *Stephanolepis diaspros* Br. Brunn. Italian waters (Osteichthyes, Filefish). *Supplemento alle Ricerche di Biologia della Selvaggina* ***21*****:** 379-382.

Corsini-Foka, M., Pancucci-Papadopoulou, M. A. & Kalogirou, S. (2010). Is the Lessepsian province in expansion? The Aegean Sea experience. Sub-regional Technical meeting on the Lessepsian migration and its impact on eastern Mediterranean fishery. Nicosia, 7-9 Dec. FAO East Med Tech. Document, pp. 50-59.

Deidun, A., Castriota, L., Falautano, M., Maraventano, G., Prazzi, E. & Andaloro, F. (2015). Documenting the occurrence of the Lessepsian fish *Stephanolepis diaspros* within the Strait of Sicily, Central Mediterranean. *Journal of Black Sea/Mediterranean Environment,* ***21***(1): 1-11.

Dulčić, J. & Pallaoro, A. (2003). First record of the filefish, *Stephanolepis diaspros* (Monocanthidae), in the Adriatic Sea. *Cybium,* ***27*****:** 321-322.

El-Ganainy, A. A. & Sabra, M. M. M. (2008). Age, growth, mortality and yield per recruit of the file fish *Stephanolepis diaspros* (Fraser-Brunner, 1940) (Pisces: Monacanthidae) in the Gulf of Suez, Egypt. *Journal of Fisheries and Aquatic Science,* ***3*****:** 252-260. https://doi.org/10.3923/jfas.2008.252.260

Erguden, D., Turan, C. & Gurlek, M. (2009). Weight-length relationships for 20 Lessepsian fish species caught by bottom trawl on the coast of Iskenderun Bay (NE Mediterranean Sea, Turkey). *Journal of Applied Ichthyology,* ***25*****:** 133-135. https://doi.org/10.1111/j.1439-0426.2008.01198.x

Froese, R. & Pauly, D. (2019). FishBase. World Wide Web electronic publication. www.fishbase.org, version (12/2019) (accessed date: 24 June 2020).

Golani, D., Öztürk, B. & Başusta, N. (2006). *The fishes of the eastern Mediterranean*. Turkish Marine Research Foundation Publication No: 24 Istanbul, Turkey. 259p.

Gücü, A. C., Bingel, F., Avşar, D. & Uysal, N. (1994). Distribution and occurrence of Red Sea fish at the Turkish coast-northern Cilician basin. *Acta Adriatica,* ***34*****:** 103- 113.

Harvey, E., Fletcher, D. & Shortis, M. (2001a). A comparison of the precision an accuracy of estimates of reef-fish lengths determined visually by divers with estimates produced by a stereo-video system. *Fisheries Bulletin,* ***99*****:** 63-71.

Harvey, E., Fletcher, D. & Shortis, M. (2001b). Improving the statistical power of length estimates of reef fish: A comparison of estimates determined visually by divers with estimates produced by a stereo-video system. *Fisheries Bulletin,* ***99*****:** 72-80.

Harvey, E., Fletcher, D. & Shortis, M. (2002). Estimation of reef fish length by divers and by stereo-video: A first comparison of the accuracy and precision in the field on living fish under operational conditions. *Fisheries Research,* ***57*****:** 255- 265.

Iglésias, S. P. & Frotté, L. (2015). Alien marine fishes in Cyprus: Update and new records. *Aquatic Invasions,* ***10*****:** 425-438. https://doi.org/10.3391/ai.2015.10.4.06

Lipej, L., Mavric, B. & Dulcic, J. (2014). Northern most record of the reticulated leather-jacket *Stephanolepis diaspros* Brunner-Fraser, 1940 in the Mediterranean Sea. In: Kapiris et al., New Mediterranean Biodiversity Records (April, 2014). *Mediterranean Marine Science,* ***15*****:** 198-212.

Oz, M. İ., Okuş, E. & Yüksek, A. (2007). Notes on the Erythrean alien fishes of Datça-Bozburun Peninsula- A specially protected area in the southeastern Aegean Sea (Turkey). Rapport du 38e Congrés de la CIESM (*Rapp. Comm. int Mer Médit.*), ***38*****:** 563.

Sangun, L., Akamca, E. & Akar, M. (2007). Weight-length relationships for 39 fish species from the north-eastern Mediterranean coasts of Turkey. *Turkish Journal of Fisheries and Aquatic Sciences,* ***7*****:** 37-40.

Servonnat, M. & Drakulic, M. (2015). New records of *Fistularia commersonii* and *Stephanolepis diaspros* around Lipsi Island, Dodacanese, Greece. In: Zenetos *et al.* New Mediterranean Biodiversity Records (April 2015). *Mediterranean Marine Science,* ***16*****:** 266-284.

Taskavak, E. & Bileceoglu, M. (2001). Length-weight relationships for 18 Lessepsian (Red Sea) immigrant fish species from the eastern Mediterranean coast of Turkey. *Journal of the Marine Biological Association of the Umited Kingdom,* ***81*****:** 895-896.

Torcu, H. & Mater, S. (2000). Lessepsian fishes spreading along the coasts of the Mediterranean and the southern Aegean Sea of Turkey. *Turkish Journal of Zoology,* ***24*****:** 139-148.

Tortonese, E. (1947). Zoological research in Rhodes Island (Aegean Sea) fish. *Bollatino di Pesca, Piscicoltura e Idrobiologia,* ***23*****:** 143-192.

Tortonese, E. (1986). Monacanthidae (pp. 1338-1339). In: Whitehead, P. J. P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J., Tortonese, E. (Eds), *Fishes of the North-eastern Atlantic and the Mediterranean*. Vol. III, UNESCO, Paris.

Türker, D., Zengin, K. & Bal, H. (2020). Length-Weight Relationships of 11 Lessepsian Migrant Fish Species Caught from Antalya Bay (Turkey). *Acta Aquatica Turcica*, ***16***(2): 301-304. https://doi.org/10.22392/actaquatr.670648

Yemisken, E., Dalyan, C. & Eryılmaz, L. (2014). Catch and discard fish species of trawl fisheries in the Iskenderun Bay (North-eastern Mediterranean) with emphasis on lessepsian and chondricthyan species. *Mediterranean Marine Science,* ***15*****:** 380-389. https://doi.org/10.12681/mms.538

Zouari-Ktari, R., Bradai, M. N. & Bouain, A. (2008). The feeding habits of the Lessepsian fish *Stephanolepis diaspros* (Fraser-Brunner, 1940) in the Gulf of Gabes (eastern Mediterranean Sea). *Cahiers de Biologie Marine,* ***49*****:** 329-335.

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RESEARCH ARTICLE

The effects of EDTA on lead accumulation in tissues of *Clarias gariepinus*

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ABSTRACT

This study was intended to determine the lead accumulation levels in liver, gill, kidney, brain, and muscle tissues of *Clarias gariepinus* (African sharp tooth catfish) under the sole effect of lead (1.0 and 2.0 ppm) and combined with EDTA (Ethylene diamine tetraacetic acid) (2.0 and 4.0 ppm) for 7, 15, and 30 days. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine tissue lead levels, and the SPSS package program was used for statistical evaluation of the experimental data. No mortality was observed in fish over the time periods of the experiments, and concentrations were determined with lead only and with lead together with EDTA. At the end of the experimental periods, the presence of lead increased the metal accumulation in the tissues and organs examined when compared to the control, and, in terms of accumulation, a relationship was determined between the tissues in the order of gill > kidney > liver > brain > muscle. The effect of lead together with EDTA was reduced lead accumulation in tissues and organs when compared to the effect of lead only.

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Introduction

The disposal and poor management of toxic chemicals such as heavy metals negatively affect ecosystem integrity, environmental quality, and human health (Banaee et al., 2013). Heavy metals such as lead, cadmium, and mercury do not have any biological functions in animal organisms, while other heavy metals, such as copper, zinc, iron, and chromium, at low concentrations, are essential for metabolic processes. However, they are also toxic above a certain concentration range and can negatively affect all trophic levels throughout the food chain (Shukla et al., 2007).

Lead is a heavy metal widely used in various industrial applications, such as paint, storage batteries, and the automotive industry, as well as being naturally found in soil, water, and rocks (Khidr et al., 2012). Lead at high concentrations leads to accumulation in tissues and organs (Cicik et al., 2004), behavioral changes (Çiftçi et al., 2008), lordoscolosis and anomalies in pigmentation (Martinez et al., 2004), collapse of the immune system in fish (Vosylienė, 1999)

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and mutagenic, teratogenic, and carcinogenic effects and infertility in humans (Eisler, 2000).

Organic and inorganic complexes, such as EDTA, NTA (Nitrilotriacetic acid), DTPA (Diethylenetriamine pentaacetic acid), DFO (Deferoxamine), DFP (Deferiprone), zeolite, clinoptilolite, and chitosan, are widely used in aquatic systems to remove pollutants from the environment. A high capacity to form complexes with heavy metals and low biological degradability in aquatic systems are the main properties of chemicals used as chelators (Kedziorek and Bourg, 2000).

In fish, the liver accumulates metals at high concentrations regardless of the intake path, and, because the metal load in the liver is close to the concentration of the metal in the environment, it is very important in monitoring metal pollution in aquatic ecosystems (Jezierska and Witeska, 2006). Gills, which have vital functions such as respiration and osmoregulation, are the main target organs for toxic chemicals because they interact directly with the environment (Poleksic and Mitrovic-Tutundzic, 1994). In fish, kidneys function in maintaining homeostasis, as well as in removing nitrogen-containing metabolic waste materials and toxic substances from the body (Cengiz, 2006). The brain is a metabolically active organ that is highly sensitive to changes in energy metabolism (Soengas and Aldegunde, 2002). Although the muscle tissue in fish is not active in terms of metal accumulation, it constitutes the main consumable part of the fish, and it functions in the transmission of metal to humans through the food chain, so it is very important in terms of environment and human health (Jezierska and Witeska, 2006).

Although there are many studies on the accumulation and toxic effects of heavy metals in aquatic organisms, the studies on the effects of complexing agents on heavy metal toxicity are limited. Therefore, this study aimed to determine the levels of lead accumulation solely and of lead accumulation together with EDTA in the liver, gill, kidney, brain, and muscle tissues of *C. gariepinus* for periods of seven, fifteen, and thirty days.

Material and Methods

The research was carried out in the basic sciences research laboratory of the Mersin University Faculty of Fisheries Application Unit under controlled ambient conditions (24±1 °C fixed temperature, 12-hour dark/12-hour daylight photoperiod, central ventilation system).

C. gariepinus specimens of 68.17 ±4.36 g weight and 21.24 ±2.08 cm total length were used as the material in the experiments. The fish were fed once daily at the same hour with commercial fish feed (Pellet No. 2, Pınar, İzmir, Turkey) at 2% of their total biomass during the study. The water-soluble salt of lead, $Pb(NO_3)_2$, was used in the study, while EDTA ($C_{10}H_{16}N_2O_8$) was used as the complexing agent. As a result of the literature reviews (Alkahemal-Balawi et al., 2011; Bawa-Allah and Saliu, 2015) and preliminary studies, non-lethal concentrations of both lead and EDTA (1.0 and 2.0 ppm Pb; 2.0 and 4.0 ppm EDTA) within the specified time periods were determined and utilized.

Five glass aquariums were used in the study to contain 120 L each of the experimental solutions. Solutions at the concentrations of 1.0 and 2.0 ppm lead were added to the first two aquariums, respectively. To the third and fourth aquariums, solutions of 1.0 ppm lead with 2.0 ppm EDTA and 2.0 ppm lead with 4.0 ppm EDTA were added, respectively. The fifth aquarium contained 120 L of lead-free tap water and constituted the control. Experiments were carried out in three replicates, and two fish were used in each replicate. Six fish were removed from each aquarium at the ends of the seven-, fifteen-, and thirty-day periods and anesthetized with phenoxy-ethanol (1 ml/L) (Morgan et al., 1997), and the liver, gill, kidney, brain, and muscle tissues of the fish were dissected separately. In total, 90 fish were used in the experiments. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Local ethics committee approval was received.

Table 1. Some physical and chemical properties of the water in the experimental aquariums

Parameter	Value
Temperature	23 ±1 °C
Dissolved Oxygen	6.47 ±0.43 ppm O_2
pH	8.07 ±0.04
Total Hardness	259.3 ±5.82 ppm $CaCO_3$
Total Alkalinity	350.41 ±1.56 ppm $CaCO_3$

Because the test solutions in the aquariums were subject to time-dependent changes in their concentrations due to evaporation, precipitation, and adhesion to surfaces, a semi-static test was applied, and the test solutions were renewed every two days by appropriate dilutions of the stock solution.

The liver, gill, kidney, brain, and muscle tissue samples that were dissected and used in the metal analysis. Samples were placed in an oven set to 105 °C for 72 hours, until a fixed weight was attained. Then, a mixture of 2 ml nitric acid (HNO_3, 65%, specific gravity 1.40, Merck) and 1 ml perchloric acid ($HClO_4$, 60%, specific gravity 1.53, Merck) (Muramoto, 1983) was added on a hot plate set at 120 °C for eight hours. After the burning process, tissue samples were transferred to polyethylene tubes, their total volumes were adjusted to 10 ml with deionized water, and they were made ready for analysis. The lead content of the tissue samples was determined by ICP-MS. Analysis was carried out in triplicate. Control samples were prepared from IAEA - 407 (International Atomic Energy Agency) fish tissue

homogenate. Detailed results on the amount of lead element obtained from the reference material and LOD-LOQ values given in Table 2. Variance analysis and the Student Newman Keul's (SNK) test were performed by using the SPSS package program for the statistical analysis of data.

Table 2. Validation parameters of the analytical method

Parameter	Value
Trace elements	Lead
LOD (ng g^{-1})	0.39
LOQ (ng g^{-1})	1.21
R^2	0.9999
Certificated Values Concentration (mg kg^{-1})	0.12
Certificated Values	
95% Confidence Interval (mg kg^{-1})	0.10-014

Note: IAEA-407 was used as reference material. LOD= Limit of Detection, LOQ= Limit of Quantification

In the present study on *C. gariepinus*, no mortality was observed in the fish due to the effects of the determined times and concentrations. At the beginning of the experiments, various behavioral and morphological changes were observed in fish, including lack of nutrients, immobility at the bottom of the aquarium, orientation toward the aquarium surface, and darkening of skin color.

The effect of lead-only concentrations after seven days was increased metal accumulation in the tissues and organs examined when compared to the control ($p<0.05$, Table 3). At the end of the 7th day, the highest accumulation was in the gill tissue, while the lowest accumulation was in the muscle tissue. The effect of high concentrations of lead combined with EDTA was reduced accumulation of lead in the tissues and organs examined, as compared to the effect of lead only ($p<0.05$).

The effect of lead-only concentrations of 1.0 and 2.0 ppm for fifteen days increased the metal accumulation in all tissues when compared to the control (Table 4). This increase was significantly higher in liver and gill tissues when compared to that on day 7 ($p<0.05$). The effect of a mixture of lead and high concentrations of EDTA for fifteen days resulted in an approximately 60% reduction in lead accumulation in liver, kidney, and gill tissues when compared to the effect of high concentrations of lead only.

Table 3. Lead accumulation in *C. gariepinus* under the sole effect of lead and together with EDTA for 7 days (µgPb/g d.w.)

Tissue / Concentration	Liver	Kidney	Gill	Brain	Muscle
	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *
Control	BSL	BSL	BSL	BSL	BSL
1 ppm Pb	2.29 ±0.09 as	14.23 ±0.46 bs	19.54 ±0.38 cs	3.39 ±0.30 ds	0.87 ±0.04 es
2 ppm Pb	4.62 ±0.28 at	14.60 ±1.15 bs	25.23 ±1.67 ct	3.65 ±0.33 as	0.84 ±0.04 ds
1 ppm Pb + 2 ppm EDTA	2.15 ±0.35 as	13.26 ±0.93 bs	16.10 ±0.81 cx	3.26 ±0.24 ds	1.37 ±0.17 at
2 ppm Pb + 4 ppm EDTA	1.40 ±0.18 ax	9.72 ±0.57 bt	6.43 ±0.86 cy	2.56 ±0.16 dt	0.21 ±0.01 ax

Note: $\bar{X} \pm S_{\bar{x}}$ = Arithmetic mean ± Standard error; BSL = Below Sensitivity Level

* = SNK; a, b, c, d, e were used to determine the differences between tissues while s, t, x, and y were used to determine the differences between concentrations. Different letters indicate statistical differences at $P<0.05$.

Table 4. Accumulation of only lead and lead together with EDTA in *C. gariepinus* tissues for 15 days (µgPb/g d.w.)

Tissue / Concentration	Liver	Kidney	Gill	Brain	Muscle
	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *
Control	BSL as	BSL as	BSL as	BSL as	BSL as
1 ppm Pb	6.38 ±0.67 at	11.84 ±0.75 bt	22.40 ±0.48 ct	3.34 ±0.23 dt	1.38 ±0.3 et
2 ppm Pb	8.29 ±0.39 ax	11.80 ±1.11 bt	57.29 ±1.73 cx	2.77 ±0.18 dt	1.58 ±0.2 dtx
1 ppm Pb + 2 ppm EDTA	4.27 ±0.55 ay	6.04 ±0.36 bx	13.51 ±0.52 cy	2.59 ±0.22 dt	2.11 ±0.2 dx
2 ppm Pb + 4 ppm EDTA	2.74 ±0.09 az	4.51 ±0.16 bx	11.71 ±0.77 cy	2.62 ±0.36 at	0.98 ±0.01 dt

Note: $\bar{X} \pm S_{\bar{x}}$ = Arithmetic mean ± Standard error; BSL = Below Sensitivity Level

* = SNK; a, b, c, d, e were used to determine the differences between tissues while s, t, x, and y were used to determine the differences between concentrations. Different letters indicate statistical differences at $P<0.05$.

Table 5. Lead accumulation in *C. gariepinus* under the sole effect of lead and under lead together with EDTA for 30 days (µgPb/g d.w.)

Tissue / Concentration	Liver	Kidney	Gill	Brain	Muscle
	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *	$\bar{X} \pm S_{\bar{x}}$ *
Control	BSL as	BSL as	BSL as	BSL as	BSL as
1 ppm Pb	10.46 ±0.61 at	13.67 ±0.43 at	47.99 ±2.53 bt	7.91 ±0.83 ct	0.96 ±0.09 dt
2 ppm Pb	14.97 ±1.24 ax	16.68 ±0.80 ax	74.02 ±4.97 bx	5.49 ±0.59 cx	1.08 ±0.08 dt
1 ppm Pb + 2 ppm EDTA	5.11 ±0.27 ay	5.92 ±0.12 ay	20.85 ±2.07 by	5.61 ±0.52 ax	0.96 ±0.09 ct
2 ppm Pb + 4 ppm EDTA	3.73 ±0.41 ay	6.51 ±0.33 by	20.69 ±2.54 cy	3.65 ±0.35 ay	0.91 ±0.02 dt

Note: $\bar{X} \pm S_{\bar{x}}$ = Arithmetic mean ± Standard error; BSL = Below Sensitivity Level

* = SNK; a, b, c, d, e were used to determine the differences between tissues while s, t, x, and y were used to determine the differences between concentrations. Different letters indicate statistical differences at $P<0.05$.

The effect of lead at higher concentrations together with EDTA for 30 days significantly increased the accumulation of lead in the tissues and organs examined in comparison to the control and to days 7 and 15 ($p<0.05$) (Table 5). While this increase was parallel to the increase in the lead concentration, the effect of lead together with EDTA reduced the metal accumulation in the examined tissues when compared to the effect of lead only. This decrease was in parallel with the increase in concentration.

Discussion

The effects of toxic substances, particularly heavy metals, on mortality rate and mortality period in aquatic vertebrates vary depending on the species, the metal, the environmental concentration of the metal, and the duration of exposure. In studies conducted on various fish species, it has been determined that the mortality rate increases in parallel with the increase in the toxin concentration in the environment and the duration of exposure (Abdullah et al., 2007; Gül et al., 2009; Alkahemal-Balawi et al., 2011). In the present study conducted on *C. gariepinus*, no mortality was observed in the fish under the conditions of time and concentration imposed. That can be explained by the fact that the lead concentrations found in the tissues are not lethal for the subject species in the specified periods.

Fish react by changing their behavior against changing environmental conditions such as pollution or changes in the physical or chemical properties of the water. Those changes include behaviors such as feeding, reproduction, growth, respiration, and movement (Alves and Wood, 2006). In this study conducted with *C. gariepinus*, behavioral changes such as indifference to food, immobility at the bottom of the aquarium, coordination disorders in swimming movements, and respiration difficulties were observed at the beginning of the lead effect, and the changes disappeared with the prolongation of the effect time. The changes observed in fish behavior at the beginning of the metal effect may result from a reaction to changing environmental conditions, while prolongation of the effect time and return to normal behaviors may be related to adaptation.

In studies conducted with various fish species under both natural and laboratory conditions, it has been found that metal accumulation varied with the tissue and organ (Alves and Wood, 2006; Shukla et al., 2007; Banaee et al., 2013; Ogbuagu et al., 2015). The liver is an important organ that functions in detoxification and biotransformation of xenobiotics, as well as in metabolic events (Van Dyk et al., 2007). It was determined that cadmium in *Oreochromis aureus* (Gül et al., 2009), lead in *Oncorhynchus mykiss* (Alves and Wood, 2006), and zinc, cadmium, and copper in *Channa punctatus* (Shukla et al., 2007) accumulate more in the liver than in other tissues and organs. In the present study conducted with *C. gariepinus*, the highest accumulation occurred in gill and kidney tissues, followed by liver tissue, under the sole effect of lead. That may be due to the liver being responsible for the detoxification process and the lead concentration failing to exceed its uptake and carrying capacity (Figueiredo-Fernandes et al., 2006; Van Dyk et al., 2007).

In fish, gills take part in respiratory, osmoregulation, and excretion functions. It was determined that lead accumulated most in gill tissue in *Carassius auratus* (Banaee et al., 2013), *C. gariepinus* (Kusemiju et al., 2012), and *C. catla* (Mohanambal and Puvaneswari, 2013), while zinc, copper, and cadmium accumulated most in gill tissue in *C. punctatus* (Shukla et al., 2007) under the effect of sublethal concentrations. In this study carried out with *C. gariepinus*, it was found that lead accumulated more in gill tissues than in the kidney, liver, brain, and muscle tissues under the effect of lead only or lead together with EDTA in the determined time periods and concentrations. This high accumulation in the gill tissue may be related to the

gills interacting directly with the environment. The gills function in excretion, as well as in respiration and osmoregulation (Poleksic and Mitrovic-Tutundzic, 1994; Mazon et al., 2002), and the retention of lead ions by binding them to the mucous membrane covering the gill surface is a defense mechanism (Tao et al., 2000).

The kidney is a multi-function organ that includes hematopoietic, reticuloendothelial, endocrine, and excretory functions in bony fishes (Karaman and Dörücü, 2017). In studies conducted with *Prochilodus lineatus* (Ribeiro et al., 2014), *Tilapia zilli* (Karataş and Kalay, 2002) and *Cyprinus carpio* (Luszczek-Trojnar et al., 2016), lead accumulates most in kidney tissues. On the other hand, in a study conducted with the mentioned species (Al -Balawi et al., 2013) and in the present study, lead was found to accumulate most in gill tissue, followed by kidney tissue. That may be due to the metal being transported to the kidney tissue to be excreted from the body (Clearwater et al., 2002) and retained by binding to metal-binding proteins such as metallothionein in the kidneys (Alves and Wood, 2006).

Unlike other metals, lead has been found to pass through the brain-blood barrier and to accumulate at high concentrations in brain tissue by inhibiting monoamine oxidase and acetylcholine esterase activity (Eisler, 2000). It also inhibits neurotransmitter functions by decreasing gamma-aminobutyric acid, cholesterol, and lipid levels (Katti and Sathyanesan, 1986). In a study conducted with *Tilapia nilotica* and *C. carpio*, it was found that lead accumulates in both types of brain tissue in higher concentrations than do cadmium, nickel, and chromium (Canlı and Kargın, 1995). As in studies on *O. mykiss* (Alves and Wood, 2006) and *C. catla* (Mohanambal and Puvaneswari, 2013), lead accumulations in the present study conducted with *C. gariepinus* were lower in the brain tissue than in the gill, liver, and kidney tissues under the effect of sublethal concentrations. Lower accumulation in brain tissue may be related to the brain-blood barrier, metal metabolism, and the carrying capacity of tissues (Eisler, 2000; Alves and Wood, 2006).

In studies carried out under various fish species and laboratory conditions (Canlı and Kargın, 1995; Alves and Wood, 2006; Kusemiju et al., 2012; Mohanambal and Puvaneswari, 2013), it was determined that the minimum lead accumulation in tissue was in the muscle tissue. In this study, both under the effect of lead only and lead together with EDTA, the lowest accumulations were in muscle tissue in comparison to other tissues and organs. Lower levels of accumulation in muscle tissue may be closely related to the metabolic function of the tissue or to a shorter exposure period for the accumulation.

Chelating agents form complexes with heavy metals in the environment by adsorption and ion exchange mechanisms, preventing their absorption and accumulation by aquatic organisms and reducing their toxic effects. In *C. carpio*, lead accumulations in tissues and organs were examined under the sole effect of lead only and of lead together with EDTA, NTA, and DTPA; with chelating agents, lead accumulation was reduced in tissues as compared to the effect of lead only (Muramoto, 1980). It was determined that calcium decreased the tissue lead accumulation in *O. mykiss* (Alves and Wood, 2006) and the tissue copper accumulation in *O. mossambicus* (James et al., 1998) as compared to the effect of metals only. In the present study conducted with *C. gariepinus*, the effect of lead together with EDTA in the experimental times and concentrations decreased lead accumulation in the tissues and organs examined as compared to the effect of lead only. The effect with a chelator may result from decreased accumulation, EDTA complexes with lead, increasing molecular size, and prevention of uptake (Shalaby, 2007).

Conclusion

As a result, both the effect of lead only and that of lead together with EDTA in *C. gariepinus* caused lead accumulation in the gill, liver, kidney, brain, and muscle tissues. The highest lead accumulation was in the gill tissue, whereas the lowest was in the muscle tissue. The effect of lead with EDTA reduced the metal accumulation in the tissues examined when compared to the effect of lead only. Therefore, it was concluded that, in *C. gariepinus*, gill tissue is an indicator organ in determining lead toxicity and that EDTA has a reducing effect on lead toxicity.

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Compliance with Ethical Standards

Authors' Contributions

All authors contributed equally to this paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The ethics committee approval was obtained from Mersin University Animal Experiments Local Ethical Committee by decision number 14/45 dated 04/11/2016.

References

Abdullah, S., Javed, M. & Javid, A. (2007). Studies on acute toxicity of metals to the fish (*Labeo rohita*). *International Journal of Agriculture and Biology*, ***9***(2): 333-337.

Alkahemal-Balawi, H. F., Ahmad, Z., Al-Akel, A. S., Al-Misned, F., Suliman, El. A. M. & Al-Ghanim, K. A. (2011). Toxicity bioassay of lead acetate and effects of its sublethal exposure on growth, haematological parameters and reproduction in *Clarias gariepinus*. *African Journal of Biotech*, ***10***(53): 11039-11047. https://doi.org/10.5897/AJB11.1463

Al-Balawi, H. F. A., Al-Akel, A. S., Al-Misned, F., El Amin, M. S., Al-Ghanim, K. A., Mahboob, S. & Ahmad, Z. (2013). Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*. *Brazilian Archives of Biology and Technology*, ***56***(2): 293-302. https://doi.org/10.1590/S1516-89132013000200015.

Alves, L. C. & Wood, C. M. (2006). The chronic effects of dietary lead in freshwater juvenile rainbow trout (*Oncorhynchus mykiss*) fed elevated calcium diets. *Aquatic Toxicology*, ***78***: 217–232. https://doi.org/10.1016/j.aquatox.2006.03.005

Banaee, M., Haghi, B. N. & Zoheiri, F. (2013). LC_{50} and bioaccumulation of lead nitrate $Pb(NO_3)_2$ in goldfish (*Carassius auratus*). *International Journal of Aquatic Biology*, ***1***(5): 233-239. https://doi.org/10.22034/ijab.v1i5.153

Bawa-Allah, K. A. & Saliu, J. K. (2015). Acute toxicity and bioaccumulation patterns of lead and zinc in juveniles of *Clarias gariepinus*. *Journal of Environmental Science Toxicology and Food Technology (IOSR-JESTFT)*, ***9***(1): 42-47. https://doi.org/10.9790/2402-09124247

Canlı, M. & Kargın, F. (1995). A comparative study on heavy metal (Cd,Cr, Pb and Ni) accumulation in the tissue of the carp *Cyprinus carpio* and the nile fish *Tilapia nilotica*. *Turkish Journal of Veterinary and Animal Sciences*, ***19***: 165–171.

Cengiz, E. İ. (2006). Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. *Environmental Toxicology and Pharmacology*, ***22***: 200–204. https://doi.org/10.1016/j.etap.2006.03.006

Cicik, B., Ay, Ö. & Karayakar, F. (2004). Effects of lead and cadmium interaction on the metal accumulation in tissue and organs of Nile tilapia *Oreochromis niloticus*. *Bulletin of Environmental Contamination and Toxicology*, ***1***(72): 141-148. https://doi.org/10.1007/s00128-003-0252-5

Clearwater, S. J., Farag, A. M. & Meyer, J. S. (2002). Review: Bioavailability and toxicity of dietborne copper and zinc to fish. *Comparative Biochemistry and Physiology - Part C*, ***132***: 269–313. https://doi.org/10.1016/S1532-0456(02)00078-9

Çiftçi, N., Cicik, B., Erdem, C. & Ay, Ö. (2008). Effects of lead concentrations on sera parameters and hematocrit levels in *Anguilla anguilla* (Linnaeus, 1758). *Journal of Fisheries Sciences*, ***2***(4): 616-622. https://doi.org/10.3153/jfscom.2008025

Eisler, R. (2000). Handbook of chemical risk assessment health hazards to humans, plants and animals: Vol 1, United States of America, 844p.

Figueiredo-Fernandes, A., Fontanhas-Fernandes, A., Rocha, E. & Reis-Henriques, M. A. (2006). The effect of paraquat on hepatic EROD activity, liver and gonadal histology in males and females of Nile tilapia, *Oreochromis niloticus*, exposed at different temperatures. *Archives of Environmental Contamination and Toxicology*, ***51***: 626–632. https://doi.org/10.1007/s00244-005-0208-3

Gül, A., Yılmaz, M. & Işılak, Z. (2009). Acute toxicity of zinc sulphate ($ZnSO_4.H_2O$) to guppies (*Poecilia reticulata* P.1859). *Gazi University Journal of Science*, ***22***(2): 59-65.

James, R., Sampath, K. & Selvamani, P. (1998). Effect of EDTA on reduction of copper toxicity in *Oreochromis mossambicus* (Peters). *Bulletin of Environmental Contamination and Toxicology*, ***60***: 487-493. https://doi.org/10.1007/s001289900651

Jezierska, B. & Witeska, M. (2006). The metal uptake and accumulation in fish living in polluted waters. *Soil and Water Pollution Monitoring, Protection and Remediation*, ***3***(23): 107-113. https://doi.org/10.1007/978-1-4020-4728-2_6

Karaman, Z. & Dörücü, M. (2017). Balıklarda bağışıklık sistemi organları ve histolojisi. *International Journal of Pure and Applied Sciences*, ***3***(1): 65-74.

Karataş, S. & Kalay, M. (2002). *Tilapia zilli*'nin solungaç, karaciğer, böbrek ve beyin dokularında kurşun birikimi. *Turkish Journal of Veterinary and Animal Sciences*, ***26***: 471-477.

Katti, S. R. & Sathyanesan, A. G. (1986). Lead nitrate induced changes in the brain constituents of the freshwater fish *Clarias batrachus* (L). *Neurotoxicology*, ***7***(3): 47–52.

Kedziorek, M. A. M. & Bourg, A. C. M. (2000). Solubilization of lead and cadmium during the percolation of EDTA through a soil polluted by smelting activities. *Journal of Contaminant Hydrology*, ***40***: 381–392. https://doi.org/10.1016/S0169-7722(99)00056-X

Khidr, B. M., Mekkawy, I. A. A., Harabawy, A. S. A. & Ohaida, A. S. M. I. (2012). Effect of lead nitrate on the liver of the cichlid fish (*Oreochromis niloticus*): A light microscope study. *Pakistan Journal of Biological Sciences*, ***15***(18): 854-862. https://doi.org/10.3923/pjbs.2012.854.862

Kusemiju, V., Patience, A. & Oluwatoyin, A. J. (2012). Accumulation of lead in the tissues of freshwater catfish *Clarias gariepinus* exposed to static nominal concentrations of lead nitrate. *Agriculture and Biology Journal of North America*, ***3***(12): 510-515. https://doi.org/10.5251/abjna.2012.3.12.510.515

Łuszczek-Trojnar, E., Sionkowski, J., Drazg-Kozak, E. & Popek, W. (2016). Copper and lead accumulation in common carp females during long-term dietary exposure to these metals in pond conditions. *Aquaculture Research*, ***47***: 2334–2348. https://doi.org/10.1111/are.12689

Martinez, C. B. R., Nagae, M. Y., Zaia, C. T. B. V. & Zaia, D. A. M. (2004). Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus*. *Brazilian Journal of Biology*, ***64***(4): 797-807. https://doi.org/10.1590/S1519-69842004000500009

Mazon, A. F., Monteiro, E. A. S., Pinheiro, G. H. D. & Fernandes, M. N. (2002). Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus Scrofa*. *Brazilian Journal of Biology*, ***62***(4A): 621-631. https://doi.org/10.1590/S1519-69842002000400010.

Mohanambal, R. & Puvaneswari, S. (2013). Bioaccumulatıon of lead in various tissues of the freshwater fish *Catla catla* (Hamilton, 1822). *International Journal of Development Research*, ***3***(8): 54-60.

Morgan, J. D., Sakamoto, T., Grau, E. G. & Iwama, G. K. (1997). Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. *Comparative Biochemistry and Physiology*, ***117A***(3): 391–398. https://doi.org/10.1016/S0300-9629(96)00261-7

Muramoto, S. (1980). Effects of complexans (EDTA, NTA and DTPA) on the exposure to high concentrations of cadmium, copper, zinc and lead. *Bulletin of Environmental Contamination and Toxicology*, ***25***: 941-946. https://doi.org/10.1007/BF01985635

Muramoto, S. (1983). Elimination of copper from Cu-contaminated fish by long term exposure to EDTA and freshwater. *Journal of Environmental Science and Health*, ***18***(3): 455-461. https://doi.org/10.1080/10934528309375113

Ogbuagu, D. H., Adebayo, E. T., Ayoade, A. A., Ugwu, O. B. & Mba, D. O. (2015). Lead accumulation in and its haematological effects on African catfish *Clarias gariepinus*. *African Journal of Aquatic Science*, ***40***(2): 201–204. https://doi.org/10.2989/16085914.2015.1028325

Poleksic, V. & Mitrovic-Tutundzic, V. (1994). Fish gills as a monitor of sublethal and chronic effects of pollution (pp. 339-352). In: Mulls, R, Llyod (Eds) on freshwater fish. Oxford, London, UK: Fishing News books.

Ribeiro, A. M., Risso, W. E., Fernandes, M. N. & Martinez, C. B. R. (2014). Lead accumulation and its effects on the branchial physiology of *Prochilodus lineatus*. *Fish Physiology and Biochemistry*, ***40***: 645–657. https://doi.org/10.1007/s10695-013-9873-8

Shalaby, A. M. E. (2007). Effect of EDTA on toxicity reduction of cadmium in relation to growth some haematological and biochemical profiles of Nile tilapia (*Oreochromis niloticus*). *Journal of Fisheries and Aquatic Science*, ***2***(2): 100-109.

Shukla, V., Dhankhar, M., Prakash, J. & Sastry, K.V. (2007). Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. *Journal of Environmental Biology*, ***28***(2): 395-397.

Soengas, J. L. & Aldegunde, M. (2002). Review. Energy metabolism of fish brain. *Comparative Biochemistry and Physiology Part B*, ***131***: 271–296. https://doi.org/10.1016/S1096-4959(02)00022-2

Tao, S., Li, H., Liu, C. & Lam, K. C. (2000). Fish uptake of inorganic and mucus complexes of lead. *Ecotoxicology and Environmental Safety*, ***46***: 174-180. https://doi.org/10.1006/eesa.1999.1902

Van Dyk, J. C., Pieterse, G. M. & Van Vuren, J. H. J. (2007). Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. *Ecotoxicology and Environmental Safety*, ***66***: 432–440. https://doi.org/10.1016/j.ecoenv.2005.10.012

Vosylienė, M. Z. (1999). The effect of heavy metals on haematological indices of fish (survey). *Acta Zoologica Lituanica. Hydrobiologia*, ***9***(2): 76-82. https://doi.org/10.1080/13921657.1999.10512290

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RESEARCH ARTICLE

Cu, Cd, As and Hg resistance levels in *Escherichia coli* isolated from Mediterranean mussel and sea snail in the Southeastern Black Sea

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ABSTRACT

Marine environment is exposed to various pollutants such as heavy metals, pesticides, and antibiotics. Bacterial resistance to these pollutants is a global problem all over the world. In this study, Mediterranean mussel (*Mytilus galloprovincialis*) and sea snail (*Rapana venosa*) were collected from 12 sampling points from Artvin, Rize, Trabzon, and Giresun Coasts of Black Sea, Turkey. A total of 54 *Escherichia coli* isolated from Mediterranean mussel and sea snail were tested for their ability to tolerate Cu, Cd, As, and Hg. For this purpose, minimum inhibitory concentration (MIC) tests for all isolates to the Cu, Cd, As, and Hg were done to determine tolerance or resistance using the broth dilution technique. MIC concentration for Cu, Cd, As, and Hg ranged between 100-400 µg/ml, 100-200 µg/ml, 25-400 µg/ml, and 3.125-25 µg/ml, respectively. All of the strains were determined as resistant to Cu, but sensitive to As. Resistance to Hg was determined as 7.4 %. The most common resistance gene in the bacteria was *nccA* and followed by *chrB* and *merA*. Tolerance or resistance of the bacteria to toxic pollutants including heavy metal(oid)s is of significant ecological importance. These bacteria could be used for monitoring environmental heavy metal(oid) pollution.

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Introduction

Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) and sea snail (*Rapana venosa* Valenciennes, 1846) are commercially important marine species in the Southeastern Black Sea. The sea snail is known as native species to the Japan Sea, the East China Sea, and the Bohai Sea (Tsi et al., 1983). It is also known as one of the most invasive species in the world. It is reported that sea snails first entered the Black Sea in 1946 (Drapkin, 1963; Saglam et al., 2015). They have a carnivorous feeding feature and are usually fed with sessile aquatic organisms such as mussels and oysters (Bat and Öztekin, 2016).

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Another species that has an important place among shellfish is *M. galloprovincialis*, known as Mediterranean mussel or black mussel. Mediterranean mussels, which are widely consumed all over the world, are known as one of the most cultivated species with economic value and rich in organic matter (Fuentes et al., 2009).

Mediterranean mussels are filter-feeding organisms in the water, and they have the potential to accumulate heavy metals, pesticides, pathogens, and radioactive materials from aquatic environments (Bat and Öztekin, 2016; Kacar, 2011). That is why they are one of the most important organisms that indicate their aquatic environment status. The mussels contaminated with pollutants pose a risk factor for both environmental, animal, and human health (Avşar and Berber, 2014; Terzi and Isler, 2019).

The gram-negative bacteria *Escherichia coli* is an indicator organism used to monitor fecal pollution of aquatic environments and seafood. The occurrence of *E. coli* in aquatic environments indicates the area or organisms polluted with feces with animal or human origin (Avşar and Berber, 2014; Terzi, 2018a). It is also known to carry multi-resistance genes like heavy metal resistance, antibiotic resistance, disinfectant resistance genes (Ture et al., 2020; Yang et al., 2020).

This study aimed to determine Cu, Cd, As, and Hg resistance levels and some resistance genes in *E. coli* isolated from sea snail (*R. venosa*) and Mediterranean mussel (*M. galloprovincialis*) in the Southeastern Black Sea using minimum inhibitory concentration test and molecular methods. Differentiation of resistance levels and metal resistance genes in isolated bacteria were determined between locations. By determining the MIC values of the isolates, the contamination status of the aquatic environment of the Southeastern Black Sea was revealed.

Material and Methods

Study Area, Sampling, and Isolation of E. coli

Mediterranean mussel and sea snail samples were collected from 12 coastal points of the Eastern Black Sea, Turkey (Figure 1). A total of 54 *E. coli* strains were isolated from Mediterranean mussel (n = 35) and sea snail (n = 19). Detailed information on sample collection, bacterial isolation, and identification have been given in our previously published articles (Terzi, 2018a; Terzi and Isler, 2019).

Minimum Inhibitory Concentration Test

Minimum inhibitory concentration (MIC) tests of all isolated *E. coli* strains were performed by the broth dilution method of Clinical and Laboratory Standards Institute (CLSI, 2018). In the tests, analytical grade metal(oid) salts of $CuCl_2.2H_2O$, $CdCl_2.6H_2O$, $NaAsO_2$, and $HgCl_2$ were used for copper, cadmium, arsenic, and mercury, respectively. The final concentration of 0 (Control), 12.5, 25, 50, 100, 200, 400, and 800 µg/ml were used for copper, cadmium, and arsenic; and 0 (Control), 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µg/ml for mercury in Luria Bertani (LB) Broth. After the inoculation of each isolate to media containing different concentrations of metal(oid)s, it is recorded whether bacteria can grow or not after incubation at 35°C for 24-36 hours and the lowest concentration that visibly inhibits bacterial growth is recorded as MIC. To determine resistance status, the isolates that can grow in the concentrations of 10 mM (750 µg/ml) for arsenic, 1 mM (112 µg/ml) for cadmium, 1 mM (63.5 µg/ml) for copper and 0.1 mM (20 µg/ml) for mercury were recorded as resistant (Nieto et al., 1987).

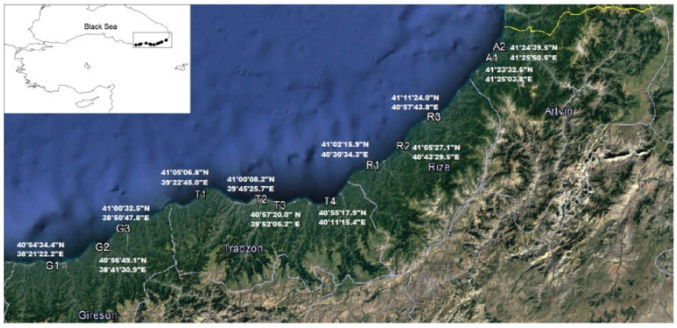

Figure 1. Sampling area

DNA Isolation and Detection of Resistance Genes by PCR

The boiling technique was used for the DNA extraction of the *E. coli* isolates for PCR assays (Boran et al., 2013; Capkin et al., 2015). The presence of *merA*, *nccA*, and *chrB* in the isolated bacterial strains was determined by PCR using the gene-specific primers shown in Table 1. Genomic DNA was used as template DNA in PCR assays.

PCR mixtures were prepared in 25 µl volumes (100 ng template DNA, 12.5 µl 2X Master Mix PCR mixture (NEB Master PCR Kit), 100 ng each of primer and sterile distilled water). Then, Thermal cycling was done with a Thermal Cycler (Biorad T100). The PCR amplification conditions consisted of initial denaturation at 95°C for 30 s; 35 cycles of denaturation at 95°C for 30 s, annealing at 55–59°C (see Table 1) for 45 s, and extension at 68°C for 45 s; and a final cycle of extension at 68°C for 90 s.

After the PCR, 10 µl of PCR mixture was subjected to electrophoresis system in 1% agarose gels prepared with 0.5 × Tris–Acetate– EDTA buffer and run at 100-110 V for 45-60 min. The size of resistance genes was estimated by 100 bp DNA

Table 1. Primers used in the PCR reactions

Target Gene	Sequencing (5′-3′)	PCR Product (bp)	Annealing Temperature (°C)	References
merA	FW"GAGATCTAAAGCACGCTAAGGC" R"GGAATCTTGACTGTGATCGGG"	1011	58	(Misra et al., 1984)
nccA	FW"ACGCCGGACATCACGAACAAG" R"CCAGCGCACCGAGACTCATCA"	450	59	(Nies et al., 1990)
chrB	FW"GTCGTTAGCTTGCCAACATC" R"CGGAAAGCAAGATGTCGATCG"	1141	55	(Abou-Shanab et al., 2007)

ladder (NEB). The gels were then stained with ethidium bromide and viewed by UV transillumination.

Results and Discussion

Metal(oid) Resistance/Sensitivity Test

A total of 54 *E. coli* strains were isolated and identified in our previous studies (Terzi, 2018a; Terzi and Isler, 2019). Of these, a total of 35 strains were isolated from Mediterranean mussels and 19 from the sea snails. The results of the MIC test showing the resistance/sensitivity levels of the isolated *E. coli* strains against Cu, Cd, Hg, and As were shown in Table 2. According to these test results, the MIC values of bacteria against copper, cadmium, arsenic and mercury ranged from 100-400 µg/ml, 100-200 µg/ml, 25-400 µg/ml and 3.125-25 µg/ml, respectively.

Table 2. Minimum inhibitory concentration results (µg/ml)

Location	Min-Max	Cu	Cd	As	Hg
Artvin	Max	400	200	200	12.5
	Min	200	100	100	3.125
Rize	Max	400	200	400	12.5
	Min	200	100	25	3.125
Trabzon	Max	400	200	400	25
	Min	100	100	50	3.125
Giresun	Max	400	200	400	25
	Min	100	100	50	3.125

According to the resistance levels of bacteria, it was determined that all the isolates were resistant to copper, followed by cadmium. No resistance to arsenic was detected in any of the bacteria. Mercury resistance (7.4%) was determined only in bacteria isolated from the stations in Trabzon and Giresun. Bacteria isolated from Rize and Artvin stations were determined to be sensitive to mercury (Table 3).

Distribution of Resistance Genes

The presence of *merA, nccA,* and *chrB* resistance genes in *E. coli* isolated from sea snail and Mediterranean mussel samples collected from the coast of Rize, Giresun, Artvin, and Trabzon provinces in the Eastern Black Sea were investigated by molecular methods. The presence of *merA, nccA,* and *chrB* genes in the isolates was shown in Table 4.

Table 3. Number of resistant (R) or susceptible (S) *Escherichia coli* strains to heavy metal(oid)s

Heavy Metal(oid)	Artvin		Rize		Trabzon		Giresun	
	R	S	R	S	R	S	R	S
Cu	4	0	13	0	18	0	19	0
Cd	1	3	5	8	8	10	11	8
As	0	4	0	13	0	18	0	19
Hg	0	4	0	13	2	16	2	17

Table 4. Distribution of resistance genes

Resistance Genes	Total (%)	Artvin (%)	Rize (%)	Trabzon (%)	Giresun (%)
N	54	4	13.0	18	19
merA	20.4	-	23.1	11.1	31.6
nccA	33.3	75	30.8	22.2	36.8
chrB	20.4	-	7.7	38.9	15.8

As a result of the research, *nccA* gene was the most common resistance gene in 33% of bacteria isolated from Mediterranean mussels and sea snails. While *nccA* gene was detected in 75% of bacteria isolated from Artvin stations, Giresun followed with 36.8%. *merA* gene responsible for mercury resistance was determined as 31.6%, 23.1%, and 11.1% at the stations in Giresun, Rize, and Trabzon, respectively and cannot be determined in Artvin station. *chrB* gene was detected the highest rate of 38.9% of *E. coli* in Trabzon. (Table 4).

Discussion

Resistance level differences against pollutants in the aquatic bacteria isolated from the different aquatic environments have been reported in various studies (Capkin et al., 2017; Terzi, 2018b). Sipahi et al. (2013) reported that all *Enterobacteriaceae* members they isolated were resistant to copper, also, almost all (99.9%) against manganese and 87.2% resistance to lead. Matyar et al. (2010) reported that all of

the 356 bacteria isolated from three different stations of Iskenderun Bay (Turkey) were resistant to copper and cadmium. Similar to these studies, in this study, the MIC values of *E. coli* strains isolated from Mediterranean mussel and sea snail against copper, cadmium, arsenic, and mercury were determined as 100-400 µg / ml, 100-200 µg / ml, 25-400 µg, 3.125-25 µg/ml, respectively. All isolates were resistant to copper and this was followed by cadmium. Moreover, in this study, arsenic resistance status was not detected in any of the bacteria. Mercury resistance was determined only in bacteria isolated from the stations in Trabzon and Giresun with rates of approximately 10%. It has been determined that the bacteria isolated from the stations in Rize and Artvin are sensitive to mercury. The toxicity of some metals like zinc, copper, manganese was lower for the bacteria than mercury and arsenic. The low toxicity of zinc, copper, and manganese could play an important role in biochemical reactions of the cell as a trace element (Nies, 1999). Gedik (2018a) reported that the samples of Mediterranean mussels (*M. galloprovincialis*) collected from the same samling points had the highest metal concentrations (Cr, Cu, Mn, Pb, Zn) at the stations in Trabzon. Similarly, in this study, the resistance levels of bacteria isolated from Trabzon stations had the highest resistance compared to the other stations. This may show that coastal areas of Trabzon are more exposed to pollution than the other stations. Besides, Akçay and Moon (2004) reported that Trabzon has local pollution in the coastal areas due to the coastal mining areas and agricultural activities. Gedik (2018b) reported that heavy metal content was found higher in sea snails in the Trabzon region compared to Artvin, Giresun and Rize. Baltas et al. (2017) measured heavy metal concentrations in soft tissues of Mediterranean mussel and sea snail collected from Artvin, Giresun, Rize, and Trabzon regions. They found that Cu and Pb concentrations in sea snails had higher than mussel but lower for Zn.

Matyar et al. (2009) found that the tolerance to heavy metals in bacteria isolated from fish gills is Cd> Cu> Mn> Cr = Pb, but in bacteria isolated from intestines it is listed as Cd> Cu> Cr> Mn = Pb. Akinbowale et al. (2007) stated that this order was Cu = Pb> Mn> Cr> Zn> Co> Cd in the bacteria isolated from *Oncorhynchus mykiss*. Abou-Shanab et al. (2007) reported the resistance frequencies of the bacteria against various metal(oid)s such as Pb, Zn, Ni, Cu, Co, Cr, Cd, Hg, and As, as 100%, 100%, 100%, 98%, 93%, 53%, 42%, 29%, and 18%, respectively. Abskharon et al. (2008) found MIC values for *E.coli* bacteria against copper, cobalt, nickel, zinc, chromium, cadmium and lead metals as 1.57, 2.55, 1.7, 9.17, 0.48, 4.4, and 3.1 mM, respectively. Toroglu and Dincer (2009) reported that *E. coli* strains isolated from Aksu River showed different levels of resistance to Ni, Cd, Cu, and Cr and especially they were more resistant to high concentrations of cadmium. Similarly, in this study, we found that all of the bacteria were resistant to copper, followed by cadmium and sensitive to arsenic. Different levels of resistance to metals may be the temporal and spatial difference of bacterial isolations.

Sipahi et al. (2013) reported that the MIC values of Enterobacteriaceae members were 200, 1600, and 1600 µg/ml for copper, manganese, and lead, respectively. Gul-Seker and Mater (2009) showed that the bacterial strains isolated from the Marmara Sea show maximum resistance to Cd, Cu, and Cr, and from the Black Sea are similarly 100%, 92.3%, and 92.3%, respectively. Matyar et al. (2009) stated that bacteria in İskenderun Bay were resistant to copper and cadmium with a rate of 50.5% and 60.2%, respectively and the lowest resistance level was determined in the lead as6.5%. It can be said that the resistance levels detected in bacteria are caused by the fact that the location, life, environment, and time of the bacteria are isolated.

The presence of resistance genes, the genetic element responsible for resistance to contaminants such as heavy metal in bacteria, has been reported in several studies. Abou-Shanab et al. (2007) found that the *mer* and *ncc* metal resistance genes detected by PCR, and the bacteria are resistant to mercury and nickel. Moreover, the co-occurrence of antibiotic resistance genes and heavy metal resistance genes has been demonstrated that antibiotic resistance could correlate with heavy metal resistance. In this study, *nccA* gene was the most common resistance gene in 33% of bacteria isolated from Mediterranean mussel and sea snail. In our previous study, we determined 9 different antibiotic resistance genes in the same bacteria and it was found that 44.4% of bacteria contained plasmids (Terzi and Isler, 2019). In our both studies, the highest antibiotic resistance gene was fond as *ampC* (98 %) responsible for ampicillin resistance and the highest metal resistance gene was *nccA* (33 %) responsible for nickel, cobalt, and cadmium. The antibiotic resistance and heavy metal resistance genes of these bacteria may be found together on plasmids (Chapman, 2003).

Conclusion

Sub-lethal concentrations of metal(oid)s in the aquatic environment might induce the antibiotic resistance of bacteria (Li et al., 2019). Discharge of pollutants such as heavy metals into the aquatic environment may lead directly to heavy metal resistance, and directly or indirectly for other antimicrobial resistance such as antibiotic and disinfectant resistance. Tolerance or resistance of the bacteria to toxic pollutants including heavy metals is of significant ecological importance. These bacteria could be used for monitoring environmental metal pollution.

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Compliance with Ethical Standards

Authors' Contributions

ET designed the study and wrote the first draft of the article. ET and FC performed laboratory analyses. Both authors read and approved the final article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Abou-Shanab, R. A. I., van Berkum, P. & Angle, J. S. (2007). Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. *Chemosphere*, ***68***(2): 360-367. https://doi.org/10.1016/j.chemosphere.2006.12.051

Abskharon, R. N. N., Hassan, S. H. A., Gad El-Rab, S. M. F. & Shoreit, A. A. M. (2008). Heavy metal resistant of *E. coli* isolated from wastewater sites in Assiut City, Egypt. *Bulletin of Environmental Contamination and Toxicology*, ***81***(3): 309. https://doi.org/10.1007/s00128-008-9494-6

Akçay, M. & Moon, C. J. (2004). The environmental impact of mining in the Pontides, Turkey: Reconnaissance sampling and GIS-based analysis. *Geochemistry: Exploration, Environment, Analysis,* ***4***(4): 317-328. https://doi.org/10.1144/1467-7873/03-052

Akinbowale, O. L., Peng, H., Grant, P. & Barton, M. D. (2007). Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *International Journal of Antimicrobial Agents*, ***30***(2): 177-182. https://doi.org/10.1016/j.ijantimicag.2007.03.012

Avşar, C. & Berber, İ. (2014). Plasmid profiling and antibiotics resistance of *Escherichia coli* strains isolated from *Mytilus galloprovincialis* and seawater. *Journal of Coastal Life Medicine*, ***2***(9): 689-693. https://doi.org/10.12980/JCLM.2.2014JCLM-2014-0069

Baltas, H., Sirin, M., Dalgic, G., Bayrak, E. Y. & Akdeniz, A. (2017). Assessment of metal concentrations (Cu, Zn, and Pb) in seawater, sediment and biota samples in the coastal area of Eastern Black Sea, Turkey. *Marine Pollution Bulletin*, ***122***(1-2): 475-482. https://doi.org/10.1016/j.marpolbul.2017.06.059

Bat, L. & Öztekin, H. C. (2016). Heavy metals in *Mytilus galloprovincialis*, *Rapana venosa* and *Eriphia verrucosa* from the Black Sea coasts of Turkey as bioindicators of pollution. *Walailak Journal of Science and Technology*, ***13***(9): 715-728.

Boran, H., Terzi, E., Altinok, I., Capkin, E. & Bascinar, N. (2013). Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. *Aquaculture*, ***396***: 8-13. https://doi.org/10.1016/j.aquaculture.2013.02.025

Capkin, E., Ozdemir, S., Ozturk, R. C. & Altinok, I. (2017). Determination and transferability of plasmid-mediated antibiotic resistance genes of the bacteria isolated from rainbow trout. *Aquaculture Research*, ***48***(11): 5561-5575.

Capkin, E., Terzi, E. & Altinok, I. (2015). Occurrence of antibiotic resistance genes in culturable bacteria isolated from Turkish trout farms and their local aquatic environment. *Diseases of Aquatic Organisms*, ***114***(2): 127-137. https://doi.org/10.3354/dao02852

Chapman, J. S. (2003). Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *International Biodeterioration & Biodegradation*, ***51***(4): 271-276. https://doi.org/10.1016/S0964-8305(03)00044-1

CLSI (Clinical and Laboratory Standards Institute). (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, (11th Ed.). Standard M07, 112

Drapkin, E. (1963). Effect of *Rapana bezoar* Linne (Mollusca, Muricidae) on the Black Sea fauna. *Doklady Akademii Nauk SRR*.

Fuentes, A., Fernández-Segovia, I., Escriche, I. & Serra, J. A. (2009). Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. *Food Chemistry*, ***112***(2): 295-302. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.05.064

Gedik, K. (2018a). Bioaccessibility of Cd, Cr, Cu, Mn, Ni, Pb, and Zn in Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) along the southeastern Black Sea coast. *Human and Ecological Risk Assessment: An International Journal*, ***24***(3): 754-766. https://doi.org/10.1080/10807039.2017.1398632

Gedik, K. (2018b). Bioaccessibility of heavy metals in rapa whelk *Rapana venosa* (Valenciennes, 1846): Assessing human health risk using an in vitro digestion model. *Human and Ecological Risk Assessment: An International Journal*, ***24***(1): 202-213. https://doi.org/10.1080/10807039.2017.1373329

Gul-Seker, M. & Mater, Y. (2009). Assessment of metal and antibiotic-resistance in marine bacteria isolated from Izmit Bay and Bosphorus entrance of Marmara and Black Sea, Turkey. *Fresenius Environmental Bulletin*, ***18***(11A): 2192-2202.

Kacar, A. (2011). Some microbial characteristics of mussels (*Mytilus galloprovincialis*) in coastal city area. *Environmental Science and Pollution Research*, ***18***(8): 1384. https://doi.org/10.1007/s11356-011-0487-3

Li, X., Gu, A. Z., Zhang, Y., Xie, B., Li, D. & Chen, J. (2019). Sub-lethal concentrations of heavy metals induce antibiotic resistance via mutagenesis. *Journal of Hazardous Materials*, ***369***: 9-16. https://doi.org/10.1016/j.jhazmat.2019.02.006

Matyar, F., Akkan, T., Uçak, Y. & Eraslan, B. (2010). *Aeromonas* and *Pseudomonas*: antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (northeast Mediterranean Sea). *Environmental Monitoring and Assessment*, ***167***(1-4): 309-320. https://doi.org/10.1007/s10661-009-1051-1

Matyar, F., Eraslan, B., Akkan, T., Kaya, A. & Dinçer, S. (2009). İskenderun Körfezi balıklarından izole edilen bakterilerde antibiyotik ve ağır metal dirençliliklerinin araştırılması. *Biyoloji Bilimleri Araştırma Dergisi*, ***2***(2), 1-5.

Misra, T. K., Brown, N. L., Fritzinger, D. C., Pridmore, R. D., Barnes, W. M., Haberstroh, L. & Silver, S. (1984). Mercuric ion-resistance operons of plasmid R100 and transposon Tn501: The beginning of the operon including the regulatory region and the first two structural genes. *Proceedings of the National Academy of Sciences*, ***81***(19): 5975-5979. https://doi.org/10.1073/pnas.81.19.5975

Nies, A., Nies, D. H. & Silver, S. (1990). Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. *Journal of Biological Chemistry*, ***265***(10): 5648-5653. https://www.jbc.org/content/265/10/5648.long

Nies, D. H. (1999). Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology*, ***51***(6): 730-750. https://doi.org/10.1007/s002530051457

Nieto, J., Ventosa, A. & Ruiz-Berraquero, F. (1987). Susceptibility of halobacteria to heavy metals. *Applied Environmental and Public Health Microbiology*, ***53***(5): 1199-1202.

Saglam, H., Kutlu, S., Dagtekin, M., Bascinar, S., Sahin, A., Selen, H. & Duzgunes, E. (2015). Population biology of *Rapana venosa* (Valenciennes, 1846) (Gastropoda: Neogastropoda) in the south-eastern Black Sea of Turkey. *Cahiers de Biologie Marine*, ***56***(4): 363-368. https://doi.org/10.21411/CBM.A.2A889E43

Sipahi, N., Mutlu, C. & Akkan, T. (2013). Antibiotic and heavy metal resistance levels of Enterobacteriaceae isolated from retail fishes in Giresun. *Gıda*, ***38***(6): 343-349. https://doi.org/10.5505/gida.2013.55264

Terzi, E. (2018a). Antimicrobial resistance profiles and tetracycline resistance genes of *Escherichia coli* in Mediterranean mussel and sea snails collected from Black Sea, Turkey. *Alinteri Journal of Agriculture Sciences*, ***33***(1): 43-49. https://doi.org/10.28955/alinterizbd.355019

Terzi, E. (2018b). Determination of antimicrobial resistance profiles of the bacteria isolated from cultured sturgeons. *Menba Kastamonu University Faculty of Fisheries Journal*, ***4***(2): 7-13.

Terzi, E. & Isler, H. (2019). Antibiotic resistance genes of *Escherichia coli* in coastal marine environment of Eastern Black Sea, Turkey. *Fresenius Environmental Bulletin*, ***28***(2A): 1594-1601.

Toroglu, S. & Dincer, S. (2009). Heavy metal resistances of Enterobacteriaceae from Aksu River (Turkey) polluted with different sources. *Asian Journal of Chemistry*, ***21***(1): 411-420.

Tsi, C., Ma, X., Lou, Z. & Zhang, F. (1983). *Illustrations of the fauna of China (Mollusca).* (2nd Ed.) Science Press, Beijing, China. 150p.

Ture, M., Kilic, M. B. & Altinok, I. (2020). Relationship between heavy metal accumulation in fish muscle and heavy metal resistance genes in bacteria isolated from fish. *Biological Trace Element Research*, https://doi.org/10.1007/s12011-020-02246-0

Yang, S., Deng, W., Liu, S., Yu, X., Mustafa, G. R., Chen, S., He, L., Ao, X., Yang, Y., Zhou, K., Li, B., Han, X., Xu, X. & Zou, L. (2020). Presence of heavy metal resistance genes in *Escherichia coli* and *Salmonella*, and analysis of resistance gene structure in *E. coli* E308. *Journal of Global Antimicrobial Resistance*, ***21***: 420-426. https://doi.org/https://doi.org/10.1016/j.jgar.2020.01.009

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RESEARCH ARTICLE

In silico characterization of *bHLH* transcription factor genes in the genome of rainbow trout *(Oncorhynchus mykiss)*

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ABSTRACT

The significance of seafood in nutrition has started to be better understood after the change in the understanding of nutrition in the world. One of the most common species cultivated in the world is rainbow trout (*Oncorhynchus mykiss*) from the origin of North America. Transcription factors are a group of proteins containing different functional components for the accomplishment of various activities. The basic helix (bHLH) domain is a highly preserved amino acid motif that characterizes a family of transcription factors. The *bHLH* gene family in the rainbow trout (*Oncorhynchus mykiss*) genome has been identified in the current study for the first-time using bioinformatics tools. According to the results, 441 *bHLH* genes (*OmybHLH*) were identified in the rainbow trout genome and the physicochemical properties of those proteins were determined. The highest number of the genes was in 7th chromosome of rainbow trout with 29 *OmybHLH* genes. 38 of *OmybHLH* genes had no intronic regions. OmybHLH proteins were divided into 4 main groups in the phylogenetic tree consistent with their motif content. The common biological function of OmybHLH proteins was the regulation of biological processes. The mode of action of OmybHLH proteins was binding activity. The *OmybHLH* gene family in the rainbow trout and the *bHLH* gene family in the Atlantic salmon (*SsabHLH*) had 95 orthologous gene relationships and average separation times of those orthologous genes were found to be 298 million years ago (MYA). Almost all the OmybHLH protein family members have dominated by the α-helix motif which is a stable conformation. Identification of the bHLH proteins and evaluation of their properties in rainbow trout can open new perspectives for aquaculture applications and fish culture to get better yield using genetic data.

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Introduction

Fish meat is a highly nutritional food and is rich in protein and unsaturated fatty acids, as well as containing essential amino acids such as methionine and lysine (İzci et al., 2009). Unsaturated fatty acids, which have benefits such as lowering blood cholesterol levels and preventing cardiovascular diseases, are abundant in fatty fish. Besides, iron, phosphorus and calcium are also abundant (Sayılı et al., 1999; Kocaman and Sayılı, 2014). The significance of seafood in nutrition has started to be better understood after the change in the understanding of nutrition in the world. Fish farming has gained importance because of the need for animal protein for the increasing population (Doğan and Güven, 2005).

In the beginning, carp cultivation was preferred because of its easy cultivation, and then the cultivation of sea bass, sea bream and trout species, which have an economic value over time, gained importance (Kocaman and Sayılı, 2014). One of the most common species cultivated in the world is rainbow trout (*Oncorhynchus mykiss*) from the origin of North America. Properties of rainbow trout, such as resistance to high temperatures and good adaptation to environmental conditions, easy to feed and good growth and having a shorter incubation period at higher temperatures than other trout types, provide easy adaptation to cultural conditions (Aydın, 2009). Trout, which is one of the most important freshwater fish, has become an important option in the market compared to marine fish in terms of both increasing the amount of production and being preferred (Yiğit and Aral, 1999).

Transcription factors are groups of proteins containing different functional components for the accomplishment of various activities such as DNA binding, activation, phosphorylation and protein oligomerization. The basic helix (bHLH) domain is a highly preserved amino acid motif that characterizes a family of transcription factors. The bHLH domain includes two regions: one of them includes about of 10–15 predominantly basic amino acids (the basic region) and other includes about 40 amino acids to construct two α-helices separated by a loop of variable length (the helix-loop-helix region) (Jones, 2004; Pires and Dolan, 2010). bHLH proteins are characterized by protein-protein interaction and highly protected areas for DNA binding (Murre et al., 1989; Atchley et al., 1999). The effects of these transcription factors are seen in many events such as neurogenesis, myogenesis, cell line detection, gender detection, cell proliferation and differentiation in organisms ranging from plants to mammals (Atchley et al., 1999).

Many of bHLH proteins have been detected in organisms ranging from yeast *Saccharomyces cerevisiae* to zebrafish and human (Robinson et al., 2000; Ledent et al., 2002; Wang et al., 2009), however, in our knowledge, there is no study about the *bHLH* gene family in rainbow trout. The *bHLH* gene family in the rainbow trout (*Oncorhynchus mykiss*) genome has been identified in the current study for the first-time using bioinformatics tools. Therefore, it was aimed to determine the properties of the bHLH proteins such as their chromosomal localization, motif regions, homology models, gene structure, the orthologous relationships between the Atlantic salmon (*Salmo salar*) and the predicated roles, molecular functions and cellular localization in the rainbow trout.

Material and Methods

Identification of bHLH Genes and Their Properties in Rainbow Trout Genome

CLC (Genomics Workbench 11) was used for the determination of rainbow trout *bHLH* proteins. BLASTp search was performed for derived protein sequences against NCBI (National Center for Biotechnology Information) database. Then, conserved domains were determined according to the Hidden Markov Model (HMM) and these regions were controlled in Pfam (https://pfam.xfam.org/) database. Repeated sequences were discarded and rainbow trout *bHLH* proteins were identified and numbered based on their chromosomal localization. Physicochemical characterization of identified proteins such as molecular weight, amino acid length, isoelectric point, etc. was determined by the ExPASy ProtParam tool (https://web.expasy.org/protparam/) (Gasteiger et al., 2005). Besides, genomic sequences of *bHLH* genes were searched against the NCBI (https://www.ncbi.nlm.nih.gov/) database to determine their chromosomal localization. The data obtained as a result of this search were visualized using MapChart (https://mapchart.net/) program (Voorrips, 2002). Gene Structure Display Server (Hu et al., 2014) was utilized to determine the exon-intron structure of rainbow trout *bHLH* genes.

Phylogenetic Tree Construction and Determination of Conserved Motifs

ClustalW algorithm was used for protein sequence alignment and the phylogenetic tree was created by the Maximum Likelihood method with bootstrap analysis for 1000 replicates in MEGAX (https://www.megasoftware.net/) program (Kumar et al., 2018).

MEME-Suite database, one of the motif determination tools, was used to define sequence motifs with short and repeating patterns in DNA that are assumed to have a biological

function (Bailey et al., 2015). The maximum motif length was selected as between 4-20 aa.

Gene Ontology Analysis

Functional data analysis of bHLH proteins belonging to rainbow trout was performed using Blast2GO software (Conesa et al., 2005). Analyses were carried out in three steps: in the first step, BLASTP search against the NCBI database was performed, in the second step, mapping according to the BLASTp results was accomplished (MAPPING) and in the third step, an information file about the sequences was prepared (ANNOTATION). At the end of those analyses, three categories have been created as predicted molecular functions, cellular locations and determination of biological functions for bHLH proteins in rainbow trout.

Identification of Orthologous Genes between Rainbow Trout and Atlantic Salmon (Salmo salar)

The amino acid sequences of bHLH proteins in rainbow trout and Atlantic salmon were aligned by the BLASTp algorithm. As a result of the BLAST query, genes meeting the ≥50 similarity condition and the expectation value of e^{-50} were selected. Orthologous of *bHLH* genes in Rainbow Trout and Atlantic salmon fish were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) software (Li, 2003). The homologous (Ks) and non-homologous (Ka) exchange rates of the aligned orthologous protein sequences were calculated, and this calculation was performed with the PAL2NAL (http://www.bork.embl.de/pal2nal/) (Suyama et al., 2006) database.

Prediction of the Three-Dimensional Structure of bHLH Proteins in Rainbow Trout

Predicted three-dimensional structures of the bHLH proteins were shown by PHYRE2 (Protein Homology/Analog/YRecognition Engine; http://www.sbg.bio.ic.ac.uk/phyre2) database (Kelley et al., 2015). BlastP search was performed to search for similar sequences and the best result was determined based on the three-dimensional structure in Protein Data Bank (PDB).

Results and Discussion

Identification of bHLH Genes and Their Properties in Rainbow Trout Genome

After multiple searches for determination of *bHLH* genes in rainbow trout genome, 441 *bHLH* genes were identified and they were named from *OmybHLH-01* to *OmybHLH-441* based on their chromosomal localization. Amino acid (aa) lengths of their protein product were between 79 and 2435 aa. The protein with the shortest aa sequence was found to be the OmybHLH-52 protein, and the protein with the longest aa sequence was OmybHLH-262. Considering the isoelectronic points (pI) of those proteins, these points varied between 4.49 and 11.02; it was found that 257 OmybHLH protein were acidic, 184 OmybHLH protein had basic properties. The percentage of the acidic OmybHLH proteins was 58.28%. The molecular weights of those proteins ranged from 9051.53 kDa to 259871.6 kDa. Besides, 97.96% of the OmybHLH proteins were unstable (Supplementary Material 1).

Considering of the previous studies, 183 in the rice (*Oryza sativa*) genome (Buck and Atchley, 2003; Wei and Chen, 2018) 35 in the worm (*Caenorhabditis elegans*) genome, 56 in Drosophila (*Drosophila melanogaster*) genome (Ledent and Vervoort, 2001), 52 in the silkworm (*Bombyx mori*) genome (Wang et al., 2007), 139 in the zebrafish (*Brachydanio rerio*) genome (Wang et al, 2009), 115 in the dog (*Canis lupus familiaris*) genome (Wang et al., 2015), 86 in the zebra finch (*Taeniopygia guttata*) genome (Liu and Zhao, 2011), 107 in the giant panda (*Ailuropoda melanoleuca*) genome (Dang et al., 2011), 57 in the ponerine ant (*Harpegnathos saltator*) genome (Liu et al., 2012), 48 in the jewel wasp (*Nasonia vitripennis*) genome (Liu et al., 2014), 109 in the pig (*Sus scrofa*) genome (Liu, 2015), 116 in the cattle (*Bos taurus*) genome (Li and Liu, 2017) were determined as bHLH proteins. When the results of this study conducted by different researchers are compared, it is seen that the number of *bHLH* genes found in organisms is close to each other, and the number of *bHLH* genes in the rainbow trout genome is higher than other organisms. This can be explained by the fact that bHLH proteins in rainbow trout can be involved in many key developmental processes and require many bHLH proteins to regulate these processes. Moreover, wide and detailed searches and high resolute genomes may be the reason for these high numbers.

Map Chart software was used to show the genomic distribution of *bHLH* genes on rainbow trout chromosomes. It was realized that the highest number of genes were carried by in the 7th chromosome of rainbow trout with 29 *OmybHLH* genes. This was followed by 8th, 5th, 4th and 3rd chromosomes with 26, 25 and 24 *OmybHLH* genes, respectively. The least number of genes were located on the 29th chromosome with 2 *OmybHLH* genes (Figure 1). Besides, chromosomal localizations of 11 *OmybHLH* genes were mapped in the scaffold level (Figure 2).

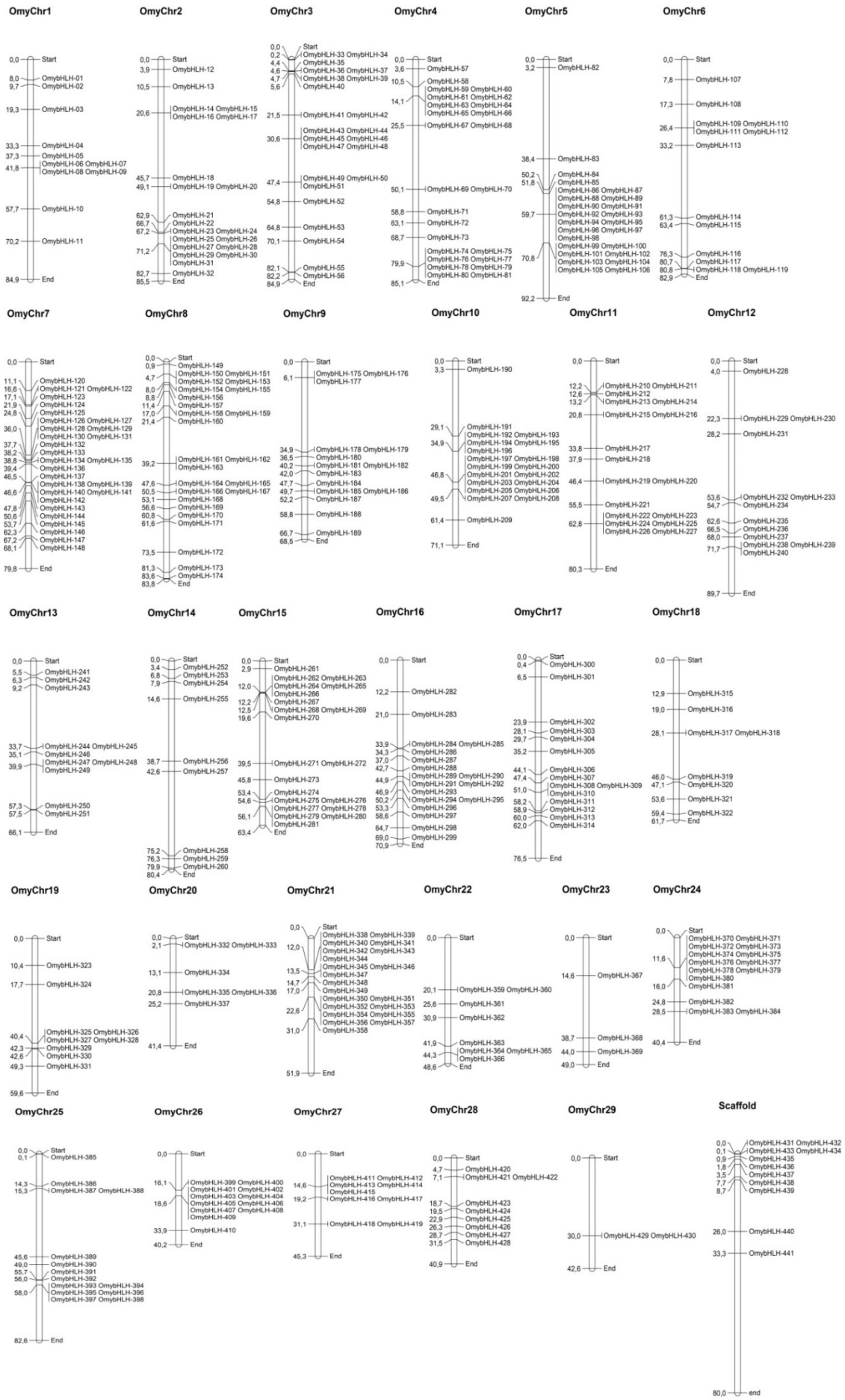

Figure 1. Presentation of OmybHLH genes localizations on rainbow trout chromosomes

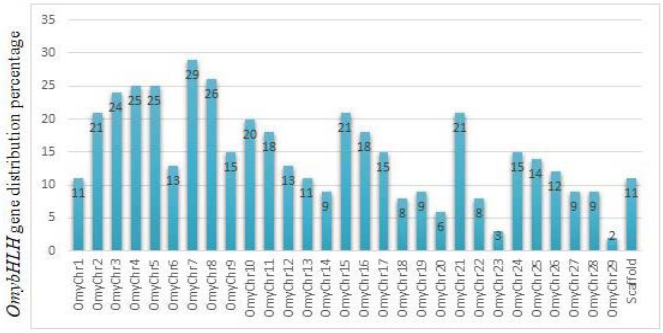

Figure 2. Chromosomal distribution of *OmybHL*H genes on rainbow trout genome

Exon regions are known as sequences encoded in DNA in eukaryotes, while intron regions appear as the parts of DNA that are not read. Exon-intron distribution of *bHLH* genes were determined by Gene Structure Display Server. According to the results, 38 of *bHLH* genes had no intron regions. Other genes had variable number of intron regions. Exon-intron of the genes were presented Supplementary Material 2.

Phylogenetic Tree and Conserved Motifs Analysis

Phylogenetic relationships are tree-like diagrams that show the kinship between species and how long the species split apart in the evolutionary process. The phylogenetic tree was drawn to evaluate evolutionary relations of OmybHLH proteins of rainbow trout. After visualization of the constructed phylogenetic tree by Interactive Tree of life (iTOL) online tool (Letunic and Bork, 2019), OmybHLH proteins are divided into 4 main groups (Class I, Class II, Class III, Class IV). It was determined that there were 63 proteins in Class I, 128 in Class II, 122 in Class III and 128 in Class IV. Class II and Class IV were found to contain an equal number of the OmybHLH proteins (Figure 3). The phylogenetic tree of OmybHLH proteins may provide information about protein sequence similarities, differences and their ancestors.

Protein sequence motifs are often used in genome research to identify and classify proteins, to determine specific binding sites in proteins and to find functional regions in proteins. Conserved motif structures in OmybHLH proteins identified in rainbow trout were determined and detailed results are shown in Supplementary Material 3. According to the MEME Suite database analysis to identify conserved motifs, 12 preserved motifs were defined for the 441 OmybHLH proteins. Those motif analyses verified the phylogenetic tree allocation. Proteins with similar motif compositions were especially in the same cluster in the phylogenetic tree.

Considering the studies on the relationship and evolutionary processes of bHLH protein families in different organisms, it was observed that atonal bHLH protein homologs in organisms such as mice, chickens, and humans are preserved throughout the evolutionary process and the coding regions are quite similar (Ben Arie, 1996). Besides, according to the phylogenetic analysis with 9 species of land plants, bHLH proteins found in these plants have come from a common ancestor (Pires and Dolan, 2010). In addition, it has been concluded that motifs of bHLH proteins found in mushrooms were preserved with aa sequences and were associated with bHLH proteins included in group B in animals (Sailsbery et al., 2012).

Gene Ontology Analysis

Gene ontology is one of the bioinformatics tools used to reveal the features and functionality of gene products in the species. Blast2GO software was utilized to define cellular localization, biological process and molecular function of OmybHLH proteins (Figure 4). According to the analysis results, OmybHLH proteins were found to have biological functions such as regulation of biological processes, roles in metabolic, cellular and developmental processes, multicellular organismal processes and response to a stimulus. Consistent with those results, in the literature, bHLH proteins are involved as positive regulators in biological processes (Norton, 2000), are involved as negative regulators (Benezra et al., 1990; Norton, 2000; Perk et al., 2005), are promoting myogenic cell proliferation and differentiation and are involved in determining flexibility in skeletal muscles and respond to mechanical or neuronal stimuli (Voytik et al., 1993; Molkentin and Olson, 1996; Puri and Sartorelli, 2000; Walters et al., 2000; Perry et al., 2001; Pownall et al., 2002; Buckingham et al., 2003; Ishido et al., 2004; Tapscott, 2005; Legerlotz and Smith, 2008). Also, they are involved in the nervous system development stages and function in neuronal cell development and differentiation in the brain (Campuzano, 1985; Guillemot et al., 1993; Guillemot, 1995; Yasunami et al., 1996; Borges et al., 1997; Miyata et al., 1999; Olson et al., 2001). Moreover, they play an important role in embryonic development and embryonic cell differentiation (Malecki et al., 1999; Norton, 2000).

When the cellular locations of OmybHLH proteins were evaluated, it was observed that they dispersed to different parts of the cell and this dispersion covered the cell, intracellular parts and as a cellular anatomical entity. There are studies in the literature about that bHLH proteins were found in embryonic cells (Nambu et al., 1991; Muralidhar vd., 1993), in muscle cells (Perry et al., 2001; Tapscott, 2005), in nerve cells (Guillemot, 2007; Jahan vd., 2010), in blood cells (Drake et al., 1997; Gering et al., 1998), in melanocyte, and mast cells (Hodgkinson et al., 1993; Steingrimsson et al., 2004; Levy et al., 2006).

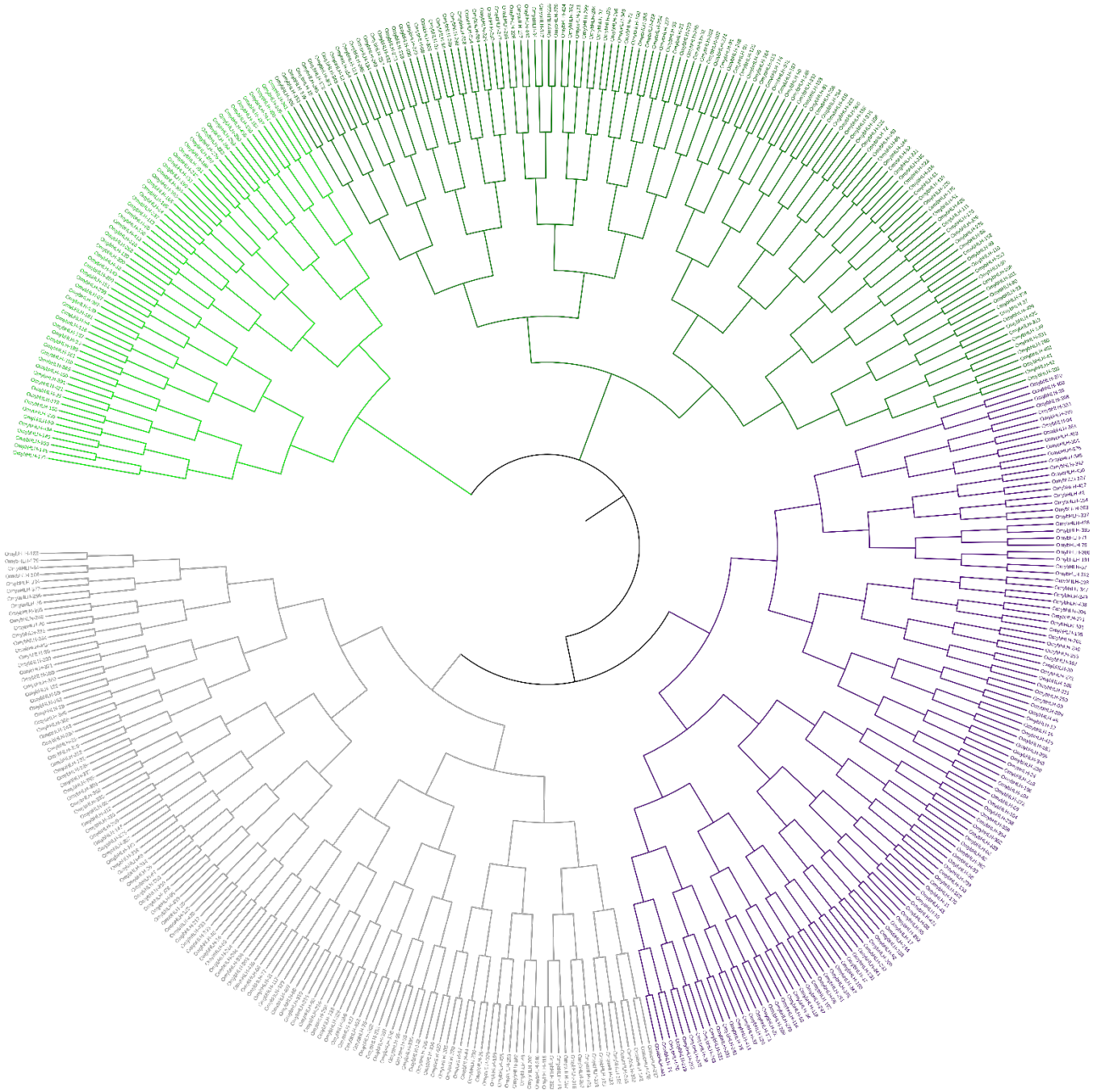

Figure 3. Phylogenetic relations of OmybHLH proteins. The phylogenetic trees were constructed by multiple sequence alignment using the ClustalW program via the MEGA X program (maximum likelihood method)

Mode of action of OmybHLH proteins was found to be the binding activity. In addition, those protein groups had transcription regulator activity which is consistent with their roles. In the literature, it has been revealed that members of the bHLH protein family show their molecular functions as binding in different studies (Ledent and Vervoort, 2001; Ledent et al., 2002; Berkes and Tapscott, 2005; Murre, 2019).

Considering the current results, OmybHLH proteins have significant roles in regulation and development processes in many cell types. High gene numbers of this protein family in rainbow trout may be explained by multiple significant roles of bHLH proteins in different cell types. Gene ontology analysis of OmybHLH proteins can draw a frame about the importance of these multifunctional proteins in the organisms and their predicated roles and localization in the cells.

Orthologous Genes between Rainbow Trout and Atlantic Salmon (Salmo salar)

Although they are found in different organisms, genes that have the same ancestral origins and have structural and

functional similarities but are separated from each other during the species formation are called orthologous genes. By comparing the rainbow trout and Atlantic salmon with the *bHLH* gene sequences, the orthologous gene analysis was performed and the separation times of the species were determined. Considering of the results, The *OmybHLH* gene family in the rainbow trout and the *bHLH* gene family in the Atlantic salmon (*SsabHLH*) had 95 orthologous gene relationships and the substitution ratio [non-synonymous (Ka) versus synonymous (Ks)] was 0.26 (Ka/Ks). Average separation times of those orthologous genes were found to be 298 million years ago (MYA) (Figure 5, Supplementary Material 4). According to the results, it can be estimated that there is a high degree of differentiation between the two fish species.

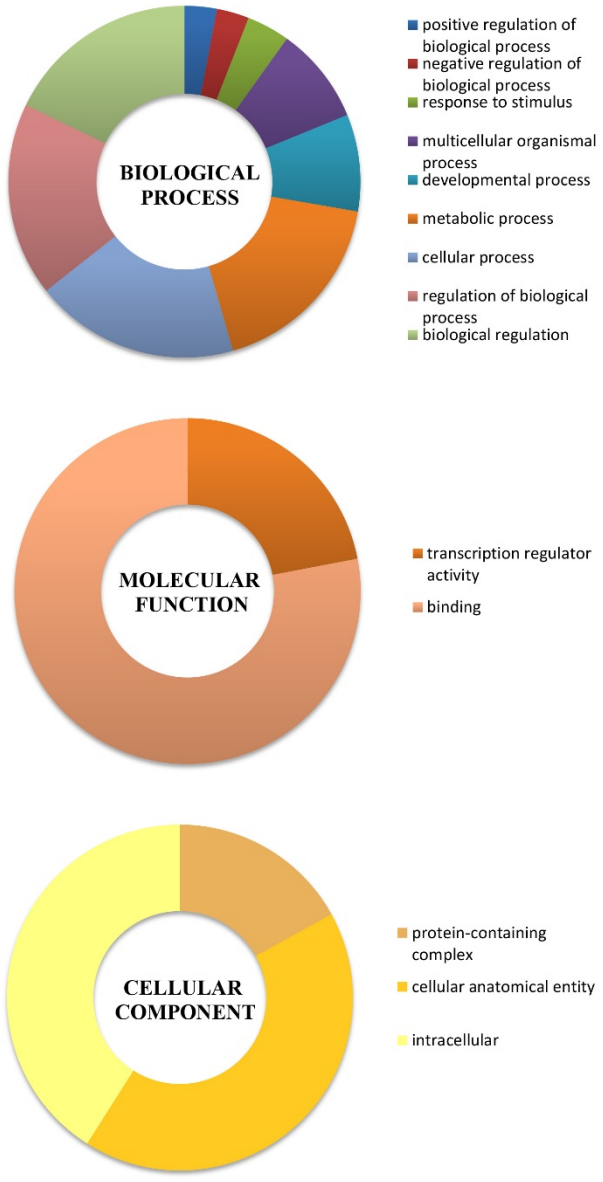

Figure 4. Predicated biological roles, molecular function and cellular localizations of OmybHLH proteins by Blast2GO analysis

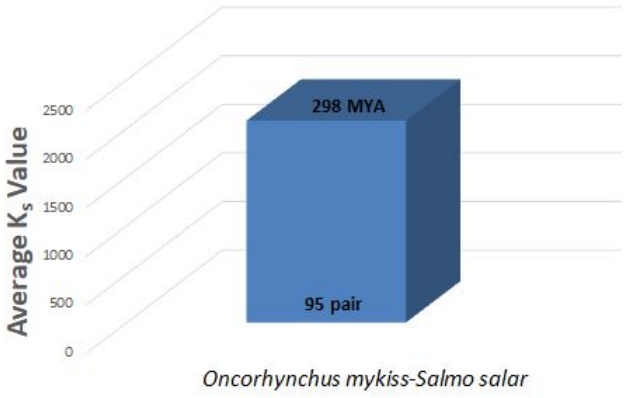

Figure 5. Presentation of orthologous gene numbers of *OmybHLH* genes and Atlantic salmon *bHLH* genes

Prediction of the Three-Dimensional Structure of bHLH Proteins in Rainbow Trout

Homology is based on the sequence similarity of one or more proteins of which structure is known, and the high sequence similarity of proteins in this context supports the hypothesis that they come from the same ancestor. Predicted three-dimensional structures of the OmybHLH proteins were determined by homology modelling in the Phyre2 database. This modeling was carried out based on >90% confidence interval under intensive mode. It was determined that the similarity level ranged between 4% and 100% and 12 OmybHLH proteins showed similarity over 90% (OmybHLH-45, 58, 145, 359, 370, 371, 372, 373, 380, 391, 424, 433) (Figure 6). According to the predicted three-dimensional structure of the proteins, it was realized that almost all of the protein family members were dominated by the α-helix motif which is a stable conformation formed by the rotation of the alpha carbon atoms of 4 to 50 amino acids in a spiral shape. This structure consists of two spirals, which include the basic region and the variable loop region (Pires and Dolan, 2010). The amino terminal end in the structure allows DNA binding, while the carboxy-terminal end provides dimerization. When modeled three-dimensional structures of OmybHLH proteins are evaluated, it is seen that these two amphipathic α-helices (H1, H2) produced by the dimerization component are present in almost all of these protein structures, which is consistent with the literature (Murre et al., 1989; Atchley et al., 1999).

Conclusion

The study evaluated the bHLH proteins in rainbow trout, which are significant transcription factor family in eukaryotes. Properties of this protein family such as their chromosomal localization, motif regions, gene structure, predicted three-dimensional structures, orthologous relationships with the

Atlantic salmon (*Salmo salar*) bHLH proteins and the predicted biological roles, molecular functions and cellular localization were determined using *in silico* analysis by bioinformatics tools. Identification of bHLH proteins and evaluation of their properties in rainbow trout can open new perspectives for aquaculture applications and fish culture to get better yield using genetic data. Besides, defined genetic data can be used in healthy and high-yield fish farming and those findings can be applied to other economically important fish species in aquaculture. From these views, the current study includes valuable data for OmybHLH proteins, which has potential usage in aquaculture implications.

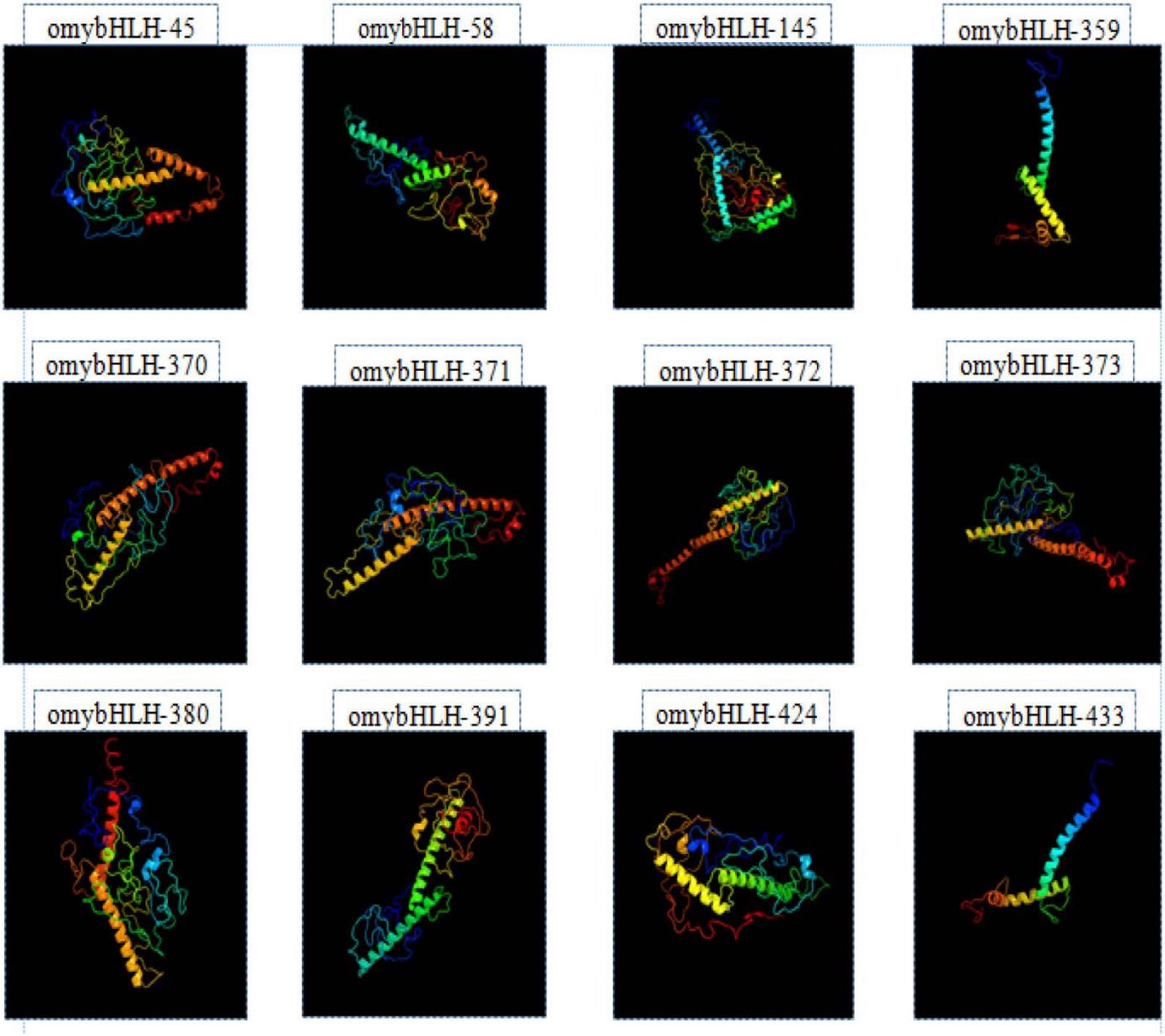

Figure 6. The predicated 3D structures of OmybHLH proteins

Compliance with Ethical Standards

Authors' Contributions

Author YCA designed the study, YCA and GD wrote the first draft of the manuscript, GD performed and managed the bioinformatics analyses. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Supplementary Materials

Supplementary data to this article can be found online at https://doi.org/10.33714/masteb.768233.

References

Atchley, W. R., Terhalle, W. & Dress, A. (1999). Positional dependence, cliques and predictive motifs in the bHLH protein domain. *Journal of Molecular Evolution*, ***48***(5): 501–516. https://doi.org/10.1007/pl00006494

Aydın, F. (2009). Alabalık biyolojisi ve yetiştirme teknikleri. Ankara Üniversitesi Ziraat Fakültesi Su Ürünleri Bölümü, Ankara, Turkey. 30p.

Bailey, T. L., Johnson, J., Grant, C. E. & Noble, W. S. (2015). The MEME suite. *Nucleic Acids Research*, ***43***(1): 39-49. https://doi.org/10.1093/nar/gkv416

Ben Arie, N. (1996). Evolutionary conservation of sequence and expression of the bHLH protein atonal suggests a conserved role in neurogenesis. *Human Molecular Genetics,* ***5***(9): 1207–1216. https://doi.org/10.1093/hmg/5.9.1207

Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990). The protein id: A negative regulator of helix-loop-helix DNA binding proteins. *Trends in Genetics,* ***61***(1): 49-59. https://doi.org/10.1016/0168-9525(90)90161-x

Berkes, C. A. & Tapscott, S. J. (2005). MyoD and the transcriptional control of myogenesis. *Seminars in Cell, Developmental Biology,* ***16***(4-5): 585–595. https://doi.org/10.1016/j.semcdb.2005.07.006

Borges, M., Linnoila, R. I., van de Velde, H. J. K., Chen, H., Nelkin, B. D., Mabry, M. & Ball, D. W. (1997). An Achaete-Scute homologue essential for neuroendocrine differentiation in the lung. *Nature,* ***386***(6627): 852–855. https://doi.org/10.1038/386852a0

Buck, M. J. & Atchley, W. R. (2003). Phylogenetic analysis of plant basic helix-loop helix proteins. *Journal of Molecular Evolution,* ***56***(6): 742–750. https://doi.org/10.1007/s00239-002-2449-3

Buckingham, M., Bajard, L., Chang, T., Daubas, P., Hadchouel, J., Meilhac, S. & Relaix, F. (2003). The Formation of Skeletal Muscle: From Somite to Limb. *Journal of Anatomy,* ***202***(1): 59–68. https://doi.org/10.1046/j.1469-7580.2003.00139.x

Campuzano, S. (1985). Molecular genetics of the Achaete-Scute gene complex of D. *melanogaster*. *Cell,* ***40***(2): 327–338. https://doi.org/10.1016/0092-8674(85)90147-3

Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M. & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics,* ***21***(18): 3674–3676. https://doi.org/10.1093/bioinformatics/bti610

Dang, C., Wang, Y., Zhang, D., Yao, Q. & Chen, K. (2011). A genome-wide survey on basic helix-loop-helix transcription factors in giant panda. *PLoS One*, ***6***(11): e26878. https://doi.org/10.1371/journal.pone.0026878

Doğan, K., & Güven, E. (2005). Ülkemizde (Türkiye) Su ürünleri yetiştiriciliği yapan işletmeler, üretim kapasiteleri, illere göre dağılımları ve ekonomik analizleri. *Su Ürünleri Mühendisleri Derneği Dergisi,* ***1***(4): 28-33. https://doi.org/1 0.22392/egirdir.414488

Drake, C. J., Brandt, S. J., Trusk, T. C., Little, C. D. (1997). TAL1/SCL Is Expressed in Endothelial Progenitor Cells/Angioblasts and Defines a Dorsal-to-Ventral Gradient of Vasculogenesis. *Developmental Biology,* ***192***(1): 17–30. https://doi.org/10.1006/dbio.1997.8751

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. *The Proteomics Protocols Handbook, 571–607.* https://doi.org/10.1385/1-59259-890-0:571

Gering, M., Rodaway, A. R. F., & Göttgens, B., & Patient, R. K. & Green, A. R. (1998). The SCL gene specifies haemangioblast development from early mesoderm. *The EMBO Journal,* ***17***(14): 4029–4045. https://doi.org/10.1093/emboj/17.14.4029

Guillemot, F. (1995). Analysis of the role of basic-helix-loop-helix transcription factors in the development of neural lineages in the mouse. *Biology of the Cell,* ***84***(1-2): 3–6. https://doi.org/10.1016/0248-4900(96)81312-8

Guillemot, F. (2007). Spatial and temporal specification of neural fates by transcription factor codes. *Development,* ***134***(21): 3771–3780. https://doi.org/10.1242/dev.006379

Guillemot, F., Lo, L. C., Johnson, J. E., Auerbach, A., Anderson, D. J. & Joyner, A. L. (1993). Mammalian Achaete-Scute Momolog 1 is required for the early development of olfactory and autonomic neurons. *Cell,* **75**(3): 463–476. https://doi.org/10.1016/0092-8674(93)90381-y

Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A. & Arnheiter, H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic- helix- loop- helix- zipper protein. *Cell,* ***74***(2): 395-404.

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J. & Gao, G. (2014). GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics,* ***31***(8): 1296–1297. https://doi.org/10.1093/bioinformatics/btu817

Ishido, M., Kami, K. & Masuhara, M. (2004). In Vivo expression patterns of MyoD, p21, and Rb proteins in myonuclei and satellite cells of denervated rat skeletal muscle. *American Journal of Physiology, Cell Physiology*, ***287***(2): 484-C493. https://doi.org/10.1152/ajpcell.00080.2004

İzci, L., Günlü, A. & Bilgin, Ş. (2009). Ülkemizde gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum, 1792)'nın değerlendirilme şekilleri. *Eğirdir Su Ürünleri Fakültesi Dergisi,* ***5***(1-2): 73-79.

Jahan, I., Kersigo, J., Pan, N. & Fritzsch, B. (2010). Neurod1 regulates survival and formation of connections in mouse ear and brain. *Cell and Tissue Research,* ***341***(1): 95–110. https://doi.org/10.1007/s00441-010-0984-6

Jones, S. (2004). An overview of the basic helix-loop-helix proteins. *Genome Biology*, ***5***(6): 226. https://doi.org/10.1186/gb-2004-5-6-226

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols,* ***10***: 845–858. https://doi.org/10.1038/nprot.2015.053

Kocaman, E. & Sayili, M. (2014). Gümüşhane ilinde gökkuşağı alabalik işletmelerinin ekonomik analizi. *Anadolu Tarım Bilimleri Dergisi*, ***29***(1): 36-45. https://doi.org/10.7161/anajas.2014.29.1.36

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, ***35***(6): 1547-1549. https://doi.org/10.1093/molbev/msy096

Ledent, V., Paquet, O. & Vervoort, M. (2002). Phylogenetic analysis of the human basic helix-loop-helix proteins. *Genome Biology,* ***3***(6): research0030.1-0030.18. https://doi.org/10.1186/gb-2002-3-6-research0030

Ledent, V. & Vervoort, M. (2001). The basic helix-loop-helix protein family: Comparative genomics and phylogenetic analysis. *Genome Research,* ***11***(5): 754–770. https://doi.org/10.1101/gr.177001

Legerlotz, K. & Smith, H. K. (2008). Role of MyoD in denervated, disused, and exercised muscle. *Muscle, Nerve,* ***38***(3): 1087–1100. https://doi.org/10.1002/mus.21087

Letunic, I. & Bork, P. (2019). Interactive tree of life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Research*, ***47***(W1): W256-W259. https://doi.org/10.1093/nar/gkz239

Levy, C., Khaled, M. & Fisher, D. E. (2006). MITF: Master regulator of melanocyte development and melanoma oncogene. *Trends in Molecular Medicine,* ***12***(9): 406–414. https://doi.org/10.1016/j.molmed.2006.07.008

Li, F. & Liu, W. (2017). Genome-wide identification, classification, and functional analysis of the basic helix–loop–helix transcription factors in the cattle, *Bos taurus*. *Mammalian Genome*, ***28***(5-6): 176-197. https://doi.org/10.1007/s00335-017-9683-x

Li, K. B. (2003). ClustalW-MPI: ClustalW analysis using distributed and parallel computing. *Bioinformatics,* ***19***(12): 1585–1586. https://doi.org/10.1093/bioinformatics/btg192

Liu, A., Wang, Y., Dang, C., Zhang, D., Song, H., Yao, Q. & Chen, K. (2012). A genome-wide identification and analysis of the basic helix-loop-helix transcription factors in the ponerine ant, *Harpegnathos saltator*. *BMC evolutionary biology*, ***12***(1): 165. https://doi.org/10.1186/1471-2148-12-165

Liu, W. (2015). Genome-wide identification, classification and functional analyses of the bHLH transcription factor family in the pig, *Sus scrofa*. *Molecular genetics and genomics*, ***290***(4): 1415-1433. https://doi.org/10.1007/s00438-015-1007-9

Liu, W. & Zhao, C. (2011). Molecular phylogenetic analysis of zebra finch basic helix-loop-helix transcription factors. *Biochemical Genetics*, ***49***(3-4): 226-241. https://doi.org/10.1007/s10528-010-9401-9

Liu, X. T., Wang, Y., Wang, X. H., Tao, X. F., Yao, Q. & Chen, K. P. (2014). A genome-wide identification and classification of basic helix-loop-helix genes in the jewel wasp, *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Genome*, ***57***(10): 525-536. https://doi.org/10.1139/gen-2014-0171

Malecki, M. T., Jhala, U. S., Antonellis, A., Fields, L., Doria, A., Orban, T. & Krolewski, A. S. (1999). Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. *Nature Genetics,* ***23***(3): 323–328. https://doi.org/10.1038/15500

Miyata, T., Maeda, T. & Lee, J. E. (1999). NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus. *Genes Development,* ***13***(13): 1647–1652. https://doi.org/10.1101/gad.13.13.1647

Molkentin, J. D. & Olson, E. N. (1996). Defining the regulatory networks for muscle development. *Current Opinion in Genetics, Development,* ***6***(4): 445–453. https://doi.org/10.1016/s0959-437x(96)80066-9

Muralidhar, M. G., Callahan, C. A. & Thomas, J. B. (1993). Single-minded regulation of genes in the embryonic midline of the drosophila central nervous system. *Mechanisms of Development,* ***41***(2-3): 129–138. https://doi.org/10.1016/0925-4773(93)90043-w

Murre, C. (2019). Helix-loop-helix proteins and the advent of cellular diversity: 30 years of discovery. *Genes Development,* ***33***: 6-25. https://doi.org/10.1101/gad.320663.118

Murre, C., McCaw, P. S. & Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and Myc proteins. *Cell,* ***56***(5): 777–783. https://doi.org/10.1016/0092-8674(89)90682-x

Nambu, J. R., Lewis, J. O., Wharton, K. A. & Crews, S. T. (1991). The drosophila single- minded gene encodes a helix-loop- helix protein that acts as a master regulator of cns midline development. *Cell,* ***67***(6): 1157-1167. https://doi.org/10.1016/0092-8674(91)90292-7

Norton, J. D. (2000). Id helix loop helix proteins in cell growth, differentiation and tumorigenesis. *Journal of Cell Science,* ***113***(22): 3897-3905.

Olson, J. M., Asakura, A., Snider, L., Hawkes, R., Strand, A., Stoeck, J. & Tapscott, S. J. (2001). NeuroD2 is necessary for development and survival of central nervous system neurons. *Developmental Biology,* ***234***(1): 174–187. https://doi.org/10.1006/dbio.2001.0245

Perk, J., Iavarone, A. & Benezra, R. (2005). Id family of helix-loop-helix proteins in cancer. *Nature Reviews Cancer,* ***5***(8): 603–614. https://doi.org/10.1038/nrc1673

Perry, R. L., Parker, M. H. & Rudnicki, M. A. (2001). Activated MEK1 binds the nuclear MyoD transcriptional complex to repress transactivation. *Molecular Cell,* ***8***(2): 291–301. https://doi.org/10.1016/s1097-2765(01)00302-1

Pires, N. & Dolan, L. (2010). Origin and diversification of basic-helix-loop-helix proteins in plants. *Molecular Biology and Evolution*, ***27***(4): 862-874. https://doi.org/10.1093/molbev/msp288

Pownall, M. E., Gustafsson, M. K. & Emerson, C. P. (2002). Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. *Annual Review of Cell and Developmental Biology,* ***18***(1): 747–783. https://doi.org/10.1146/annurev.cellbio.18.012502.1

Puri, P. L. & Sartorelli, V. (2000). Regulation of muscle regulatory factors by DNA-binding, interacting proteins, and post-transcriptional modifications. *Journal of Cellular Physiology*, ***185***(2): 155–173. https://doi.org/10.1002/1097-4652(200011)185:2<155::aid-jcp1>3.0.co;2-z

Robinson, K. A. & Lopes, J. M. (2000). Survey and summary: Saccharomyces cerevisiae basic helix–loop–helix proteins regulate diverse biological processes. *Nucleic Acids Research*, ***28***(7): 1499-1505. https://doi.org/10.1093/nar/28.7.1499

Sailsbery, J. K., Atchley, W. R. & Dean, R. A. (2012). Phylogenetic analysis and classification of the fungal bHLH domain. *Molecular Biology and Evolution,* ***29***(5): 1301–1318. https://doi.org/10.1093/molbev/msr288

Sayılı, M., Karataş, M., Yücer, A. & Akça, H. (1999). Tokat ilinde alabalık yetiştiriciliği yapan işletmelerin yapısal ve ekonomik analizi. *Ekin Dergisi,* ***7***: 66-72.

Steingrimsson, E., Copeland, N. G. & Jenkins, N. A. (2004). Melanocytes and the microphthalmia transcription factor network. *Annual Review of Genetics,* ***38***(1): 365–411. https://doi.org/10.1146/annurev.genet.38.072902.09

Suyama, M., Torrents, D. & Bork, P. (2006). PAL2NAL: Robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Research,* ***34***(Web Server): W609-W612. https://doi.org/10.1093/nar/gkl315

Tapscott, S. J. (2005). The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. *Development,* ***132***(12): 2685–2695. https://doi.org/10.1242/dev.01874

Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity,* ***93***(1): 77–78. https://doi.org/10.1093/jhered/93.1.77

Voytik, S. L., Przyborski, M., Badylak, S. F. & Konieczny, S. F. (1993). Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscles. *Developmental Dynamics,* ***198***(3): 214-224. https://doi.org/10.1002/aja.1001980307

Walters, E. H., Stickland, N. C. & Loughna, P. T. (2000). The expression of the myogenic regulatory factors in denervated and normal muscles of different phenotypes. *Journal of Muscle Research, Cell Motility,* ***21***(7): 647-653. https://doi.org/10.1023/A:1005683825960

Wang, X. H., Wang, Y., Liu, A. K., Liu, X. T., Zhou, Y., Yao, Q. & Chen, K. P. (2015). Genome-wide identification and analysis of basic helix-loop-helix domains in dog, *Canis lupus familiaris*. *Molecular Genetics and Genomics*, ***290***(2): 633-648. https://doi.org/10.1007/s00438-014-0950-1

Wang, Y., Chen, K., Yao, Q., Wang, W. & Zhu, Z. (2007). The basic helix-loop-helix transcription factor family in *Bombyx mori*. *Development Genes and Evolution,* ***217***(10): 715-723. https://doi.org/10.1007/s00427-007-0184-x

Wang, Y., Chen, K., Yao, Q., Zheng, X. & Yang, Z. (2009). Phylogenetic analysis of zebrafish basic helix-loop-helix transcription factors. *Journal of Molecular Evolution,* ***68***(6): 629-640. https://doi.org/10.1007/s00239-009-9232-7

Wei, K. & Chen, H. (2018). Comparative functional genomics analysis of bHLH gene family in rice, maize and wheat. *BMC Plant Biology,* ***18***(1): 309. https://doi.org/10.1186/s12870-018-1529-5

Yasunami, M., Suzuki, K., Maruyama, H., Kawakami, H., Nagai, Y., Hagiwara, M. & Ohkubo, H. (1996). Molecular Cloning and Characterization of a cDNA Encoding a Novel Basic Helix- Loop- Helix Protein Structurally Related to Neuro-D/BHF1. *Biochemical and Biophysical Research Communications,* ***220***(3): 754-758. https://doi.org/10.1006/bbrc.1996.0476

Yiğit, M. & Aral, O. (1999). Gökkuşağı alabalığı'nın (*Oncorhynchus mykiss* W., 1792) tatlısu ve denizsuyundaki büyüme farklılıklarının karşılaştırılması. *Turkish Journal of Veterinary and Animal Sciences,* ***23***: 53-59.

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RESEARCH ARTICLE

Length-weight relationship and condition factor of freshwater blenny *Salaria fluviatilis* (Asso, 1801) in Asi River (Hatay, Turkey)

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ABSTRACT

In this study, a total of 109 specimens (57 female and 52 male) of *Salaria fluviatilis* were captured by tulle net and scooped net between October 2017 and March 2018 from the Asi River system, Turkey. The length-weight relationship was $W = 0.0013L^{3.054}$ ($r^2 = 0.967$) for both sexes with isometric growth. The values of the exponent *b* of the length-weight relationships (LWRs) were 3.055 for females and 3.052 for males. The b values for females, males, and both sexes were not significantly different from 3.0 ($P>0.05$). Fulton condition factor (CF) was calculated 1.3955±0.034 for females, 1.4495±0.042 for males and 1.4212±0.027, for both sexes. Estimations of LWRs and CF for both sexes of *S. fluviatilis*, captured from Asi River, were provided for the first time.

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Introduction

The freshwater blenny *Salaria fluviatilis* (Asso, 1801) is a member of the family Blenniidae and is well-known in the Mediterranean Region. *S. fluviatilis* is mainly a riverine species, naturally found in drainages around the northern Mediterranean and from Turkey, Israel to Portugal, and in Morocco and Algeria (Oliveira et al., 1992; Crivelli, 1996; Kottelat and Freyhof, 2007; Froese and Pauly, 2020). *S. fluviatilis* was reported from Seyhan Reservoir (Alagoz, 2005), Ceyhan River (Alp and Kara, 2007) and drainage of the Black Sea (Bostancı et al., 2016), Aegean and some Mediterranean regions of Turkey (Geldiay and Balık, 1999; İlhan et al., 2013; Erguden, 2016; İnnal, 2019; Çiçek et al., 2020).

S. fluviatilis a common inhabitant of stony and gravel bottoms of rivers and brooks (Bath, 1986). It is generally preferred rubble and gravel substrate. Adults feed on small benthic organisms such as small snails and shrimps, and all kinds of insect larvae (Wilson, 2009). This species can reach up to 15.4 cm in total length (Laporte et al., 2012), and the maximum age is 5 (Kottelat and Freyhof, 2007). Females

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deposit a layer of eggs on the underside of the nest stone and the eggs are guarded against predators by the males (Cote et al., 1999). *S. fluviatilis* individuals start breeding at the end of the first year and a female can lay around 1200 eggs at one time (IUCN, 2020). Females lay an average of 200-300 eggs at a time, 2-30 times (Çoker et al., 2019). The number of eggs in a nest is around 500-8000 (Gasith and Goren, 2009).

S. fluviatilis is considered as Least Concern (LC) in the IUCN Global Red List for inland waters (IUCN, 2020), this species has affected and decreased from large parts of the Asi River drainage due to pollution, habitat destruction, and water abstraction. Thus, it is considered a locally endangered species listed by the IUCN Red List (Crivelli, 2006; Fricke et al., 2007).

Length-weight relationships (LWRs) are calculated for estimating the weight of a given length. LWRs are also useful for comparing life history and morphological aspects of populations inhabiting different habitats. Besides, LWRs are necessary for stock assessment and fisheries management (Pauly, 1983; Petrakis and Stergiou, 1995; Goncalves et al., 1997; Koutrakis and Tsikliras, 2003). Because the LWR values; in the length values for estimation of biomass, in the estimation of weight from a given length, and in stock estimation models transforming growth in length and growth in weight equations to each other for comparison of life cycle of a fishes from different areas are widely used by many researchers (Binohlan and Pauly, 1998; Froese, 2006).

Condition factor (CF) is used for comparing the fish's health or the fatness of the fish (Froese, 2006). The condition factor is an index reflecting interactions between biotic and abiotic factors in the physiological condition of the fishes. The condition of the fish is calculated from the estimation of the length-weight relationships. Thus, CF is also an essential parameter for the evaluation of fish stocks, and this parameter is widely used for studies of fisheries and fish biology.

Figure 1. *Salaria fluviatilis* (Asso, 1801) captured in the Asi River

To date, a few studies regarding length-weight relationships of *S. fluviatilis* were in the eastern Mediterranean and the Aegean Sea for the inland waters of Turkey (İlhan et al., 2013; Erguden, 2016; İnnal, 2019). However, LWRs and condition factors according to sexes for *S. fluviatilis* have not been reported from Turkey. Besides, there is no information on LWRs and CF for *S. fluviatilis* from the Asi River system (Southeastern Anatolia, Turkey). In the present paper, author reported on the estimations of LWRs and CF for both sexes of *S. fluviatilis*, captured from Asi River, were provided for the first time.

Material and Methods

A total of 109 (57 female and 52 male) specimens were captured from Asi (Orontes) River system using tulle net and scooped net at 0-2 m depths between October 2017 and March 2018 (Coordinates: 36° 48' N, 36° 10' E – 36° 53' N, 36° 15' E). Species identification follows Geldiay and Balık (1999) and confirmed based on FishBase (Froese and Pauly, 2020) (Figure 1). Total length (TL) was measured to the nearest 0.1 cm by means of a vernier caliper and total weight was recorded to the nearest 0.01 g on a precision balance. The sex of each specimen was determined by examining the gonads macroscopically. Chi-square (χ^2) analysis was used to test the significant differences between the sex ratio.

The relationship between the length and weight of the fish samples were estimated using the equation

$$W = aL^b \text{ (Ricker, 1975)}$$

where W is the weight of the fish, TL is the total length, and a and b are constants. The relationship between total length, TL, and weight was calculated for females, males, and all individuals. The significance of the regression was assessed by ANOVA. The student's t-test was applied to determine the significance of differences (95% level) between the isometric growth (b=3) and the estimated b value of the equation.

Fulton's coefficient of condition factor (CF) was calculated by the formula given below.

$$CF = \frac{W}{L^3} \times 100$$

where; L is length (cm) and W is weight (g) (Bagenal, 1978; Sparre and Venema, 1992).

The relationships among the variables were identified using the regression analysis. The observed differences were evaluated statistically using SPSSv. 22.0 package program and Student's t-test (Sokal and Rohlf, 1969).

Results

Of the 109 specimens, 57 were (52.30%) females, 52 were males (47.70%). The sex ratio for female and male individuals

(F:M) was 1.00:0.91. The χ^2 test showed (χ^2= 3.819, P>0.05) that there was no significant difference in sex ratio from the expected 1:1. Total length in females ranged from 1.7-7.1 cm with a mean of 3.23±1.32 cm. Total length in males ranged from 2.0-6.8 cm with a mean of 3.29±1.33 cm. The mean total weights of females and males values were 0.78±1.10 g and 0.81±1.09 g, respectively (Table 1). The t-test showed that there was significant difference between sexes in overall total length and total weight (t=1.477, P<0.05). Females were more abundant length class (in 3.5-2.5 cm) than males (in 3.0-2.0 cm) in the length class. The dominant length class in the total samples was 2-2.5 cm (Figure 2).

The *b* values of length-weight relationship, which were not significantly different between sexes (ANOVA, P>0.05), indicated isometric growth for females (b=3.055), males (b=3.052) and both sexes (b=3.054). The estimated parameters of the length-weight relationship parameters are given in Table 2. The LWRs were described as $W = 0.0012xL^{3.055}$ ($r^2 = 0.969$) for females, $W = 0.0012xL^{3.052}$ ($r^2 = 0.965$) for males, and $W = 0.0013xL^{3.054}$ ($r^2 = 0.967$) for both sexes. Estimation of length-weight relationship for female, male, and both sexes are given in Figure 3.

The length-weight relationships were isometric growth in *S. fluviatilis* samples for females, males, and both sexes. The *b* values for females, males, and both sexes were not significantly different from 3.0 (*b*=3, P>0.05), (Table 2). In addition to the present study, a comparison of published length-weight relationship parameters in different regions for *S. fluviatilis* is given in Table 3.

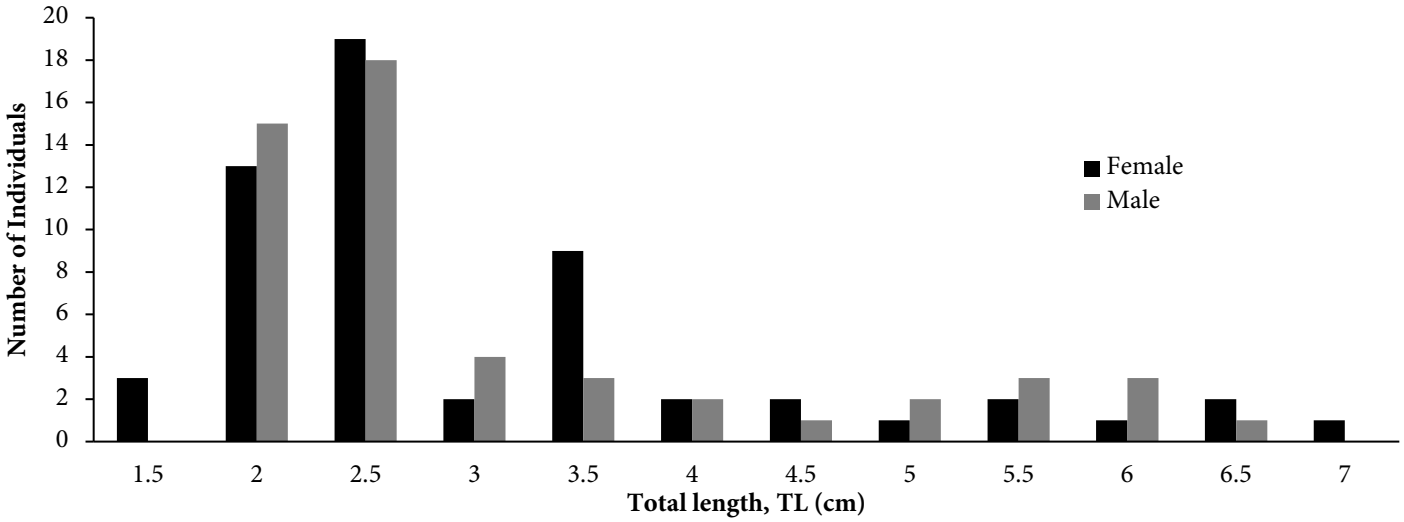

Figure 2. Length frequency distributions of *S. fluviatilis* collected in Asi River

Table 1. Mean and standard deviation, minimum, maximum, for total length (TL) and weight (W) characteristics according to sexes of *S. fluviatilis* occurring in the Asi River

Sex	N	L_{min}-L_{max} (cm)	Mean±SD	W_{min}-W_{max} (cm)	Mean±SD
Female	57	1.70-7.10	3.23±1.32	0.07-4.85	0.78±1.10
Male	52	2.00-6.80	3.29±1.33	0.10-4.37	0.81±1.09
Both	109	1.70-7.10	3.26±1.32	0.07-4.85	0.78±1.10

Note: N: Sample Size; S.D: Standard Deviation Min: Minimum; Max: Maximum

Table 2. Length-weight relationship parameters for *S. fluviatilis* in Asi River (Southeastern Anatolia)

Sex	N	a	b	±S.E(b)	95% C.I. of b	r^2	t-test	P	Growth
Female	57	0.00120	3.055	0.073	2.908-3.201	0.969	0.587	>0.05	Isometry
Male	52	0.00120	3.052	0.082	2.888-3.216	0.965	0.974	>0.05	Isometry
Both	109	0.00130	3.054	0.054	2.947-3.154	0.967	1.097	>0.05	Isometry

Note: N: Sample Size, a: Intercept of the relationship; b: Slope of the relationship; S.E.(b): Standard Error of b; r^2: Coefficient of determination, C.I.: Confidence Interval

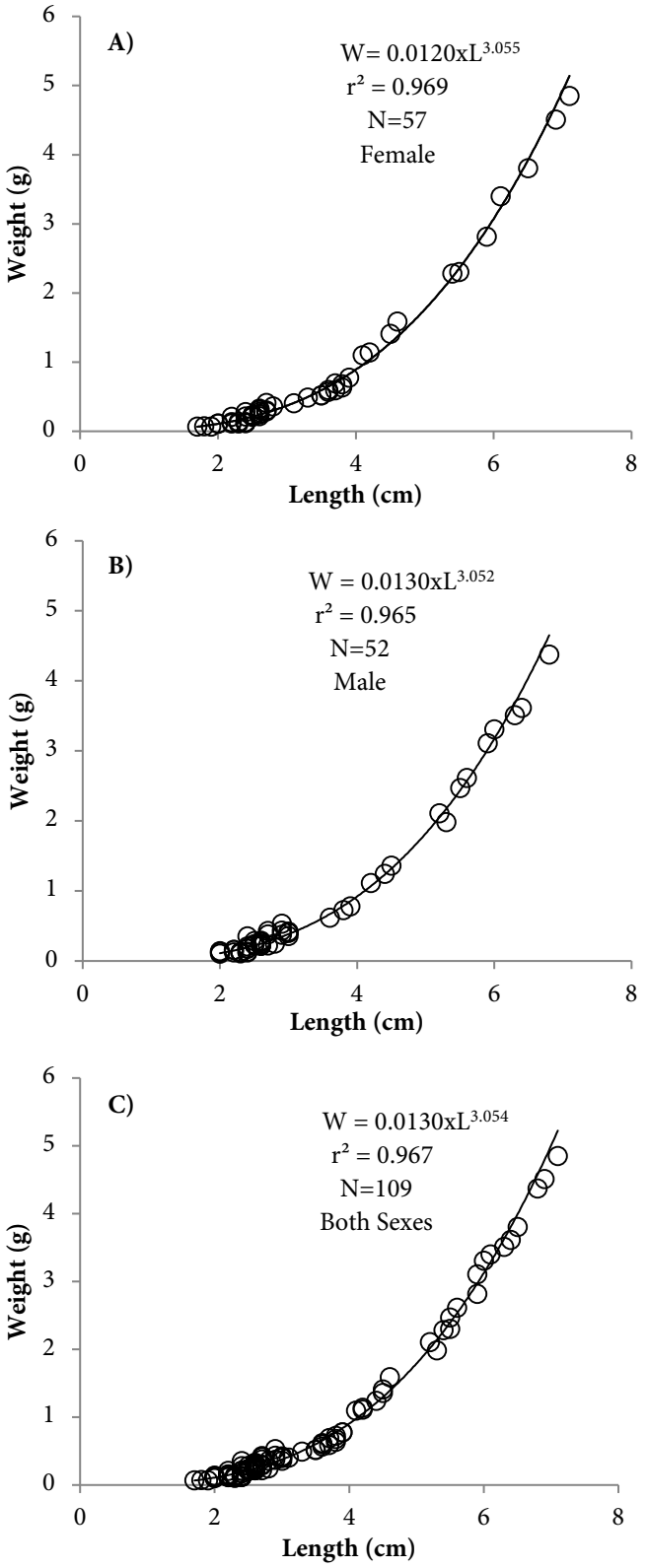

Figure 3. Length-weight relationship of *S. fluviatilis*. (A), female (B), male and (C), both sexes in Asi River (Southeastern Anatolia, Turkey)

The present study, Fulton condition factor (CF) value was estimated as 1.3955±0.034, for females, as 1.4495±0.042 for males and as 1.4212±0.027 for both sexes. Condition factor values showed no significant variations (P>0.05) of *S. fluviatilis* for between sexes.

Discussion

Investigations carried out on 109 specimens of *S. fluviatilis*, captured in the Asi River, revealed a sex ratio (males: females) of 1.00:0.91. Besides, it was also observed that females were more dominant in the population (Figure 2). In a study conducted in Ceyhan River by Alp & Kara (2007) reported the female/male ratio (F:M) as 0.69:1.00. Neat et al. (2003) determined as 0.73 in Lake Kournas, 0.58 in the Fango River, and 0.61 in Lake Garda (Italy). In the present study, the sex ratio of *S. fluviatilis* in the Asi River was slightly different from those in these previous reports, but similarly, females are dominant than males. According to Cote et al. (1999) The differences in the sex ratio of *S. fluviatilis* may be due to the egg guarding behavior of the males.

Total lengths of examined specimens of *S. fluviatilis* ranged between 1.7 and 7.1 cm. This finding is similar to the studies from different regions, e.g., 2.0-7.0 cm Kleanthidis et al. (1999) from Greece (Lake Trichonis); 2.0-7.5 cm (Erguden, 2016) from the Southeastern Anatolia, Turkey; 2.1-7.5 cm from western Mediterranean brackish water of Turkey (İnnal, 2019). Other results having considerably lower length range were also reported from different locations such as 2.4-6.5 cm from Rhios estuary, NW Aegean, Greece (Koutrakis and Tsikliras, 2003), 2.3-4.8 cm from Lake Iznik, Turkey (Tarkan et al., 2006). However, as a different result, İlhan et al. (2013) from Turkey Inland waters (7 drainage basin) declared maximum length (TL= 12.9 cm) for *S. fluviatilis*. The length differences in values might be explained with the habitat differences, environmental factors and catch period. Moreover, the size of fishes may fluctuate due to gender, season, feeding rate, gonadal development, water flow, and their behavior (Tarkan et al., 2006).

The values of the exponent *b* provide information on fish growth. When *b*=3, the increase in weight is isometric. When the value of *b* is higher than 3, the weight increase is allometric (positive if *b*>3, negative if *b*<3). In the present study, the *b* values ranged from 3.055 for female to 3.052 for male individuals of *S. fluviatilis*.

Based on previously reported studies, positive allometry and isometry were observed in *S. fluviatilis* from the west and the east Mediterranean inland waters of Turkey (Table 3). The presented paper, the *b* values were generally in similar results for *S. fluviatilis* reported from other geographical areas (Kleanthidis et al., 1999; Tarkan et al., 2006; İlhan et al., 2013; Erguden, 2016). The observed small differences could be due to the fishing equipment, season, fishing pressure, sampling

Table 3. Length-weight relationships of *S. fluviatilis* from different regions

Author(s)	Locality	Sex	N	Lt	Length (cm)	a	b	r^2
Kleanthidis et al. (1999)	Lake Trichonis, Greece	Female	441	TL	2.0-7.0	0.01020	3.080	0.941
		Male	409	TL	2.0-7.0	0.00680	3.330	0.980
Koutrakis and Tsikliras (2003)	Rhios estuary, NW Aegean, Greece	Mixed	5	TL	2.4-6.5	0.01220	2.986	0.986
İlhan et al. (2013)	Turkey Inland waters (7 drainage basin), Turkey	Mixed	652	TL	2.0-12.9	0.0135	3.004	0.986
Tarkan et al. (2006)	Lake İznik, Marmara	Mixed	92	TL	2.3-4.8	0.00960	3.060	0.933
Erguden (2016)	Seyhan Reservoir, Turkey	Mixed	97	TL	2.0-7.5	0.01170	3.084	0.962
İnnal (2019)	Western Mediterranean Brackish water, Turkey	Mixed	100	TL	2.1-7.5	0.00620	3.308	0.960
This study	Asi River, Turkey	Mixed	109	TL	1.7-7.1	0.00130	3.054	0.967

Note: Lt: Length type

procedure, sample size, length range, reproduction season and environmental or habitat factors (Petrakis and Stergiou, 1995). However, the previous two reports of positive allometric growth of *S. fluviatilis* has also been reported by Kleanthidis et al. (1999) (male samples; b=3.330 for Lake Trichonis, Greece) and by İnnal (2019) (b=3.308 for W. Mediterranean brackish water). On the contrary, Koutrakis and Tsikliras (2003) from Rhios estuary of Greece has reported negative allometric growth (b=2.986). These differences could be the result of environmental variations or habitat factors (Hossain et al., 2015), including seasonal effect, degree of stomach fullness, gonad maturity, food richness (Gonzalez et al., 2004; Ruiz-Campos et al., 2006), sex, health, reproductive period, and seasonal variation (Bagenal and Tesch, 1978; Alp et al., 2005).

According to Bagenal and Tesch (1978) and Goncalves et al. (1997), the parameters of *b* generally do not vary significantly throughout the year, unlike parameter *a,* which may vary seasonally, daily, and between habitats. Besides, several factors are known to influence the LWRs in fish, including habitat, general fish condition, gonad maturity, season, and preservation of fish (Tesch, 1971; Bagenal and Tesch, 1978; Wootton, 1990), these factors were not accounted in the present study.

The coefficient of determination (r^2) was found to be >0.96, a highly significant value of the result. The regression analysis has demonstrated that fish length had a highly significant correlation with weight (P<0.001).

Le Cren (1951) and Ricker (1975), reported the condition factor of fish populations may show variations with gonad development, feeding activity and seasonal changes in growth. In this study, CF values were also calculated, where the lowest and highest estimations were found to be 1.3955 and 1.4495, respectively, with a mean value of 1.4212±0.027 for both sexes. Le Cren (1951) declared that CF values greater than 1 indicated the good condition of the fish whereas a value <1 is indicative of the reverse nature. The present results revealed that both males and females indicate that both of the sexes are in good condition for *S. fluviatilis.*

Conclusion

The present study provides the first reference on LWRs and CF, of *S. fluviatilis*. Besides, the CF value of *S. fluviatilis* calculated for both sexes showed that it was in a very good condition in terms of its optimum growth in the Asi River. This study is also useful for fishery biologist, sustainable fishery management, and conservation.

S. fluviatilis is considered a locally endangered species listed by the IUCN Red List (Crivelli, 2006; IUCN, 2020). Besides, this species is locally threatened inland waters of Turkey due to dams built on rivers, habitat destruction, pollution, and water abstraction in the next years (Fricke et al., 2007). Today, *S. fluviatilis* has affected and decreased from large parts of the Asi drainage due to water pollution habitat destruction and introduction of exotic species. Therefore, a conservation action should be taken as a high priority for this species.

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Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

This study was conducted in accordance with ethics committee procedures of animal experiments. All procedures were performed in accordance with the Law on Veterinary and Medical Activities and National Animal Welfare Act. Therefore, ethics approval was not required.

References

Alagoz, S. (2005). Determination of ichthyofauna in the Seyhan Dam Lake (Adana). MSc Thesis. Cukurova University, Adana, Turkey.

Alp, A., Kara, C. H., Büyükçapar, M. & Bülbül, O. (2005). Age, growth and condition of *Capoeta capoeta angorae*, Hanko 1924 from the upper water systems of the River Ceyhan, Turkey. *Turkish Journal of Veterinary and Animal Sciences,* ***29***: 665-676.

Alp, A. & Kara, C. H. (2007). Distribution pattern and morphological differences between the sexes of river blenny, *Salaria fluviatilis* (Asso, 1801), in the Ceyhan River Basin, Turkey. *Turkish Journal of Zoology*, ***31***: 113-120.

Bagenal, T. B. (1978). *Methods for Assessment of Fish Production in Fresh Waters*. (3rd ed.), Handbook No: 3, Blackwell Scientific Publication, Oxford, 365p.

Bagenal, T. B. & Tesch, F. W. (1978). Age and growth (pp. 101-136). In: Bagenal, T. (Ed.), *Methods for assessment of fish production in fresh waters*. (3rd ed.), IBP Handbook No. 3, UK: Blackwell Scientific Publications, Oxford.

Bath, H. (1986). Blenniidae (pp. 355-357). In: Daget, J., Gosse, J. P., Thys van den Audenaerde, D. F. E. (Eds.), *Checklist of the freshwater fishes of Africa (CLOFFA)*. Vol. 2. ISNB, Brussels, MRAC, Tervuren; and ORSTOM, Paris.

Binohlan, C. & Pauly, D. (1998). The length-weight table (pp. 121-123). In: Froese, R., Pauly, D. (Eds.), *FishBase 1998: Concepts, design and data sources*. ICLARM, Manila.

Bostancı, D., Darçın, M. & Helli, S. (2016). A study on the investigation of fish fauna of Yalıköy Stream (Ordu). *Ordu University Journal of Science and Technology*, ***6***(2): 146-157.

Cote, I. M., Vinyoles, D., Reynolds, J. D., Doadrio, I. & Perdices, A. (1999). Potential impacts of gravel extraction on Spanish populations of river blennies *Salaria fluviatilis* (Pisces, Blenniidae). *Biological Conservation*, ***87***: 359-367.

Crivelli, A. J. (1996). The freshwater fish endemic to the Mediterranean region. An action plan for their conservation. Tour du Valat Publication, 171p.

Crivelli, A. J. (2006). *Salaria fluviatilis*. *The IUCN Red List of Threatened Species* 2006: e.T60764A12407160. Retrieved on 11 September 2020 from https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T60764A12407160.en.

Çiçek, E., Sungur, S. & Fricke, R. (2020). Freshwater lampreys and fishes of Turkey; a revised and updated annotated checklist 2020. *Zootaxa*, ***4809***(2): 241-270. https://doi.org/10.11646/zootaxa.4809.2

Çoker, T. (2019). Morphological characteristics of eggs and larvae of *Salaria fluviatilis* (Asso, 1801) (Family: Blenniidae) Collected from Akyaka Azmak Creek. *LIMNOFISH-Journal of Limnology and Freshwater Fisheries Research*, ***5***(3): 220-225. http://doi.org/10.17216/LimnoFish.484462

Erguden, S. A. (2016). Length-weight relationships for six freshwater fish species from the Seyhan Reservoir (south-eastern Anatolia, Turkey). *Journal of Applied Ichthyology,* ***32***: 141-143. https://doi.org/10.1111/jai.12905

Fricke, R., Bilecenoglu, M. & Sarı, H. M. (2007). Annotated checklist of fish and lamprey species (Gnathostomata and Petromyzontomorphi) of Turkey, including a Red List of threatened and declining species. *Stuttgarter Beitragezur Naturende Serie A,* ***706***: 1-172.

Froese, R. (2006). Cube law, condition factor and weight length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology,* ***22***(4): 241-2530. https://doi.org/10.1111/j.1439-0426.2006.00805.x

Froese, R. & Pauly, D. (2020). Fishbase.Worldwide Web Electronic Publication. Retrieved on August 16, 2020 from https://www.fishbase.se/summary/54714

Geldiay, R. & Balık, S. (1999). *Freshwater Fishes in Turkey,* (in Turkish). Ege University Fisheries Faculty Press, No: 46, (3. Ed.), Bornova, İzmir, Turkey. 532p.

Goncalves, J. M. S., Bentes, L., Lino, P. G., Ribeiro, J., Canario, A. V. M. & Erzini, K. (1997). Weight-length relationships for selective fish species of the small scale demersal fisheries off the South and Southwest coast of Portugal. *Fisheries Research*, ***30***: 253-256. https://doi.org/10.1016/S0165-7836(96)00569-3

Gonzalez, A, De La Cruz Aguero, A. F. G. & De La Cruz Aguero, J. (2004). Length weight relationships of fish species caught in a mangrove swamp in the Gulf of California (Mexico). *Journal of Applied Ichthyology*, ***20***: 154-155.

Gasith, A. & Goren, M. (2009). Habitat availability, reproduction and population dynamics of the freshwater blenny *Salaria fluviatilis* (Asso, 1801) in Lake Kinneret, Israel. *Electronic Journal of Ichthyology*, ***2***: 34-46.

Hossain, M. Y., Sayed, S. R. M., Rahman, M. M., Ali, M. M., Hossen, M. A., Elgorban, A. M. & Ohtomi, J. (2015). Length-weight relationships of nine fish species from the Tetulia River, southern Bangladesh. *Journal of Applied Ichthyology*, ***31***: 967-969. https://doi.org/10.1111/j.1439-0426.2011.01900.x

IUCN, (2020). The IUCN Red List of Threatened Species. Version 2020-2. Retrieved on September 14, 2020 from https://www.iucnredlist.org

İlhan, A., Ustaoğlu, M. R. & Berberoğlu, S. (2013). The length-weight relationship of freshwater blenny, *Salaria fluviatilis* (Asso, 1801) in 7 drainage basin of Turkey. *Ege Journal of Fisheries Aquatic Sciences*, ***30***(1): 41-43.

İnnal, D. (2019). Diversity and length-weight relationships of Blenniid species (Actinopterygii, Blenniidae) from Mediterranean brackish waters in Turkey. *Aquatic Sciences and Engineering,* ***34***(3): 96-102. https://doi.org/10.26650/ASE2019573052

Kleanthidis, P. K., Sinis A. I. & Stergiou, K. I. (1999). Length-weight relationships of freshwater fishes in Greece. *Naga, ICLARM Quarterly*, ***22***(4): 37-41.

Kottelat, M. & Freyhof, J. (2007). *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin. 646p.

Koutrakis, E. T. & Tsikliras, A. C. (2003). Length-weight relationships of fishes from three northern Aegean estuarine systems (Greece). *Journal of Applied Ichthyology*, ***19***: 258-260.

Laporte, M., Mattei, J., Perret, P., Roché, B., Vinyoles, D., Goren, M., Bacha, M., Mourad, Z., Pou, I., Roviram, Q., Berrebi, P. & Magnan, P. (2012). New maximum lengths for the freshwater blenny (*Salaria fluviatilis* Asso, 1801) and length comparison between continental and island rivers. *Cybium*, ***37***(4): 309-313.

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, ***20***: 201-219.

Neat, F. C., Lengkeek, W., Westerbeek, E. P., Laarhoven, B. & Videler, J. J. (2003). Behavioural and morphological differences between lake and river populations of *Salaria fluviatilis*. *Journal of Fish Biology*, ***63***: 374-387.

Oliveira, R. F., Almada, V. C., Almeida, A. J., Santos, R. S. & Gonçalves, E. J. (1992). A checklist of the blennioid fishes (Teleostei, Blennioidei) occurring in Portuguese waters. Arquipélago. *Ciencias da Natureza*, ***10***: 23-37.

Pauly, D. (1983). *Some simple methods for the assessment of tropical fish stocks*. FAO Fisheries Technical Report, No: 234, FAO, Rome. 52p.

Petrakis, G. & Stergiou, K. I. (1995). Weight length relationships for 33 fish species in Greek water. *Fisheries Research*, ***21***: 465-469. https://doi.org/10.1016/0165-7836(94)00294-7

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin Fisheries Research Board of Canada,* ***191:*** l-382.

Ruiz-Campos, G., González Acosta, A. F. & De La Cruz Aguero, J. (2006). Length–weight and length–length relationships for some continental fishes of northwestern Baja California, Mexico. *Journal of Applied Ichthyology,* ***22***: 314-335. https://doi.org/10.1111/j.1439-0426.2006.00780.x

Sokal, R. R. & Rohlf, F. J. (1969). *Introduction to Biostatistics.* (2nd Ed.), New York, USA: W.H. Freeman and Company. 363p.

Sparre, P. & Venema, S. C. (1992). *Introduction to Tropical Fish Stock Assessment, Part I*. FAO Fisheries Technical Paper 306/1, Rome. 376p.

Tarkan, A. S., Gaygusuz, O. Acipinar, H. Gürsoy C. & Ozulug, M. (2006). Length-weight relationship of fishes from the Marmara region (NW-Turkey). *Journal of Applied Ichthyology,* ***22***: 271-273. https://doi.org/10.1111/j.1439-0426.2006.00711.x

Tesch, F. W. (1971). Age and growth (pp. 98-103). In: Ricker, W. E. (Ed.), *Methods for assessment of fish production in fresh waters*. (1st ed.). Oxford, UK: Blackwell Scientific Publications. 348p.

Wilson, S. K. (2009). Diversity in the diet and feeding habits of Blennies (pp. 139-162). In: Patzner, R. A., Gonçalves, E. J., Hastings, P. A. and Kapoor, B. G. (Eds.), *The biology of blennies*. Enfield, NH: Science Publishers. 482p.

Wootton, R. J. (1990). *Ecology of Teleost Fishes*. Fish and Fisheries Series 1, Chapman & Hall, London. 404p.

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RESEARCH ARTICLE

Current status of critically endangered fan mussel *Pinna nobilis* (Linnaeus 1758) population in Çanakkale Strait, Turkey

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ABSTRACT

The population of *Pinna nobilis* (Linnaeus, 1758) has been severely damaged, especially by the effect of the disease, in addition to the causes such as fishing activities, overexploitation, environmental pollution, habitat degradation, tourism, and human impacts. Particularly, *Haplosporidium pinnae* caused gigantic destruction in the *P. nobilis* population. Accordingly, the present study aimed to determine the current status of the *P. nobilis* populations in the Çanakkale Strait. Samplings were carried out between July and August 2020 at 9 different stations in the Çanakkale Strait. The results revealed that mass mortality was observed at the two stations (Abide Beach and Kumkale Village Beach) located near the Aegean Sea. On the other hand, it has been determined that the lowest mortality rate (9.62%) has been found at Ilgardere station. Juvenile individuals have been observed in healthy and uninfected populations with high survival rates. This paper is the first document on the spatial distribution of *P. nobilis* in Çanakkale Strait. Therefore, the *P. nobilis* population should be continuously monitored to ensure the sustainability of the species.

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Introduction

The fan mussel *Pinna nobilis* is an endemic species and the largest marine bivalve in the Mediterranean Sea. It can reach to 1.2 m (Zavodnik et al., 1991) and live up to 45 years (Rouanet et al., 2015). It is distributed in the coasts covered by the seagrass meadows, mud, sandy mud, or gravel, and it is buried in the soft bottom (or holds on to soft bottoms) by its byssus (Tebble, 1966).

P. nobilis are filter-feeder organisms that feed suspended organic/inorganic matters. Due to this characteristic, it is a good and sensitive bio-indicator for Mediterranean littoral quality and water quality (Vicente et al., 2002; Natalotto et al., 2015). In addition to the hard surface of this species, it serves as potential living habitat for many species such as annelids, ascidians, bivalves, bryozoans, cnidarians, crustaceans, echinoderms, macroalgae, gastropods, sponges (Rabaoui et al., 2009, Acarli et al., 2010). On the other hand, the population of ecologically important fan mussel has been greatly reduced due to recreational and commercial fishing activities to supply food, shell usage for decorative purposes, and incidental killing by trawling and/or anchoring. The fan mussel has been announced as an endangered and protected species under Annex IV of the Habitats Directive (Council Directive 92/43/EEC). Particularly, species of Haplosporidium parasite, *Haplosporidium pinnae* caused great destruction to the *P. nobilis* population for the last four years (Vázquez-Luis et al., 2017; Dariba et al., 2017; Carella et al., 2019; Katsanevakis et al., 2019). Moreover, the status of the species has been updated from "Vulnerable" to "Critically Endangered" by the Spanish Sectoral Environmental Conference on 17 July 2017, at the national level.

In Turkey, some characteristics of the species have been investigated such as growth (Acarli et al., 2011a; Demirci and Acarli, 2019), spat settlement (Acarli et al., 2011b; Kurtay et al., 2018), and gonad development (Acarli et al., 2018). However, there is an exclusive study on the determination of *P. nobilis* population in the Aegean Sea and the Marmara Sea (Acarlı et al., 2020; Öndes et al., 2020a). However, there is no study on the *P. nobilis* population in the Çanakkale Strait. Therefore, the present study aimed to determine the *P. nobilis* population in the Çanakkale Strait and to provide recent knowledge to the scientific literature about the current status of the species.

Material and Methods

This study was carried out at 9 stations in the Çanakkale Strait (Figure 1). Çanakkale Strait is located in the north-western part of Turkey and is a part of the Turkish Straits System including Bosporus Strait, the Marmara Sea, and Çanakkale Strait (Kale, 2020). It is a transition zone between the Aegean Sea and the Black Sea. The region has a typical transition climate type characterized by rainy and cold winters and dry and hot summers (Kale, 2020). Cengiz and Akbulak (2009) reported that July is the warmest month and January is the coldest month. Kale (2017a) documented that air temperature had an increasing trend in the region similar to the report of Kale (2017b) which indicates that trends in the evaporation were increasing.

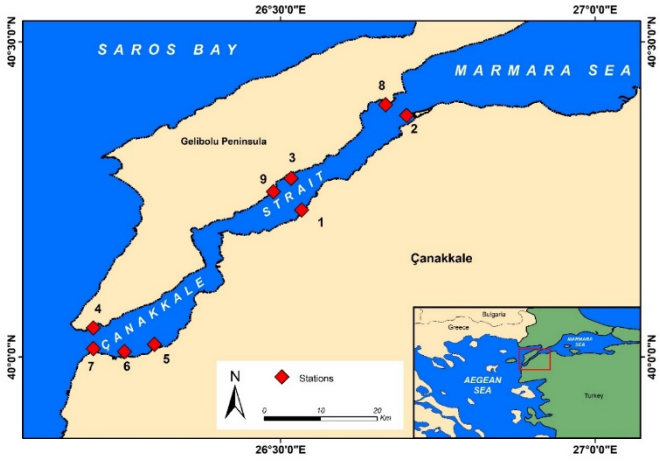

Figure 1. Study area (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

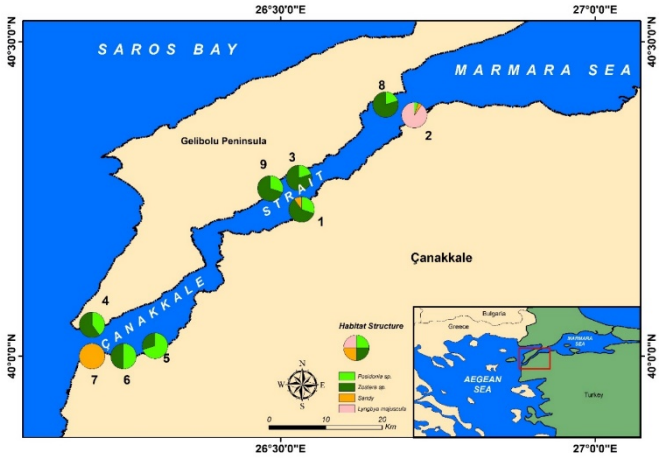

Figure 2. Habitat structure of the sampling stations in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Monitoring fish and underwater habitats require non-destructive investigation techniques and this is usually succeeded by divers carrying out underwater visual census (UVC) (Pelletier et al., 2011). UVC is the most common approach to estimate the abundance, diversity, and size of fishes

Table 1. Descriptive information of the sampling locations

Station	Coordinates	Surveyed Area (m^2)	Depth Range(m)	Habitat Structure
Yapıldak	40°14'017"N 26°32'460"E	1500	2-6	*Posidonia* sp. (30%) *Zostera* sp. (60%) Sandy (10%)
Çardak Kum Adası	40°23'003"N 26°42'542"E	3500	5	*Lyngbya majuscula* (90%) Dead *Posidonia* sp. + Sandy (10%)
Cennet Bay	40°17'544"N 26°31'128"E	2000	2-4	*Posidonia* sp. (20%) *Zostera* sp. (80%), (from shore to 2.5 m depth)
Abide Beach	40°03'148"N 26°12'497"E	2500	3-5	*Posidonia* sp. (40%) *Zostera* sp. (60%)
İntepe	40°01'025"N 26°18'861"E	2500	0-7*	*Posidonia* sp. (70%) *Zostera* sp. (30%)
Kumkale Harbour	40°00'053"N 26°15'624"E	3500	0-5*	*Posidonia* sp. (50%) *Zostera* sp. (50%)
Kumkale Village Beach	40°00'446"N 26°12'505"E	3000	4-6	Sandy (100%)
Hamza Bay	40°24'649"N 26°40'833"E	3000	4-5	*Posidonia* sp. (20%) *Zostera* sp. (80%)
Ilgardere	40°16'683"N 26°29'631"E	1500	3-4	*Posidonia* sp. (30%) *Zostera* sp. (70%)

Note: * indicates that no specimen was observed.

in clear waters. On the other hand, the estimation of the abundance by UVC approach is exclusively problematic in species that highly aggregated and/or observing at low densities due to their high mobility at both temporal and spatial scales (Irigoyen et al., 2018). The statistical strength of investigations including UVC approaches may be improved by increasing the number of replicates or the area explored. Alternatively, video-based techniques have become commonly used tools to observe underwater macrofauna and habitats (Pelletier et al., 2011). Therefore, SCUBA diving equipment was used to determine the current status of the *P. nobilis* population. Transect and visual census methods were used to estimate the abundance of the populations of *P. nobilis*. Depending on underwater visibility (2.5-10 m), divers detected the number of alive and dead *P. nobilis* individuals in a certain area underwater. At the same time, the UVC observation methodology was also supported with video-based tools (GoPro Silver model, and Nikon Coolpix 5600 model). The depths and temperature were measured with the Oceanic Geo 2 dive computer. The divers used an underwater tablet as an approximate estimator to measure the shell lengths of *P. nobilis* (dead or alive) underwater. Then, all measurements were photographed by the divers and all taken photos were analysed to determine unburied shell lengths by using an image processing toolbox in Matlab. The actual lengths of *P. nobilis* were calculated by the formula between the unburied shell length and total shell length given in Equation 1. Raw data provided by Acarli et al. (2018) were used in order to calculate this relationship.

$$a = 0.8061b + 28.61 \ (r^2 = 0.717) \quad (1)$$

In this equation, a is calculated total length, b is unburied shell length.

Descriptive information about the sampling locations such as habitat characteristics, depth, and surveyed area are given in Table 1. Since the underwater habitat differs in each region, the divers first determined the transect distance, and then the surveyed area was calculated separately for the number of dead, alive and total (dead + alive shell) individuals per 1000 m^2. Shell lengths were measured by UVC techniques.

Results

During the underwater observations, the temperature was recorded as 27°C for Abide beach and Kumkale Harbour, 26.8°C for Kumkale Village Beach, 26.5°C for İntepe, 26.3°C for

Figure 3. Illustrations of alive *P. nobilis* observed during underwater observations in different habitats in the Çanakkale Strait

Ilgardere, 26°C for Cennet Bay Yapıldakaltı, Hamza Bay, and Çardak Kum Adası. It has been observed that *P. nobilis* individuals in small size groups were between 108 mm and 200 mm in length and they distributed throughout 1.5-2 m water depth. It has been determined that the majority of the individuals in the large size groups (>30 cm) are distributed between 4 and 6 m water depth. In addition, 90% of the individuals were observed in seagrass meadows of *Posidonia* sp. and *Zostera* sp. while the rest were completely found on the sandy habitat (Table 1). Habitat structure of the sampling stations in the Çanakkale Strait is presented in Figure 2.

Minimum and maximum shell length values were given in Table 2. A total of 494 fan mussel individuals were observed during underwater observations. Some illustrations taken during the underwater observations were shown in Figure 3. Total numbers of individuals observed at all stations in the Çanakkale Strait are demonstrated in Figure 4. It has been determined that 71.25% of *P. nobilis* individuals were dead. The spatial distribution of dead and alive individuals in the Çanakkale Strait was given in Figure 5. No individuals were observed at İntepe and Kumkale Harbour stations located near the Aegean Sea. Mass mortality was observed in Abide Beach and Kumkale Village Beach nearly located to the Aegean Sea whereas the least mortality was found in Ilgardere station (9.62%) located in the central part of the strait.

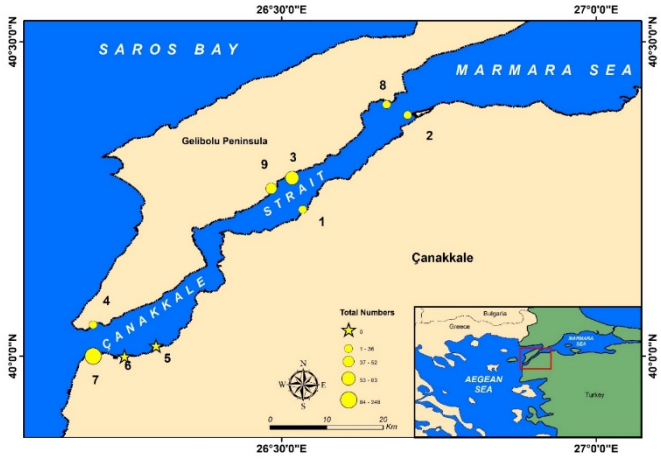

Figure 4. Total numbers of individuals observed in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Table 2. Minimum and maximum shell length values of *Pinna nobilis* individuals measured by the underwater visual census in the Çanakkale Strait

Station	Alive		Dead		Total	
	N	Min-Max (cm)	N	Min-Max (cm)	N	Min-Max (cm)
Yapıldak	9	12.5-22.5	27	22.6-36.0	36	12.5-36.0
Çardak Kum Adası	7	22.4-28.2	17	28.3-35.3	24	22.4-35.3
Cennet Bay	65	11.3-30.0	18	30.1-37.4	83	11.3-37.4
Abide Beach	0	-	27	28.8-38.2	27	28.8-38.2
İntepe	0	*	0	*	0	*
Kumkale Harbour	0	*	0	*	0	*
Kumkale Village Beach	0	-	248	24.0-42.7	248	24.0-42.7
Hamza Bay	14	13.6-15.8	10	15.9-24.1	24	13.6-24.1
Ilgardere	47	10.8-29.1	5	29.2-34.7	52	10.8-34.7

Note: No *P. nobilis* individual was observed in this station.

On the other hand, the mortality rate was lower in Hamza Bay station, and Çardak Kum Adası stations located near the Marmara Sea. However, in the central part of the strait, one station (Yapıldak station) has a very high mortality, whereas the other two stations (Cennet Bay station, Ilgardere station) that very close to this station (Yapıldak station) have a very low mortality rate. Table 3 provides further information about the findings on the mortality at all stations.

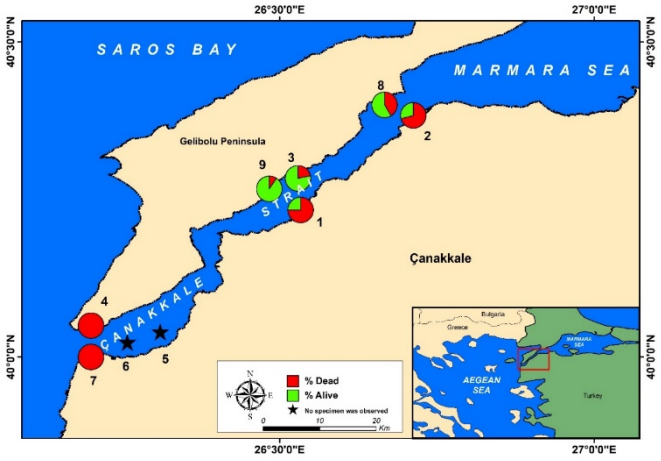

Figure 5. Spatial distribution of dead/alive individuals in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Discussion

This study was carried out in 9 stations in order to determine the fan mussel population and to provide recent knowledge on the current status of the species in the Çanakkale Strait. The current status of the population should be monitored since the *P. nobilis* species could not preserve its existence in the Mediterranean and had high mortality rates. Cabanellas-Reboredo et al. (2019) indicated that the main reasons are higher salinity and water temperature (>13.5°C) for the increase of the parasite infections in *P. nobilis*. The authors also documented that mortality was found at the temperature of 26.5°C. Šarićet al. (2020) reported mass mortality between 13°C and 25°C temperature in the Adriatic Sea. However, non-infected areas where less exposed to major sea currents have also been detected by the authors. Trigos (2015) indicated that the high temperatures of the seawater during the summer months could have decreased the physiological capacity of *P. nobilis* to resist parasite infections since respiratory requirement will be increased at high temperatures. In the present study, the temperature was recorded between 26°C and 27°C during the underwater observations in July and August 2020. The temperature (when combined with other stressors) may have a fatal impact on the population of *P. nobilis* particularly at stations that mass mortality (100%) has occurred. However, the mortality rate was found in other

stations. Šarić et al. (2020) reported that areas less exposed to sea current were at lower risk of pathogen transmission. Although the transmission of the disease is commonly through the currents, the present study reports a different case for this common phenomenon. The spread of the disease occurs in the opposite direction to the currents in the strait. The current in the Çanakkale Strait has a two layer structure. The upper layer flows from the Marmara Sea to the Aegean Sea while the lower layer flows from the Aegean Sea to the Marmara Sea. The upper layer of the current sometimes reverses because of the meteorological conditions and the shape of the coasts (Başar, 2010). Similarly, Jarosz et al. (2012) estimated that large amounts of lower part of the current (approximately 43%-45% of the flow) reversed back to the Aegean Sea. Therefore, this inverse relationship between the spread and expected route of the disease could be related to the currents directions. The decrease of the mass mortality and lower spread of the disease could be associated with the reverse current.

Mass mortality (100% death) was observed in Kumkale Village Beach station and Abide Beach station located near the Aegean Sea similar to Öndes et al. (2020a). On the other hand, the mortality rate was found lower in the stations located near the coasts of the Gelibolu Peninsula (Cennet Bay, Hamza Bay, and Ilgardere stations). It is thought that the stations on these coasts are less affected by human activities since the Gelibolu Peninsula is under protection by legislative regulations. Spatial distribution, abundance and mortality of *P. nobilis* are affected by several factors such as environmental stress including variations of temperature, salinity, depth, wave height (García-March et al., 2007; Kurihara et al., 2018; Maoxiao et al., 2019) and human impacts (Deudero et al., 2015; Öndes et al., 2020b). Moreover, Deudero et al. (2015) claimed that anthropogenic impacts much more affected the coastal benthic communities than global environmental changes.

Acarli et al. (2018) indicated that the spawning period of *P. nobilis* was ranged from May to September (primarily in July) in İzmir Bay, Turkey. The settlement of *P. nobilis* was determined in August in the same region (Acarli et al., 2011b). Some authors reported that *P. nobilis* has reached 150 mm in length after one-year growth experiments (Kožul et al., 2011; Acarli et al., 2011a; Demirci and Acarli, 2019). In this study, individuals have generally been assumed to be over 1 year old in lights of the previously conducted researches and investigations on the reproduction and growth of the species, length measurements, and the prominence of the serrated protrusions on it. . During the underwater observations, healthy and uninfected juvenile individuals were determined. Newly attached individuals of the *P nobilis* species are resistant to manipulation and accordingly their survival rate is quite high (Acarli et al 2011b). Šarić et al. (2020) indicated that mortality in juveniles was lower, better reaction to *H. pinnae* in Sakarun (Dugi Otok) station. *Haplosporidans parasitize* infect marine and freshwater invertebrates such as mollusc and crustacean (Stentiford et al., 2013; Arzul and Carnegie, 2015). *Bonamia ostreae* and *H. pinnae* belong to the Haplosporiidae family (Catanese et al., 2018). *B. ostreae* has distributed in many countries where oyster stocks are depleted (Culloty and Mulcahy, 2007). It was first detected in 1979 in Brittany-France and later distributed in Spain, Denmark and England. Deaths (up to 90%) have been detected in uninfected populations. Factors such as high stocking density, handling, lack of nutrients, high temperature, and salinity have caused the spread of bonamiosis (Crawford, 2016). Mortality increases particularly at high water temperature (Engelsma et al., 2010). Arzul et al. (2009) declared that the mortality is more effective at higher temperature (25°C) compared to the lower temperature values (4°C and 15°C). Engelsma et al. (2010) indicated that a positive relationship was found between prevalence of *B. ostreae* and salinity. The authors indicated that higher salinity is more suitable for *B. ostreae*. Cáceres-Martínez et al. (1995) pointed out that a significant positive correlation between the mean total length and the presence of *B. ostreae* could be detected in an oyster at all sizes but the mortality rate is lower in individuals smaller than 2 cm shell length. Thus, infection increased as the size increased. Disease susceptibility seems to be age-related. Robert et al. (1991) and Culloty and Mulcahy (1996) noted that the age of 2 years comes out to be the critical age for the development of the disease. It can be concluded that the anamnesis of *B.* ostreae and *H.* pinnae shows similarities. Since the survival rate is high in juvenile individuals and the mortality rate is higher in older individuals, it is thought that it may be in the same situation in *P. nobilis* as in *O. edulis*. Therefore, continuously monitoring of surviving juvenile and adult individuals is essential to ensure the sustainability of the critically endangered *P. nobilis* stocks in order to make more accurate assessments regarding the distribution and degree of impact of the parasite, and to rehabilitate other populations which the disease has observed.

It is very important to identify uninfected populations in coastal waters of Turkey to continuously monitor and to protect these regions in terms of ensuring the continuity of stocks. In previous studies, healthy *P. nobilis* populations were observed in the Erdek-Ocaklar coasts and Marmara Island (in the Marmara Sea), which are relatively close to the stations located near the Marmara Sea in the Çanakkale Strait (Pers. obser. in the Marmara Sea by SA and DA). Similarly, Öndes et al. (2020a) reported the existence of healthy individuals on the Erdek

coasts. Juveniles can be collected with the help of collectors from uninfected areas and transported to new areas. These areas are important for rehabilitating and revitalizing damaged fan mussel populations. Again, it can be aimed to create restocking programs from resistant individuals (resilient juveniles and adults) by transplantation and monitoring. The information provided on artificial recruitment, experimental juvenile growth, and re-implantation in nature, may serve in the future to help reinforce or even re-establish populations in locations devastated by the recent mass mortality event.

Conclusion

In conclusion, mortality has been observed in *P. nobilis* population in the Çanakkale Strait despite the fact that the mortality decreased gradually from the Aegean Sea to the Marmara Sea. In this context, it is known that the Marmara Sea has healthy populations, considering our personal observations. The Çanakkale Strait currently acts as a barrier. The monitoring of the populations distributed in this area is very important in terms of the course of the disease. Particularly, it is of great importance to transplantation of resilient individuals in the Çanakkale Strait in new areas (the natural population of fan mussel has occurred or not) that are infected and/or not infected for the rehabilitation of these areas. Alternatively, new healthy populations might be established by transplanting juvenile individuals from non-infected populations from the Marmara Sea to suitable areas in the Çanakkale Strait. Marine protected areas should be established for protecting and monitoring the healthy populations and/or infected (but has lower mortality) populations. Thus, the sustainability of the population could be ensured. In addition, the natural populations might be improved by aquaculture approaches (breeding program, larval culture, spat collecting, rearing, etc.) starting from the larval stage.

Compliance with Ethical Standards

Authors' Contributions

Author SA designed the study, DA carried out underwater surveys. SK performed GIS-related analyses. All authors wrote the first draft of the manuscript together and equally contributed for preparing the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Acarlı, D., Acarlı, S. & Öktener, A. (2020). Mass mortality report of critically endangered fan mussel (*Pinna nobilis*, Linnaeus 1758) from Cunda Island, Ayvalık (Aegean Sea, Turkey). *Acta Natura et Scientia,* ***1***(1): 109-117.

Acarli, S., Lök, A. & Acarli, D. (2011a). Preliminary spat settlement of fan mussel *Pinna nobilis* Linnaeus 1758 on a mesh bag collector in Karantına Island (Eastern Aegean Sea, Turkey). *Fresenius Environmental Bulletin*, ***20***(10): 2501-2507.

Acarli, S., Lok, A., Yigitkurt, S. & Palaz, M. (2011b). Culture of fan mussel (*Pinna nobilis*, Linnaeus 1758) in relation to size on suspended culture system in Izmir Bay, Aegean Sea, Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, ***17***(6): 995-1002. https://doi.org/10.9775/kvfd.2011.4922

Acarli, S., Lök, A., Acarli, D. & Kırtık, A. (2018). Reproductive cycle and biochemical composition in the adductor muscle of the endangered species fan mussel (*Pinna nobilis*, Linnaeus 1758) from the Aegean Sea. Turkey. *Fresenius Environmental Bulletin*, ***10***: 6506-6518.

Acarli, S., Lök, A., Acarli, D., Serdar, S., Küçükdermenci, A., Yiğitkurt, S., Kırtık, A. & Güler, M. (2010). Urla karantina adası civarında dağılım gösteren pina (*Pinna nobilis*, Linnaeus 1758) kabukları üzerine tutunan makrobentik türler. *Türkiye'nin Kıyı ve Deniz Alanları (KAY) VIII. Ulusal Konferansı Bildiriler Kitabı*, Trabzon, Türkiye. pp. 741-746.

Arzul, I. & Carnegie, R. B. (2015). New perspective on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology*, ***131***: 32-42. https://doi.org/10.1016/j.jip.2015.07.014

Arzul, I., Gagnaire, B., Bond, C., Chollet, B., Morga, B., Ferrand, S., Robert, M., Renault, T., 2009. Efects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting fat oysters *Ostrea edulis*. Diseases. *Aquatic Organisms*, ***85***: 67–75.

Başar, E. (2010). Investigation into marine traffic and a risky area in the Turkish straits system: Canakkale strait. *Transport*, ***25***(1): 5-10. https://doi.org/10.3846/transport.2010.01

Cabanellas-Reboredo, M., Vázquez-Luis, M., Mourre, B., Álvarez, E., Deudero, S., Amores, Á., Addis, P., Ballesteros, E., Barrajón, A., Coppa, S., García-March, J. R., Giacobbe, S., Casalduero, F. G., Hadjioannou, L., Jiménez-Gutiérrez, S. V., Katsanevakis, S., Kersting, D., Mačić, V., Mavrič, B., Patti, F. P., Planes, S., Prado, P.,

Sánchez, J., Tena-Medialdea, J., de Vaugelas, J., Vicente, N., Belkhamssa, F. Z., Zupan, I. & Hendriks, I. E. (2019). Tracking a mass mortality outbreak of pen shell Pinna nobilis populations: A collaborative effort of scientists and citizens. *Scientific Reports*, ***9***: 13355. https://doi.org/10.1038/s41598-019-49808-4

Cáceres-Martínez, J., Robledo, J. A. F. & Figueras, A. (1995). Presence of *Bonamia* and its relation to age, growth rates and gonadal development of the flat oyster, *Ostrea edulis*, in the Ría de Vigo, Galicia (NW Spain). *Aquaculture*, ***130***: 15-23.

Carella, F., Aceto, S., Pollaro, F., Miccio, A., Iaria, C., Carrasco, N., Prado, P. & de Vico, G. (2019). A mycobacterial disease is associated with the silent mass mortality of the pen shell *Pinna nobilis* along the Tyrrhenian coastline of Italy. *Scientific Reports*, ***9***: 2725. https://doi.org/10.1038/s41598-018-37217-y

Catanede, G., Grau, A., Valencia, K. J. M., Garcia-March, J. R., Vázquez-Luis, M., Alvarez, E., Deudero, S., Darribba, S., Carballal, M. J. & Villalba, A. (2018). *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with mass mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea. *Journal of Invertebrate Pathology*, ***157***: 9-24. https://doi.org/10.1016/j.jip.2018.07.006

Cengiz, T. & Akbulak, C. (2009). Application of analytical hierarchy process and geographic information systems in land-use suitability evaluation: A case study of Dümrek village (Çanakkale, Turkey). *International Journal of Sustainable Development & World Ecology*, ***16***(4): 286-294. https://doi/10.1080/13504500903106634

Crawford, C. (2016). National review of Ostrea angasi aquaculture: Historical culture, current methods and future priorities. Hobart, Australia: Institute of Marine and Antarctic Studies, 43 p.

Darriba, S. (2017). First haplosporidan parasite reported infecting a member of the Superfamily Pinnoidea (*Pinna nobilis*) during a mortality event in Alicante (Spain, Western Mediterranean). *Journal of Invertebrate Pathology*, ***148***: 14-19. https://doi.org/10.1016/j.jip.2017.05.006

Demirci, A. & Acarli, S. (2019). Estimation growth parameters of endangered the fan mussel species (*Pinna nobilis* L.) by using different growth models from Izmir Bay, Aegean Sea, Turkey. *Fresenius Environmental Bulletin*, ***28***(10): 7368-7374.

Deudero, S., Vázquez-Luis, M. & Álvarez, E. (2015). Human stressors are driving coastal benthic long-lived sessile fan mussel *Pinna nobilis* population structure more than environmental stressors. *PLOS One*, ***10***(7): e0134530. https://doi.org/10.1371/journal.pone.0134530

García-March, J. R., Pérez-Rojas, L. & García-Carrascosa, A. M. (2007). Influence of hydrodynamic forces on population structure of *Pinna nobilis* L., 1758 (Mollusca: Bivalvia): The critical combination of drag force, water depth, shell size and orientation. *Journal of Experimental Marine Biology and Ecology*, ***342***(2): 202-212. https://doi.org/10.1016/j.jembe.2006.09.007

Gosling, E. (2003). Bivalve molluscs biology ecology and culture. London, UK: Fishing News Books, 443 p.

Irigoyen, A. J., Rojo, I., Calò, A., Trobbiani, G., Sánchez-Carnero, N. & García-Charton, J. A. (2018). The "Tracked Roaming Transect" and distance sampling methods increase the efficiency of underwater visual censuses. *PLoS ONE*, ***13***(1): e0190990. https://doi.org/10.1371/journal.pone.0190990

Jarosz, E., Teague, W. J., Book, J. W. & Beşiktepe, Ş. T. (2012) Observations on the characteristics of the exchange flow in the Dardanelles Strait, *Journal of Geophysical Research*, ***117***(C11): C11012. https://doi.org/10.1029/2012JC008348

Kale, S. (2017a). Climatic trends in the temperature of Çanakkale city, Turkey. *Natural and Engineering Sciences*, ***2***(3): 14-27. https://doi.org/10.28978/nesciences.348449

Kale, S. (2017b). Analysis of climatic trends in evaporation for Çanakkale (Turkey). *Middle East Journal of Sciences*, ***3***(2): 69-82. https://doi.org/10.23884/mejs.2017.3.2.01

Kale, S. (2020). Development of an adaptive neuro-fuzzy inference system (ANFIS) model to predict sea surface temperature (SST). *Oceanological and Hydrobiological Studies*, ***49***(4): 354-373 https://doi.org/10.1515/ohs-2020-0031

Katsanevakis, S., Tsirintanis, K., Tsaparis, D., Doukas, D., Sini, M., Athanassopoulou, F., Kolygas, M., Tontis, D., Koutsoubas, D. & Bakopoulos, V. (2019). The cryptogenic parasite *Haplosporidium pinnae* invades the Aegean Sea and causes the collapse of *Pinna nobilis* populations. *Aquatic Invasions*, ***14***(2): 150-164. https://doi.org/10.3391/ai.2019.14.2.01

Kožul, V., Glavić, N., Bolotin, J. & Antolović, N. (2011). The experimental rearing of fan mussel *Pinna nobilis* (Linnaeus, 1758). *Proceedings of the 46th Croatian and 6th International Symposium on Agriculture*, Opatija, Croatia. pp. 803-806.

Kurihara, T., Nakano, S., Matsuyama, Y., Hashimoto, K., Yamada, K., Ito, A. & Kanematsu, M. (2018). Survival time of juvenile pen shell *Atrina pectinata* (Bivalvia: Pinnidae) in hyposaline water. *International Aquatic Research*, ***10***: 1-11. https://doi.org/10.1007/s40071-017-0183-0

Kurtay, E., Lok, A., Kirtik, A., Kucukdermenci, A. & Yigitkurt, S. (2018). Spat recruitment of endangered Bivalve *Pinna nobilis* (Linnaeus, 1758) at two different depths in Izmir Bay, Turkey. *Cahiers de Biologie Marine*, ***59(***6): 501-507. https://doi.org/10.21411/CBM.A.43183913

Natalotto, A., Maisano, M., Mauceri, A. & Deudero, S. (2015) Biomarkers of environmen-tal stress in gills of *Pinna nobilis* (Linnaeus 1758) from Balearic Island. *Ecotoxicology and Environmental Safety*, ***122***: 9-16. https://doi.org/10.1016/j.ecoenv.2015.06.035

Öndes, F., Alan, V., Akçalı, B. & Güçlüsoy, H. (2020a). Mass mortality of the fan mussel, *Pinna nobilis* in Turkey (eastern Mediterranean). *Marine Ecology*, ***00***: e12607. https://doi.org/10.1111/maec.12607

Öndes, F., Kaiser, M. J. & Güçlüsoy, H. (2020b). Human impacts on the endangered fan mussel, *Pinna nobilis*. *Aquatic Conservation: Marine and Freshwater Ecosystems*, ***30***: 31-41. https://doi.org/10.1002/aqc.3237

Pelletier, D., Leleub, K., Mou-Thamb, G., Guillemotb, N. & Chaban, P. (2011). Comparison of visual census and high definition video transects for monitoring coral reef fish assemblages. *Fisheries Research*, ***107***(1-3): 84-93. https://doi.org/10.1016/j.fishres.2010.10.011

Rabaoui, L., Tlig-Zouari, S., Cosentino, A. & Hassine, O. K. B. (2009). Associated fauna of the fan shell *Pinna nobilis* (Mollusca:Bivalvia) in the northern and eastern Tunisian coasts. *Scientia Marina,* ***73***(1): 129-141. https://doi.org/10.3989/scimar.2009.73n1129

Rouanet, E., Trigos, S. & Vicente, N. (2015). From youth to death of old age: The 50-year story of a *Pinna nobilis* fan mussel population at Port-Cros Island Port-cros National Park, Provence, Mediterranean Sea. *Scientific Reports of the Port-Cros National Park*, ***29***: 209-222.

Šarić, T., Župan, I., Aceto, S., Villari, G., Palić, D., De Vico, G. & Carella, F. (2020). Epidemiology of noble pen shell (*Pinna nobilis* L. 1758) mass mortality events in Adriatic Sea is characterised with rapid spreading and acute disease progression. *Pathogens*, ***9***(10): 776. https://doi.org/10.3390/pathogens9100776

Stentiford, G. D., Bateman, K. S., Stokes, N. A. & Carnegie, R. B. (2013). *Haplosporidium littoralis* sp. nov.: A crustacean pathogen within the Haplosporida (Cercozoa, Ascetosporea). *Dieases and Aquatic Organisms*, ***105***(3): 243-252. https://doi.org/10.3354/dao02619

Tebble, N. (1966). British bivalve seashells a hand book identification. London, UK: Trustees of the British Museum. 213p.

Vázquez-Luis, M., Álvarez, E., Barrajón, A., García-March, J. R., Grau, A., Hendriks, I. E., Jiménez, S., Kersting, D., Moreno, D., Pérez, M., Ruiz, J. M., Sánchez, J., Villalba, A. & Deudero, S. (2017). S.O.S. *Pinna nobilis*: A mass mortality event in western Mediterranean Sea. *Frontiers in Marine Science*, ***4***: 220. https://doi.org/10.3389/fmars.2017.00220

Vicente, N., de Gaulejac, B. & Avon, M. (2002) *Pinna nobilis* biological indicator of the Mediterranean littoral quality. *Proceedings of the Premier Sémi-naire International Sur La Grande Nacre de Méditerranée : Pinna nobilis*. Île des Embiez Var, France. 111-126.

Zavodnik, D., Hrs-Brenko, M. & Legac, M. (1991) Synopsis on the fan shell *Pinna nobilis* L. in the eastern Adriatic Sea. In: C .F. Boudouresque, M. Avon, V. Gravez (Eds.) Les Espèces Marines à Protéger en Méditerranée. GIS Posidonie, Marseille, pp. 169-178.

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REVIEW

A review on turmeric (*Curcuma longa* L.) and usage in seafood

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ABSTRACT

Seafood stands out as an important protein source for human nutrition. Providing food safety, increasing food production and processing, preventing nutrient losses, preserving the quality of food, and extending shelf life have gained importance. Thus, the use of food additives has been a technological must. Turmeric (*Curcuma longa* L., Zingiberaceae) is obtained from the root of the *Curcuma longa* plant, a fiber plant from the ginger family. It is a plant with polyphenolic effects. Curcumin (diferuloylmethane) is the most active ingredient of turmeric. It has an antioxidant effect. Turmeric has been found to have anti-inflammatory, anti-carcinogenic, and antiatherogenic effects. Turmeric is used in smoked meats, pickles and some cakes, seafood, fish soup and other soups, rice, cold cuts, and various vegetable dishes. Turmeric has a very important role in maintaining the nutritional quality of seafood products, extending the shelf life with its antioxidant and antimicrobial effect, increasing the attractiveness of seafood by adding color and flavor and obtaining healthy products.

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Introduction

Today, one of the most important problems of our world, which is in a rapid change in social, cultural and economic terms, is nutrition with adequate, balanced and healthy foods. It is stated that seafood is the only dietary food that contains protein, fat, carbohydrates, vitamins and minerals in a balanced and proportionate form for healthy nutrition, growth, development and survival (Koral, 2006).

Seafood is one of the most valuable food substances in terms of the nutrient components it contains. In addition to the fact that its diabetic characteristics, especially due to its low energy value, containing high amount of unsaturated fatty acid, very high protein rate and being easy to digest, it contains almost most of the amino acids in nature, is rich in minerals and vitamins and has high biological value. These factors include seafood among the valuable and quality foods and make it an important raw material in catering technology. Fish is preferred by consumers due to its low fat, cholesterol and calories. Since it is abundant and cheap, and also can be replaced with the decreasing natural stocks by aquaculture, it has become more

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and more important in the world day by day (İnal, 1988; Gülyavuz and Ünlüsayın, 1999).

Seafood is an important source of protein for human nutrition. They contain about 11-25% protein. The total muscle protein has consisted of 30% sarcoplasmic protein, 60-40% myofibrillar protein and 10% stroma (connective tissue) protein (Çaklı, 2007).

It is stated that seafood meat contains less common essential amino acids in other foods such as valine, leucine, isoleucine, lysine, threonine, methionine, phenylalanine, tryptophan, arginine, histidine in the most convenient amounts in addition to amino acids commonly found in other foods such as aspartic acid, serine, proline, alanine, taurine, glycine, tyrosine (Gülyavuz and Ünlüsayın, 1999).

Apart from protein, fish meat also contains non-protein nitrogenous substances. These substances are responsible for both flavor and spoilage. Fish meat is a food rich in fat-soluble vitamins (A, D, E, K), especially vitamins A and D (Göğüş and Kolsarıcı 1992; Gülyavuz and Ünlüsayın, 1999).

Fish meat is easy to digest and is excellent food in terms of the high protein (rich in lysine and isoleucine) and fat (due to ω3 and ω6 unsaturated fatty acids, EPA and DHA in fish oils, especially in oily fish such as salmon, mackerel, tuna, pike, garfish, trout, anchovy) (Altun et al., 2004).

Fish and seafood are shown as healthy food by health institutions in many parts of the world. It is stated that especially omega-3 and omega-6 fatty acids protect against some important diseases such as coronary heart diseases and cancer (Atar and Alçiçek, 2009).

The food industry, with its changing consumption habits, is turning onto methods to produce healthier and safer foods (Barazi and Erkmen, 2010).

Compared to other meat products, seafood can spoil faster due to its high water content and weak connective tissue. Many seafood processing techniques have been applied to slow this deterioration. These processing technologies are increasingly diversified through advancing knowledge and experience. However, while this increase is being realized, traditional methods are not abandoned completely, but are evaluated and developed (Varlık et al., 2004; Alçiçek and Bekcan, 2009).

In addition to the fresh consumption of seafood today, it has an important place in the processing sector due to being processed with various processing technologies. Technologies such as drying, salting, smoking, cold preservation, freezing and marinating are some of the processing methods used for preserving seafood. It is known that the demand for processed products has increased due to reasons such as developing technology, rapidly increasing human population and the increasing number of working women. Moreover, Turkish people are more selective in terms of healthy nutrition. They show a tendency to both processed and healthy products. While extending shelf life is an important criterion, it is equally important to preserve the nutritional content of the product, to add flavor to the product and also to obtain a healthy product.

General Information

Since the existence of mankind, food preservation has been necessary for the continuity of life. Due to the changing eating habits over time and the increase in the number of employees, the development of ready-to-eat foods has become mandatory. Along with the developments in food processing methods and the acquisition of new products, it is aimed to extend the shelf life of the products obtained and to maintain their quality. In this way, seafood that is hunted abundantly in certain periods can be offered to human consumption when they are less (Gram et al., 2002).

Due to many requirements such as increasing need for long-term preservation of seafood without spoiling in accordance with hygiene and sanitation rules, processing product when it is abundant and consuming in other seasons, processing it with suitable methods and making use of the wastes in terms of economy, making it ready to use the product and providing convenience for consumer and diversity for products, it has become important to utilize seafood by processing in recent years (Anonymous, 2001).

Today, increase in consumption and processing of food based on the relationship between the development of the industry and consumption and production of food has made the use of food additives a technological must. Increase in the number of people working outside the home, changing eating habits, having less time for preparing food or the desire to spend little time for preparing have encouraged the production of semi-ready or commercially completely ready food and this situation has made the use of food additives inevitable (Toprak et al., 2002; Angiş and Oğuzhan, 2008).

Providing food safety and security is one of the most important issues of today. In providing food security, increasing food production and preventing nutrient losses, preserving their quality during the period between when the food is abundant and less and extending their shelf life have gained importance. The use of food additives has become inevitable in this case, too (Yurttagül and Ayaz, 2008).

One of the Food Additives: Colorants

The definition of food additive included in the Turkish Food Codex regulation published in the Official Gazette dated 16 November 1997 is as follows: They are substances which cannot be consumed as food alone or used as a food raw

material and either has nutritional value alone or not; their residue or derivatives can be found in the product during the process or manufacturing used by the technology chosen; they are allowed to be used to preserve the taste, smell, look, structure and other qualifications of the food during the period of production, classification, processing, preparation of the food or to prevent the unwanted changes in the given qualifications and stages (TGKY, 2010).

When historical developments regarding the use of chemicals in foods are examined, it is understood that salt and wood smoke is the oldest known additive use methods. It is seen that the use of food dyes dates back to the Egyptians in 3500 BC, around 3000 BC salt was used to store meat and seafood, and around 900 BC both salt and wood smoke were used as food storage methods. In addition to salt and wood smoke in the middle ages, nitrate is added to the meats to prevent botulism and it has been noticed it changes the color of the meat in a positive way and makes it look healthier. 50 BC spices have been used as flavors and in parallel with the rapid urbanization in Istanbul during the 19th century, the use of additives has become widespread, especially to protect food against spoilage. Today, these substances have formed an indispensable part of developing food technology (Altuğ, 2009).

Ensuring nutritional quality, achieving a reduction in residual rate, and helping processing by performing quality and endurance are the characteristics sought in food additives used in foods. A food additive should not hide processing and production errors, deceive the consumer, and reduce the nutritional value of a food. The categories of food additives according to their intended use are indicated on the packages of convenience foods with their special names and "E (European)" numbers according to the category. To indicate that a food additive is allowed to be used in the European Union (EU) countries, it is assigned the number "E". "E" numbers are introduced by European Union countries as a practical coding method for food additives. "E" at the beginning of the number symbolizes the EU (European Union). "E" numbers and special names allow food to be easily recognized during export and import. In the classification of food additives according to the basic functions with the "E" number system, Colorants are numbered with E 100180 (Sağlam, 2000).

Codex Alimantarius Comission (CAC) has been founded with the cooperation of World Health Organization (WHO) and Food and Agriculture Organization (FAO). Every year in the meetings they hold about food additives, after the approval by international organizations such as Joint Expert Committee on Food Additives, Scientific Committee on Food of European Commission and Food and Drug Administration of USA, Joint Expert Committee on Food Additives decides which foods can be added to which food and to what extent. They prepare suggestive standards for all countries (Çalışır et al., 2003; Yurttagül and Ayaz, 2008).

Colorants and sweeteners are defined by the Turkish Food Codex in the "Declaration of Communicators Used in Foods (2002)". According to this declaration, colorants are defined as substances that are not consumed as food alone or used as the main ingredient in foods and are added to the food as color enhancers or color regulators (Anonymous, 2002).

Colorants, dyes and pigments are used to gain consumer admiration, strengthen natural color, to regain color lost during processing of food or to color a product that is colorless. Some of the colorants have been found to be toxic and carcinogenic and their use is prohibited. Health problems associated with coloring agents that are allowed for use are hypersensitivity reactions (Yurttagül and Ayaz, 2008).

Use of plants by mankind as paint dates back centuries. Therefore, dye plants have become the main dyestuff of industrial products such as textiles, food, leather, etc. Our country is one of the richest countries in Europe and the Middle East in terms of vegetation with its nearly 10,000 plant species. Parallel to this rich flora, the number of plants used in natural dyeing is quite high. There are nearly 150 plant species used in natural dye production in our country. Some of these plants are turmeric, elecampane, licorice, common juniper, and sage (Mert et al., 1992).

Food dyes, which constitute an important group in food additives, are used in the industry for various purposes such as preserving, increasing, or modifying the existing and typical color available, controlling color change and deterioration, standardizing the appearance, adding decorative features or creating new products. Food dyes should not be used to raise low quality and mislead the consumer and should not be harmful to health. Use for these and similar purposes have been brought under control through legal regulations (Newsome, 1990; Karaali and Özçelik, 1993; Yentür et al., 1996).

Food dyes are additives used in confectionery, food eaten between meals, soft drinks, pastries, and many foods such as gelatin desserts (Furia, 1980; Yentür et al., 1996).

Color is one of the first characteristics of food that attract people. A conventional color is desired in foods to be consumed. There is more or less loss of color when processing raw materials. Colorants are substances added in food production to correct changes in a color loss that occur during processing or at the end of the process, that is, to correct the color of food or to color food. Color substances are also important for creating a standard color in the product technologically (Anonymous, 2002; Batu and Molla, 2008).

Color is the first sensory parameter about food quality and taste. In this sense, synthetic dyes constitute an important class of food additives. It has been known for years that the use of dyes as food additives makes food ingredients more aesthetically and psychologically attractive. Besides, dyes are widely used to provide the desired color in foodstuffs that lose their natural color during production and storage (Altınöz and Toptan, 2003; Tripathi et al., 2007; Yentür et al., 2009).

The colorants are used to regain the natural color lost during processing and storage, to strengthen the weak color, to color the actually colorless food, and to gain consumer appreciation by hiding low quality (Topsoy et al., 1991).

As the additives are chemicals, their excess is harmful to health. Adding these colorants more than the allowed amounts may increase health risks (Batu and Molla, 2008).

According to the way they are obtained, the colorants are divided into two as natural and artificial colorants. Natural colorants are obtained from microbial, vegetable, animal, and mineral sources. The color stability of natural colorants is very low against physical and chemical effects. The majority of natural colorants are low in water solubility. Anatto, anthocyanin, canthaxanthin, plain caramel, carotenes and chlorophylls are examples of natural colorants. The substances that are not found in nature due to their chemical structure and obtained by chemical synthesis are artificial colorants. Artificial colorants are more preferred in the food industry in terms of their physicochemical properties. Artificial colorants are easily soluble in water and oil (Altuğ, 2009).

In recent years, synthetic and natural colorants are used in some foods to eliminate the discoloration applied to food processing techniques and related color disorders. Moreover, color materials are used to provide homogeneous color distribution in the product, to make its appearance attractive and to color the food with new formulations (Saldamlı and Uygun, 2004).

Turmeric (Curcuma longa L.), One of the Spices Used in Foods

People have used spices and aromatic herbs not only to add flavor and smell to food, but also to mask the impaired taste and smell of spoiled meat, to obtain body odors, to treat wounds, to have a clear mind. In addition to using spices for color (turmeric, saffron, paprika, red pepper), fragrance (clove, cinnamon, cumin, black pepper, rosemary, sage), they are also used in some cases to extend the shelf life of foods with their antioxidant and antimicrobial effects (Wilson, 1993).

International Organization for Standardization (ISO) defines spices and condiments as natural herbal products or mixtures used to add color and fragrance to foods (Abbas and Halkman, 2003).

It is known that most of the spices used in foods are contaminated with varying degrees of bacteria, yeast and mold. Since the plants from which spices are obtained are in contact with the soil and water, which are the source of many bacteria and fungi, they are naturally contaminated with these microorganisms (İnal, 1969).

Another reason for contamination is that many spices are grown and harvested in regions where hygiene conditions are not sufficient. Spices are usually laid and let to in areas such as fields and stream beds that are exposed to high levels of contamination and dried. Additionally, growing spices in hot and humid areas also increase the risk of mold and bacteria contamination (Tainter, 1992).

If contamination in the contaminated spice is not eliminated, the deterioration caused by the lack of sanitation in processed foods causes food poisoning and foodborne diseases (Hayashi et al., 1994).

Spices are usually cooked with food, but microorganisms in the form of spores remain alive during the cooking process, causing a proliferation in products stored in improper conditions during storage and distribution. The microflora in the spice shortens the shelf life of the products, moreover, it causes spoilage and foodborne diseases. These bacteria generally cause spoilage in products such as pickles, salami, sausage and canned food (Abbas and Halkman, 2003).

The place of turmeric (*Curcuma longa L.*) in the systematic is shown in Table 1 (Aggarwal et al., 2005).

Table 1. The place of Turmeric (*Curcuma longa* L.) in the systematic

Systematic Classification	Nomenclature
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaccae
Genus	*Curcuma*
Species	*Curcuma longa* L.

Turmeric (*Curcuma longa* L., Zingiberaceae) is obtained from the root of the Curcuma longa plant, a fiber plant from the ginger family. It is a plant with polyphenolic properties. Curcumin (diferuloyl methane; 1,7-bis- (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione) is generally known in the literature as turmeric (*Curcuma longa*) (Aggarwal et al., 2005).

The Latin name of turmeric is *Curcuma longa*, in English, Indian Saffron (turmeric) and in Persian "zerd-çubi". Other names are saffron root, yellow dye, curcuama domestica and

Indian saffron. Turmeric is a fragrant, perennial herbaceous plant with fleshy rhizomes, large and pointed leaves, yellow flowers (Mert et al., 1992). The plant can grow up to 1-2 meters in height. It has long dotted leaves and funnel-shaped flowers. The main rhizomes of the plant under the ground (finger-shaped roots) are egg-or pear-shaped. Its side rhizomes are finger-shaped. The upper side of the rhizomes is yellowish and the inner side is yellow. It has a bitter taste. Turmeric is often used as a coloring agent in foods. It also contains tetrahydrokurcumin, an odorless and heat-resistant antioxidant compound. It has a mild aroma and a sharp taste similar to ginger (Craig, 1999).

It is available on the market in the form of fingers (rhizome) and powder. The rhizomes (root tubers) of the plant are used. The active ingredients of these rhizomes are: Curcumin, eugenol, cinnamic acid, limonene, linalool, turmerone, vanillic acid, calcium, iron, manganese, potassium, phosphorus, zinc, vitamins B, B2, B3 and C. Curcumin (diferuloylmethane) is the most active ingredient of turmeric (Aggarwal et al., 2003).

It contains essential oil, resin, and curcumin. But its active ingredient is curcumin (contains 36%) and its color is bright yellow. Curcumin is isolated from turmeric, which has been used as a spice that gives yellow color in dishes for a long time, and is produced from the yellow powder of *Curcuma longa* which is a tropical plant. It is a polyphenolic herbal compound consisting mainly of small molecules. It has an antioxidant effect and no toxic effects (Ammon et al., 1991).

The part of the turmeric used is its powdered root (Figure 1). Turmeric powder contains curcumin in the ratio of approximately 1:30 to 1:100. The use of 1 dessert spoon of turmeric, with its top wiped, (an average of 3 grams) contains an average of 30-90 mg of curcumin. Turmeric has been found to have anti-inflammatory, anti-carcinogenic and antiatherogenic effects at 200 mg/day doses (about 2-4 dessert spoon of powder with its top wiped) (Ammon and Wahl, 1991; Aggarwal et al., 2003).

Figure 1. Plant and powder form of turmeric (*Curcuma longa*)

It is known that turmeric has been used worldwide for 4000 years. Its miraculous features have not been reflected in past literature, including Ottoman records. While tea, tincture and extract of almost all plants in nature can be made, this process has not been possible in turmeric. After a European scientist discovered curcumin, the active ingredient of turmeric in the early 1900s, it has been noticed in the last 20 years as a result of technological researches even though it was late and intensive clinical researches have been started (Aggarwal, 2013).

Although turmeric, which is one of the basic elements of curry powder, is used as a spice in the west, it has been used as a natural remedy in Asia for a long time. Turmeric has also been used to treat stomach and liver problems in Asia. Turmeric, which entered Turkish cuisine in the 16th century, was used as a natural dye to give yellow color to the saffron rice dessert called *zerde*, a name similar to turmeric in Turkish. Even though the first purpose in history was dyestuff for fabrics and yarns, its use for health purposes has largely surpassed its use for spice today (Karaman and Köseler, 2017).

It is also called "Indian saffron" since dyed substance resembling saffron is obtained from the stems of this plant. Turmeric is generally used in making *zerde* instead of saffron due to its high price. Dried turmeric is used as a spice and in making curry, it gives the curry yellow color. With its bright yellow color, turmeric has been used as paint, medicine and spice since the 600s BC. Marko Polo has described turmeric as "a vegetable that replaces saffron, but is not saffron". Its homeland is South Asia. It grows in the tropical regions of Asia, primarily Pakistan, India, China, Bangladesh and Indonesia. Indonesians used to use this spice to paint parts of their bodies during wedding ceremonies. It is cultured in tropical countries. Turmeric is also grown in Turkey. Although it is mostly used as a spice in the West, it has been used as a natural medicine for a long time in Asia. Turmeric, which we know as a household remedy and spice, actually plays an important role in the prevention and even treatment of many diseases. Its healing properties have been proven in the Indian and Chinese medical systems. It is widely used in the treatment of many diseases (Ammon and Wahl, 1991).

Turmeric is applied locally in skin diseases, insect bites and chickenpox in India (Nadkarni, 1976). It has been used as a supplement to alternative medicine in wound healing for many years (Sidhu et al., 2002). Apart from the purpose of coloring in food and clothing products, it was recommended because it shows bactericidal activity against *Escherichia coli* and *Staphylococcus aureus*, and this activity has been proven microbiologically (Shinyoung, 2005). It is still used in India as an antimicrobial agent (Negi et al., 1999).

The benefits of turmeric can be listed as follows:

It has an antioxidant effect. The antioxidant effect of curcumin is stronger than vitamins E and C. It has anti-inflammatory properties. Turmeric is beneficial for the liver. It strengthens the liver and helps remove toxins from the liver. It

is used in the treatment of respiratory infections. Curcumin protects against cancer and has an anti-proliferative feature. Studies have shown that it can be beneficial for skin, colon, esophagus and breast cancer. Another of the benefits of turmeric is its effect against the functional diseases of the gallbladder and biliary tract. Experimental studies have shown that turmeric has a cholesterol-lowering effect. In the study, it was also seen that it can prevent heart diseases. Using turmeric facilitates digestion and helps flatulence. It has been seen in a study that it can significantly reduce the harm caused by smoking. It has been used in the treatment of conditions such as calcification and dementia. It is externally useful for skin conditions (Aggarwal, 2013).

Turmeric is used in smoked foods, pickles, and some cakes. It is used in some dishes, the mixture of curry, mustard, sauces for chicken meat, in some desserts, especially *zerde*, a dessert served at weddings in Anatolia, and gives it its yellow color. It is also used in seafood, fish soup, egg dishes, soups, rice, cold cuts, and various vegetable dishes. It is used especially in Indian and South Asian cuisine. On the other hand, turmeric is used for dyeing silk fabrics and thin leathers and as a colorant in henna. Moreover, turmeric paper was used instead of litmus paper in the past. The parts of turmeric used are their fruits. To use it as a spice, the turmeric plant must be boiled in water after being cleaned, or dried after steaming, and the dark yellow root stems must be ground. After powderize the dried fruits, it can be used as a spice or turmeric tea, which can be prepared by boiling in water. It should be stored in cool, dry, and dark places (Özer, 2010).

The Effects of Turmeric on Health

Turmeric has been shown to have strong antioxidant and anti-inflammatory effects. However, the use of doses of turmeric to provide the same therapeutic effect for this effect should be accurate daily, it is not advisable to use a high dose in once. Turmeric is generally a reliable food material as long as there is not much consumption in healthy individuals and there are no side effects (Karaman and Köseler, 2017).

Even if all the nutrients and substances in nature are beneficial for human health, they can turn into a harmful substance when consumed too much. There is an amount that each substance affects and this amount should not be exceeded. Studies have shown that the tolerability and safety of polyphenol is not toxic even at doses up to 8 grams per day, but a clear dose and active ingredient to illuminate this subject have not been determined. Nutritional Content of Turmeric is shown in Table 2. (Karaman and Köseler, 2017).

Oxygen Radical Absorption Capacity (ORAC) value, which shows the antioxidant capacity of foods, is 44.776 in turmeric. With this value, turmeric ranks first in the list of spices with the highest antioxidant capacity. ORAC is a scale that is called free radicals in our body that indicates the absorption of substances that cause many diseases, especially cancer, that is, it is used for nutrients that indicates the absorption value. A high ORAC value indicates that food is more antioxidant, protects against cancer and delays aging. ORAC is a scale that indicates the absorption value of the substances called free radicals in our body that cause many diseases, especially cancer, and is used for foods. A high ORAC value indicates that food is more antioxidant, protects against cancer and delays aging (Karaman and Köseler, 2017).

Turmeric can be used in the form of capsules, liquid essence and tincture, containing powder for adults. The cut root of turmeric can be used 1.5-3 g daily, dried powdered turmeric root 1-3 g daily can be used. Also, a standard powder (curcumin), 400-600 mg, can be taken 3 times a day. Liquid extract (1:1) is recommended to consume 30 to 90 drops per day, 1 dose in the morning and 1 dose in the evening (1 part is 5ml). Fresh turmeric can be stored for several weeks in a cool dry place. When olive oil is consumed together with black pepper and chili pepper, its absorption is much higher. It can be preferred in Indian dishes as a sauce, stews, Turkish dishes, and in all cuisines such as noodles and pasta. It can be used especially in salads, rice and meat dishes to increase the flavor of the food and the consistency of the sauce, to give the food a yellow color. It can be added to the dishes by mixing with honey. It is also added to fish soup, cold cuts and various vegetable dishes as a seasoning. It is used in the famous "paella" dish of the Spanish, and in the "curry" sauce of the Indians. Turmeric can also be used as tea. Its use as tea is popular in Asian countries, especially in Japan (Aggarwal, 2013).

Curcuma, the active ingredient of turmeric, prevents cancer and Alzheimer's, protects from heavy metals, heals the liver, and is also a powerful antioxidant. But for this effect of turmeric, the doses that will provide the same therapeutic effect in daily times should be correct. Curcuma is great when it is pure and in small quantities, but its taste is bitter and less enjoyable when taken in larger doses. It is not recommended to use high doses at once (Änderung, 2017).

Curcumin is absorbed very little in the human body when taken alone and is rapidly excreted from the intestines. For this reason, many studies have been conducted on the substances that will increase the bioavailability of curcumin, and it has been suggested that the piperine contained in black pepper can increase the absorption of curcumin by 2000% (20 times). Due to the rapid metabolism of turmeric in the liver and intestinal walls, its bioavailability is tried to be improved through piperine, which increases the absorption of all nutrients. Very

Table 2. Nutritional content of turmeric

Nutritional Ingredient	Spice Powder (100gr)	Vitamins	Content	Minerals	Content
Water	12.85gr	Vitamin C, total ascorbic acid	0.70 mg	Calcium, Ca	168 mg
Energy	312.00 kcal	B^1, thiamine	0.058 mg	Iron, Fe	55.00 mg
Protein	9.68 gr	B^2, riboflavin	0.150 mg	Magnesium, Mg	208 mg
Total fat	3.25 gr	B^3, niacin	1.360 mg	Phosphor, P	299 mg
Carbohydrate	67.14 gr	B^6, pyridoxine	0.107 mg	Potassium, K	2080 mg
Total fiber	3.25 gr	B^9, folic acid	0.00	Sodium, Na	27 mg
Total sugar	3.21 gr	Folate (total)	20 qg	Zinc, Zn	4.5 mg
Sucrose	2.38 gr	B^{12}	0.00	Copper, Cu	1.300 mg
Dextrose	0.38 gr	Vitamin D	0.00	Manganese, Mn	19.800 mg
Fructose	0.45 gr	Vitamin A, IU	0.00	Selenium, Se	6.2ug
FATS		Vitamin E, (α tocopherol)	4.43 mg		
Total fatty acid saturated	1.838 gr	Vitamin K	13.40 qg		
Total fatty acid monounsaturated	0.449 gr	Others			
Total fatty acid polyunsaturated	0.756 gr	Caffeine	0.00		
Total fatty acid trans fatty	0.58 gr				
Cholesterol	0.00				

little of curcumin is absorbed if such an improvement is not used, and even doses up to 4,000 mg can be completely inactive. Scientific researches show that the active ingredient of turmeric, curcumin, is a difficult substance to absorb, and accordingly, it has poor bioavailability as well. Scientific researches also show that piper, the active ingredient of black pepper, increases the absorption of substances that are difficult to absorb. Taking these two substances together with an oil rich in unsaturated fatty acids further strengthens this benefit (Rajinder et al., 2002).

With the widespread use of natural additives in the food industry, the interest in natural antioxidants in plants around the world is increasing day by day. Our country has an important potential in terms of production and export of some herbs and spices. With the widespread use of natural additives in the food industry, the interest in natural antioxidants in plants around the world is increasing day by day. Our country has an important potential in terms of production and export of some herbs and spices. Knowing the antioxidant and antimicrobial properties of these herbs and spices will make an important contribution in extending the shelf life of food products (Çoban and Patır, 2010).

Lipid oxidation is an important problem that limits the shelf life of foods and causes quality loss. Synthetic antioxidants have been used for many years to control lipid oxidation. However, many studies have found that the use of these substances has negative effects on human health. Hence, consumer preferences have shifted to natural products, and the use of herbs and spices as antioxidants has come to the fore. Foods rich in polyunsaturated fatty acids are exposed to oxidative deterioration. Oxidative deterioration is one of the important factors that limit the shelf life of food products and cause quality loss. In industrial processes, synthetic antioxidants are mainly used to prolong the preservation of nutrients. However, many researchers point out that some synthetic antioxidants used in food processing for a long time have carcinogenic and teratogenic effects in the living organism. Consumers generally prefer natural antioxidants over synthetic ones. Thus, spices and natural aromatic herbs that are used as additives to increase the properties of nutrients such as smell and taste have become increasingly important. The antioxidant effect of phenolic compounds found in the structures of these plants derives from their properties such as cleaning free radicals, compounding with metal ions and preventing the formation of single oxygen. Some of these herbs and spices have been proven to have more antioxidant capacities than synthetic antioxidants. Because of the flavors and aromas peculiar to them and antimicrobial and antioxidant properties, herbs and spices that have a wider bioactivity profile are natural antioxidant substances that can be used as an alternative in the food industry. Prevention of lipid oxidation in foods with such natural substances is very important for the producer and consumer. In this context, turmeric has a very important place among natural antioxidants with its strong antioxidant effect (Altun et al., 2004).

Turmeric is used for dyeing silk fabrics and thin leathers and as a colorant in henna. Besides, turmeric paper used to be used instead of litmus paper in the past. It is added as a seasoning to fish soup, rice, sauces, chicken bouillon, pickled cheese, cold cuts and various vegetable dishes. It is used in the

famous "paella" dish of the Spanish, and the "curry" sauce of the Indians. It is reported that turmeric, which has great importance in Indian medicine, is used in the treatment of cold, cough, liver disorders, rheumatism, sinusitis, and anorexia. It is also used in Ayurveda for blood purifiers, tonic, and skin diseases (Çoban and Patır, 2010).

Turmeric, which is generally used as a coloring agent in foods, contains tetrahydro-curcumin as an active ingredient, which is an odorless, heat-resistant, antioxidant compound. The molecular formula of curcumin, which melts at 184 degrees, is $C_2H_2O_6$ and makes up 3-5% of turmeric. It is soluble in acetone and ethanol, but insoluble in water. The chemical structure of the curcuminoids is shown in Figure 2. (Çoban and Patır, 2010).

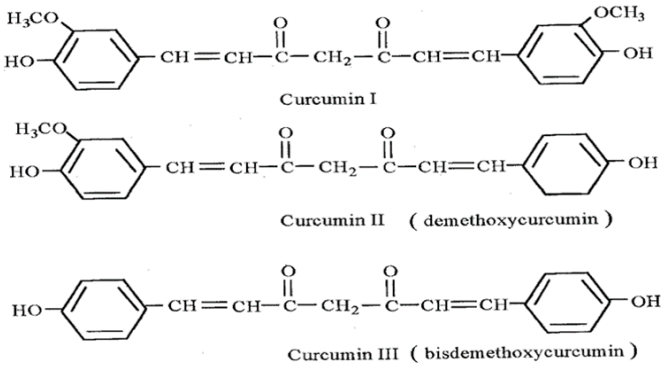

Figure 2. The chemical structure of the curcuminoids (Jayaprakasha et al., 2005)

Studies on Turmeric

In study, hot smoked needlefish (*Belone belone euxini*, Günther 1866) marinated in brine which was added turmeric (*Curcuma longa* L.) and sunset yellow FCF, storage in the refrigerator conditions. The determination of these product shelf lives was aimed at using freshness control methods like chemical, microbiological and sensorial. The amount of Total volatile basic nitrogen (TVB-N) and Thiobarbituric Acid (TBA), the number of Total mesophilic aerobic bacteria, Total psychrophilic bacteria, Total yeasts and molds increased during the cold storage. Effects of turmeric and sunset yellow FCF uses on the sensory freshness control methods like appearance, smell, taste, texture, and saltiness scores were significantly ($p<0.05$). According to the sensory freshness control methods, the shelf life of hot smoked Needlefish kept in the refrigerator has been determined as 17 days. It has been observed that the use of turmeric instead of Sunset yellow FCF can provide positive changes in the appearance, taste, and texture of the product that may appeal to the consumer. It has also been found to have a positive effect on the increase in the consumption of smoked garfish. Thus, the use of turmeric, which is a natural colorant where more positive results are obtained, instead of artificial colorants that may be harmful to human health, has been suggested in terms of both making the color that is impulsive to the consumer in the product attractive and increasing the consumption of the smoked product (Özer, 2010).

In a study conducted in Scotland, the content of mold and yeast in the ground spice mixture was analyzed and no mold was found only in cloves. It has been found that the least mold in the spice examined was turmeric with 50 cfu/g and the highest was black pepper with $6.4x10^5$ cfu/g (Flannigan and Hui, 1976).

In another study conducted in India, it has been observed that the dominant microflora in turmeric, red pepper, and ginger samples consist especially of Bacillus species such as *B. cereus, B. subtilis, B. polymyxa* and *B. coagulans* (Seenappa and Kempton, 1981).

Researchers have done microbiological analyzes in commonly used spices such as packaged and unpackaged turmeric, red pepper, black pepper and coriander. While the total number of bacteria in the packaged samples is 1.3×10^5 cfu/g on average, they have not detected a significant change in the total number of bacteria in the packaged and unpackaged samples. In the vast majority of the samples analyzed, they have found coliforms in numbers ranging from 0>1100/g. *Aspergillus niger* and *Aspergillus flavus* were identified as the dominant molds isolated from both groups of samples and no yeast has been found in any sample (Shamshad et al., 1985).

Researchers have investigated the development of *Aspergillus parasiticus* in black pepper, turmeric, red pepper, dried ginger and cardamom, which are autoclaved whole, ground, surface sterilized, and forming aflatoxin. They have found that black pepper and turmeric is an insufficient substrate for fungal growth and aflatoxin production (Madhyastha and Bhat, 1985).

In another study, the researchers have investigated the distribution of microorganisms in 15 samples of selected spices. They have reported that the total number of bacteria in turmeric, black pepper, white pepper, rosemary and basil is between 3×10^3-5×10^7 cfu/g. They have also stated that coliforms are determined between 2×10^2-2×10^6 cfu/g in 8 samples and *Bacillus pumilus* and *Bacillus subtilis* are the aerobic spore-forming bacteria (Muhamad et al., 1986).

Turmeric, red pepper, "Garammasala", ground black pepper, "Tandori masala", coriander, ginger, mustard, garlic, paprika and curry have been checked for the presence of bacteria that cause food poisoning. The results have shown that the total bacterial load in all spices, except garlic and mustard, is more than 5×10^6 cfu/g at 37°C. The dominant microflora *Bacillus spp*. consists of *B. subtilis* and *B. lichenif ormis*.

Escherichia coli, *Salmonella spp*., *Clostridium perfringens*, *Bacillus cereus* and *Staphylococcus aureus* could not be isolated (Chattopadhyay et al., 1986; Abbas and Halkman, 2003).

It has been observed that the ground and prepackaged turmeric, black pepper, red pepper and coriander spices are heavily contaminated with bacteria and molds, and the total number of bacteria is 10^5-10^7 cfu/g, the total number of molds is 10^2-10^6 cfu/g (Munasiri et al.,1987).

Irradiated (10 kGy) and non-irradiated, pre-packaged whole and ground black pepper, red pepper and turmeric have been analyzed in 6 different laboratories in India to determine their microbiological quality. In 3 of 6 laboratories, it has been stated that no colony development detected while in the other 3 laboratories at a level of 0-90 cfu/g counting has been noted in irradiated samples. All of the laboratories have specified that no *E. coli* and *B. cereus* found in irradiated spice (Sharma et al., 1989).

In the study, in which the effect of irradiation at 0.5 and 10 kGy dose on the color value of dry turmeric and 3 red pepper samples are investigated, it has been observed that gamma radiation does not cause any change in the color values of turmeric and red pepper when stored for up to 1 year in room conditions (Chatterjee et al., 1998).

The researchers state that about curcumin, which is the main component of turmeric, has therapeutic properties in many chronic diseases in which inflammation plays a major role, as well as its preventive and curative effects in various types of cancer. In addition to neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's diseases, they have stated that curcumin has a protective effect in Crohn's Disease, Helicobacter pylori infection, after kidney transplantation from a cadaver, gallbladder function and cognitive disorders such as cognitive performance, learning and verbal memory. It is noted that curcumin has a wide range of anti-inflammatory and anticancer properties, and extensive controlled studies are needed to better understand the health effects of curcumin. They have reported that turmeric has become a promising natural remedy in diseases with its reliability, low cost and proven efficacy (Delikanlı Akbay and Pekcan, 2016).

In the study, the combined effect of turmeric powder and salt (dry) along with sun-drying process on physico-chemical (physical characteristics, proximate and chemical analysis), mineral and bacteriological quality of three freshwater fish products (shol, taki and tengra) has been identified. Sensory characteristics, moisture, protein, fat, ash, salt, TVB-N, FFA, pH, some mineral contents (Ca, Mg, Fe, Cu, Zn, Mn) and bacterial load (SPC and HBC) were analyzed of freshly processed turmeric and salt-treated sun-dried fishes using standard methods of analyses. The lowest moisture content of these three dried fish-products indicated that it was more resistant to enzymatic and microbial activities (Farzana et al., 2016).

The study was carried out on raw meat samples derived from pigs fed with a control diet and a diet supplemented with daily 4.5 g of turmeric powder per pig. After slaughter raw meat was stored for 7 days at 4°C. At day 0 and day 7 samples were cooked in a preheated oven at 163°C to the internal temperature of 71°C. Color parameters, Warner Bratzler shear force, TBARS and antioxidant capacity (ABTS, DPPH and FRAP) were determined at day 0 and day 7. Dietary turmeric powder induced an increase in cooked meat of L* value ($P<0.001$) and reductions in a*, b* indexes and in C* value ($P<0.01$, $P<0.001$ and $P<0.001$, respectively). Color modifications in cooked meat were correlated with color parameters of raw samples. The *Curcuma longa* powder dietary supplementation did not affect lipid oxidation, Warner Bratzler shear force and antioxidant capacity of cooked meat ($P>0.05$) (Mancini et al., 2017).

In this study was to evaluate the effects of turmeric powder and ascorbic acid on lipid oxidation and antioxidant capacity in cooked rabbit burgers. The burgers were derived from 3 different formulations (C, control, with no additives; Tu with 3.5% of turmeric powder and AA with 0.1% of ascorbic acid) and were stored at 4°C for 0 and 7 d and cooked. The lipid oxidation (thiobarbituric acid reactive substances [TBARS]) and antioxidant capacity (2,2-azinobis-[3 ethylbenzothiazoline-6-sulfonic acid] {ABTS}, 1,1-diphenyl-2-pircydrazyl [DPPH] and ferric reducing ability [FRAP]) were evaluated. A significant interaction between storage time and formulation ($P<0.001$) was observed for DPPH, FRAP and TBARS in cooked burgers. At day 0 and day 7, the DPPH value was higher in Tu and AA compared to C burgers. At day 0, C showed a lower level of FRAP than the Tu and AA burgers. At day 7, the FRAP values tended to decrease but remained significantly higher in Tu and AA compared to C burgers. Lipid oxidation at day 0 in Tu and AA showed lower TBARS values compared to C burgers. The addition of 3.5% turmeric powder in rabbit burgers exerts an antioxidant effect during storage and it seems more effective in controlling lipid oxidation than ascorbic acid after cooking (Mancini et al., 2016).

In the study, 180 Nile tilapia fish were used in 3 months growth trial to study the effect of turmeric on growing tilapia. Fish were divided into three treatment groups. The first group T1 was given the basal diet without any supplementation of turmeric and served as the control group. The second group T2 was given a diet supplemented by 0.25% turmeric powder. The third groupT3 was given a diet supplemented by 0.50% turmeric powder. At the end of the growth trial, fish were

challenged with pathogenic *Pseudomonas fluorescence.* Turmeric supplementation non-significantly improved growth performance. There was a trend of higher values with increasing the turmeric supplementation level, and significant improvement in feed consumption in T3 compared to T1 and T2. Fish body composition was affected by turmeric supplementation. Crude protein content was significantly increased in T3 compared to T1. Ether extract content was significantly decreased with increasing the turmeric supplementation level as T1 was the highest in ether extract content and T3 was the lowest, this was significantly reflected on the gross energy (GE) content of the fish. The clinical signs in the challenged fishes were observed on the second-day post-injection. Fish showed loss of balance, excessive mucus secretions on skin and gills, ascites with slightly protruded reddish vent and hemorrhages all over the body surface, frayed and torn tail and fins, with no mortalities in the 0.50% turmeric supplemented group. We concluded that 0.50% turmeric supplementation may improve growth performance and significantly protect fish against *P. fluorescens* (Manal et al., 2014).

Turmeric increases the duration of storage by preventing peroxide formation in foods. Turmeric has been reported to be more effective than vitamin E in preventing lipid oxidation. It is determined that the components isolated from *Curcuma longa* have a strong antioxidant effect and are very important on lipid oxidation (Jayaprakasha et al., 2005).

In a study, 400 ppm turmeric extract is added to chicken mince and its antioxidant properties are investigated. The results of the research have revealed that turmeric extract is significantly effective when compared with the control group. It is stated that turmeric is caused by the phenolic components contained in its antioxidant properties (Sharma, 1976).

In another study, antioxidant properties of curcuminoids have been investigated and it is determined that the antioxidant capacity of these extracts is equivalent to ascorbic acid. It is noted that compared to 100 ppm BHT, curcumin has higher antioxidant activity. Turmeric roots have been reported to have aromatic and antiseptic properties (Khana, 1999).

The antioxidant properties of curcumin, which is a large proportion of turmeric and is a phenolic component, have been investigated and it has been determined that curcumin is an antioxidant that can be used safely in the food industry (Ak and Gülçin, 2008).

In this study is to evaluate the effects of turmeric powder (*Curcuma longa*) as a dietary supplement for the ornamental fish Green Terror (*Andinocara rivulatus*) on growth and feed performance, survival rate, and hematologic parameters. In this regard, 144 specimens with an average weight of 1.53±0.22 (g) were obtained and the hypotheses were studied with four iso-caloric and iso-nitrogenous diets containing 0.1, 0.2 and 0.3 percent of turmeric powder, formulated with Win feed 2.8 software. Along the period of 100 days, the fish were biometry every 20 days, and at the end of the trial, a blood examination test was performed. Results showed that the fish fed with diet contains 0.3% turmeric powder (T3) had better growth performance, FCR, condition factor and survival rate specification, but no significant differences observed between the treated and control groups (p>0.05). RBC, PCV, hemoglobin, MCHC were increased not significantly (p>0.05), whereas WBC increased significantly in T3 compared to the other groups (p<0.05). MCH and MCV were decreased non-significant in groups fed by supplemented diets compared to the control group (p>0.05). Applying turmeric powder at the level of 0.3 percent of the basal diet could not alter the growth indices significantly but could alter the hematological parameters with emphasis on WBC (Mooraki et al., 2019).

The effect of Turmeric has been evaluated on the Immune stimulatory response of fish *Labeo rohita* as an effective compound (Behera et al., 2011). In another study reported that curcumin had a protective effect on Bloch tissue and increase the growth performance (Manju et al., 2011). In this regard, the consumption of turmeric by fantail guppy (*Poecilia reticulate*) caused the reduction of FCR and improvement of growth performance (Mukherjee et al., 2009). Sand Goby (*Oxyeleotris marmoratus*) also showed a positive reaction to the consumption of turmeric powder by an increment in amylase, lipase, trypsin and chemotrypsin secretions (Rojtinnakon et al., 2012). The effectiveness of turmeric powder on Immunity response of *Labeo rohita* to *Aeromonas hydrophila* and white shrimp (*Litopenaeus vannamei boone)* has been evaluated by Sahu et al. (2008), Vanichkul et al. (2010) and Lawhavinit et al. (2011), respectively. Moreover, it has been evaluated the effect of turmeric on hematological and immunological parameters of *Mugil cephalus* vaccinated with *Aeromans hydrophila* bacterin (El-Bahr and Saad, 2008). This synergistic effect was also investigated on Japanese flounder (Ji et al., 2007) and Nile Tilapia fingerlings (El-Maksoud et al., 2002) fed by a diet supplemented with turmeric.

In another study, the effect of turmeric Curcuma longa on *Cyprinus carpio* was studied. Fish were divided into four groups being fed for 45 days with 0.3, 0.6 and 0.9gm with add-on commercial diet as the control. After the Groups fed with copepods mediate treated with *C. carpio* using by the different concentration at 0.3gm, 0.6gm and 0.9gm when compare to the high dose, were differential leukocyte counts in *C. carpio* Neutrophils, Lymphocytes, Monocyctes, Esnophils and

basnophils analysis was showed a highly significant difference compared to controls (Palanisamy et al., 2016).

Conclusion

Seafood is among the most valuable nutrients in terms of the nutritional components it contains. Preserving food is a necessity for the continuity of life. Along with the developing technology, it should be aimed to extend the shelf life of new products obtained with the developments in food processing methods and to preserve the nutritional content of the product, to add flavor to the product and most importantly, to obtain a healthy product besides preserving their quality. At the stage of obtaining healthy products, consumers started to prefer natural products and the use of natural additives in the food industry has become widespread. Turmeric is a plant with polyphenolic properties, Curcumin is the most active ingredient. Turmeric has been determined to have antioxidant, anti-inflammatory, anticancer and antiatherogenic effects. Turmeric is used in fish soup, rice, sauces, pickles, pastas, salads, cold cuts and various vegetable dishes. When it is consumed with olive oil, black pepper and chili pepper, its absorption is much higher. In addition to being the most valuable nutrient with the nutritional components it contains, seafood can deteriorate much faster. In this context, turmeric has a very important role in extending the shelf life of seafood products by preserving the nutritional value with its powerful antioxidant and antimicrobial effect, and also in obtaining healthy products with its natural coloring and flavoring aspect.

Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Abbas, N. M. S. & Halkman, K. (2003). The effect of irradiation on spice microflora. *Orlab On-Line Journal of Microbiology*, ***1***(3): 43-65.

Aggarwal, B. B., Kumar, A. & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Research*, ***23***(1A): 363–398.

Aggarwal, B. B., Kumar, A., Aggarwal, M. S. & Shishodia, S. (2005). Curcumin derived from turmeric (*Curcuma longa*): A spice for all seasons (pp. 349-387). In: Preuss, H. (Ed.), *Phytopharmaceuticals in Cancer Chemoprevention*. Boca Raton: CRC Press.

Aggarwal, B. B. (2013). Curcumin-free turmeric exhibits anti-inflammatory and anticancer activities: Identification of novel components of turmeric. *Molecular Nutrition & Food Research*, *57*(9): 1529-1542. https://doi.org/10.1002/mnfr.201200838

Ak, T. & Gülçin, İ. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, ***174***: 27–37. https://doi.org/10.1016/j.cbi.2008.05.003

Akbay, G. D. & Pekcan, A. G. (2016). Turmeric: evaluation in terms of health and nutrition, *Journal of Nutrition Diet*, ***44***(1): 68-72.

Alçiçek, Z. & Bekcan, S. (2009). The effects of different liquid incense on the sensory quality of vacuum packed rainbow trout (*Oncorhynchus mykiss,* Walbaum, 1792) fillets stored in refrigerator conditions. *Journal of Agricultural Engineering*, ***353***: 18-21.

Altınöz, S. & Toptan, S. (2003). Simultaneous determination of Indigotin and Ponceau-4R in food samples by using Vierordt's method, ratio spectra first order derivative and derivative UV spectrophotometry. *Journal of Food Composition and Analysis,* ***16***: 517–530.

Altuğ, T. (2009). Food Additives. Ege University Press Faculty of Engineering Food Engineering Department, İzmir, 268 p.

Altun, T., Usta, F., Çelik, F. & Danabaş, D. (2004). The benefits of seafood for human health. *National Water Days 2004*, İzmir, Turkey. pp. 11-18.

Ammon, H. P. & Wahl, M. A. (1991). Pharmacology of *Curcuma longa*. *Planta Medica,* ***57***(1): 1–7.

Änderung, L. (2017). Curcuma–Sechs Tipps zur richtigen Anwendung. Retrieved on July 22, 2020 from https://www.zentrum-der-gesundheit.de/curcuma-anwendung-ia.html

Angiş, P. & Oğuzhan, P. (2008). Additives used in seafood. *Turkey 10. Food Congress.* Erzurum, Turkey. pp. 603-606.

Anonymous. (2001). Fisheries and aquaculture industry specialization commission report. VIII. Five-year development plan. Ankara, Turkey. 142 p.

Anonymous. (2002). Communiqué on colorings used in foods. Official Newspaper: 25.08.2002- 24857. Communiqué No: 2002/55.

Arunkumar, P., Ramasubramanian, V. & Munirasu, S. (2016). Effect of curcuma longa enriched mesocylops thermocyclopoides on fresh water fish, *Cyprinus carpio,*

International Journal of Research and Development in Pharmacy and Life Sciences, ***6***(1): 2484-2492.

Atar, H. H. & Alçiçek, Z. (2009). Seafood Consumption and Health. *TAF Preventive Medicine Bulletin*, ***8***(2): 173-176.

Barazi, A. Ö. & Erkmen, O. (2010). *Food preservation with modified atmosphere methods* (pp 266-287). In Erkmen, O. (Ed.), Food Microbiology. Ankara, Turkey: Eflatun Publishing.

Batu, A. & Molla, E. (2008). Additives used in Turkish delight production. *Journal of Food Technologies Electronics*, (1): 33-36 Publishing, Ankara, 270 p.

Behera, T., Swain, P., Sahoo, S. K., Mohapatra, D. & Das, B. K. (2011). Immuno-stimulatory effects of Curcumin in fish *Labero rohita* (H*). Indian Journal of Natural Products and Resources*, ***2***(2): 184-188.

Çaklı, Ş. (2007). *Seafood processing technology* (*Basic subjects in seafood processing technology*). Ege University Faculty of Fisheries Publishing, İzmir, Turkey. 696 p.

Çalışır, E. Z. & Çalışkan, D. (2003). Food additives and their effects on human health. *Journal of Ankara Faculty of Pharmacy*, ***32***(3): 193-206.

Chatterjee, S., Padwal-Desai, S. R. & Thomas, P. (1998). Effect of γ-irradiation on the colour power of turmeric (*Curcuma longa*) and red chillies (*Capsicum annum*) during storage. *Food Research International,* ***31***(9): 625-628.

Chattopadhyay, B. & Teli, J. C. (1986). Bacterial contamination of spices. *Environmental Health*, ***94***(4): 106-107.

Çoban, Ö. E. & Patır, B. (2010). Use of cereals and spices with antioxidant effects in foods. *Journal of Food Technologies Electronics*, ***5***(2): 7-19.

Craig, W. J. (1999). Health-promoting properties of common herbs. *The American Journal of Clinical Nutrition*, ***70***: 4919.

Delikanlı Akbay G. & Pekcan, A. G. (2016). Turmeric: Evaluation in terms of health and nutrition. *Beslenme ve Diyet Dergisi*, ***44***(1): 68-72.

El-Bahr, S. M. &. Saad, T. T. (2008). Effect of black cumin seeds (*Nigella sativa*) and/or Turmeric (*Curcumin*) on hematological, biochemical and immunological parameters of *Mugil cephalus* fish vaccinated with *Aeromonus hydrophila* bacterin. *Proceedings of the 13 Scientific Congress.* pp. 365-388.

El-Maksoud, A. M. S., Hassouna M. M. E., Said, A. M. A. & El-Gendy, H. (2002). The response of Nile tilapia fingerlings to animal protein free diets supplemented with some free amino acids and some medicinal plants. *Proceedings of the 1st Scientific Conference of the Egyptian Aquaculture Society,* El-Arrish, North Sinai, Egypt. pp. XX-XX

Farid, F. B., Latifa, G. A., Chakraborty, S. C., Nahid M. N. & Begum, M. (2016). Combine effect of *Curcuma longa* (turmeric) powder and dry-salt with sun-drying in quality changes of three freshwater fish species of Meghna River, *European Journal of Biotechnology and Bioscience*, ***4***(10): 42-47.

Flannigan, B. & Hui, S. C. (1976). The occurrence of aflatoxin-producing strains of *Aspergillus flavus* in the mould floras of ground spice. *Journal of Applied Bacteriology*, ***41***: 411-418.

Furia, E. T. (1980). Handbook of Food Additives. (2nd ed.) C.R.C. Press Inc., London, pp. 339-382.

Göğüş, A. K. & Kolsarıcı, N. (1992). *Seafood processing technology*. Ankara University Faculty of Agriculture Publications No: 1243. Ankara, Turkey. 261 p.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B. & Givskov, M. (2002). Food spoilage–Interactions between food spoilage bacteria. *International Journal of Food Science and Technology*, ***78***: 79-97.

Gülyavuz, H. & Ünlüsayın, M. (1999). Seafood processing technology. Süleyman Demirel University Eğirdir Fisheries Faculty. Ankara, Turkey: Sahin Printing Press. 366 p.

Hayashi, T., Todoriki, S. & Kohyama, K. (1994). Irradiation effects on pepper starch viscosity. *Journal of Food Science*, ***59***(1): 118-120.

İnal, T. (1969). Spice sterilization and its importance in food industry. *Turkish Journal of Veterinary Medicine,* ***35***(5-6): 296-301.

İnal, T. (1988). Fish storage methods, food hygiene. İ.U. Faculty of Veterinary Medicine Press, pp. 356- 442.

Jayaprakasha, G. K., Jagan, L. & Sakariah, K. K. (2005). Chemistry and biological activities of *C. longa. Trends in Food Science & Technology*, ***16***: 533–548.

Ji, S. C., Jeong, G. S., Gwang-Soon, I. M., Lee, S. W., Yoo, J. H. & Takii, K. (2007). Dietary medicinal herbs improve growth performance, fatty acid utilization and stress recovery of Japanese flounder. *Fisheries Science*, ***73***: 70-76.

Karaali, A. & Özçelik, B. (1993). Natural and synthetic paints as food additives. *Food Journal*, ***18***(6): 389-396.

Karaman, B. E. & Köseler, E. (2017). The relationship between turmeric and chronic diseases, *Başkent University Journal of Health Sciences Faculty*, ***2***(2): 96-112.

Khanna, N. M. (1999). Turmeric: Nature's precious gift. *Current Science*, ***76***: 1351-1356.

Koral, S. (2006). *Determination of quality changes of fresh and smoked mullet (Mugil soiuy, Basilewski, 1855) and bonito (Sarda sarda, Bloch, 1838) fish in room and refrigerator conditions*. MSc. Thesis, Karadeniz Technical University, Trabzon, Turkey. 67 p.

Lawhavinti, O. R., Sineharoenpokai, P. & Sunthornandh, P. (2011). Effect of ethanol turmeric (*Curcuma longa linn*) extract against shrimp pathogenic *vibrio spp*. on growth performance and immune status of white shrimp (*Litopenaeus vannamei*). *Kastesart Natural Science*, ***45***: 70-77.

Madhyastha, M. S. & Bhat, R. V. (1985). Evaluation of substrate potentiality and inhibitory effects to identify high-risk spices for aflatoxin contamination. *Journal of Food Science*, ***50***: 376-378.

Mahmoud, M. M. A., El-Lamie, M. M. M., Dessouki, A. A. & Yusuf, M. S. (2014). Effect of turmeric (*Curcuma longa*) supplementation on growth performance, feed utilization, and resistance of Nile tilapia (*Oreochromis niloticus*) to *Pseudomonas fluorescens* challenge. *Global Research Journal of Fishery Science and Aquaculture*, ***1***(12): 26-33.

Mancini, S., Paci, G. & Preziuso, G. (2017). Effect of dietary turmeric powder (*Curcuma longa* L.) on cooked pig meat quality. *International Food Research Journal*, ***24***(6): 2460-2465.

Mancini, S., Preziuso, G. & Paci, G. (2016). Effect of turmeric powder (*Curcuma longa* L.) and ascorbic acid on antioxidant capacity and oxidative status in rabbit burgers after cooking. *World Rabbit Science*, ***24***: 121-127.

Manju, M., Akbarsha, M. A. & Oommen, O. V. (2011). In vivo protective effect of dietary curcumin in fish *Anabas testudineus* (Bloch). *Fish Physiology Biochemistry,* ***38***(2): 309-318.

Mert, H. H., Doğan, Y. & Başlar, S. (1992). Some plants used in natural dye production. *Çevre Magazine*, ***5***: 14-17.

Mooraki, N., Batmany, Y., Zoriehzahra, S. J. & Kakoolaki, Sh. (2019). Evaluating the effect of using turmeric (*Curcuma longa*) on growth performance and hematological parameters of the ornamental fish, green terror (*Andinocara rivulatus*). *Journal of Survey in Fisheries Sciences*, ***5***(2): 37-47.

Muhamad, L. J., Ito, H., Watanabe, H. & Tamura, N. (1986). Distribution of microorganisms in spices and their decontamination by gamma-irradiation. *Agricultural and Biological Chemistry*, ***50***(2): 347-355.

Mukherjee, A., Mandal, B. & Banerjee, S. (2009). Turmeric as a carotenoid source on pigmentation and growth of fantail guppy (*Poecilia reticulata*). *Proceedings of the Zoological Society*, ***62***(2): 119-123.

Munasiri, M. A., Parte, M. N., Ghanekar, A. S., Sharma, A., Padwaldesai, S. R. & Nadkarni, G. B. (1987). Sterilization of ground prepacked Indian spices by gamma irradiation. *Journal of Food Science*, ***52***(3): 823-826. https://doi.org/10.1111/j.1365-2621.1987.tb06736.x

Nadkarni, K. M. (1976). *Curcuma longa* (pp. 414-416). In: Nadkarni, K. M. (Ed.), Indian Materia Medica. Bombay, India: Popular Prakashan Publishing Company.

Negi, P. S., Jayaprakasha, G. K., Jagan Mohan Rao, L. & Sakariah, K. K. (1999). Antibacterial activity of turmeric oil: A byproduct from curcumin manufacture. *Journal of Agricultural and Food Chemistry*, ***47***(10): 4297–4300.

Newsome, L. R. (1990). Natural and synthetic food colours (pp. 327-345). In: Branen, A. L., Davidson, M. P. (Eds.). Food additives. New York, USA: Marcel Deeeer Inc.

Özer, Ö. (2010). Determination of some quality criteria hot smoked zargana fish (*Belone Belone* euxini Günther, 1866) preserved in refrigerator conditions by using natural and artificial colors. MSc. Thesis, Sinop University, Sinop, Turkey. 108 p.

Rajinder, K., Bhardwaj, Hartmut, G., Laurent, B., Ulrich, K., Suresh, K. & Gupta, Martin, F. (2002). Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *Journal of Pharmacology and Experimental Therapeutics*, ***302***(2): 645-650. https://doi.org/10.1124/jpet.102.034728

Rojtinnakorn, J., Rittiplang, S., Tongsiri, S. & Chaibu, P. (2012). Turmeric extract inducing growth biomarker in Sand Goby (*Oxyeleotris marmoratus*). *Proceedings of the 2nd International Conference on Chemical, Biological and Environment Sciences* (ICCEBS'2012). pp. 41-43.

Sağlam, Ö. F. (2000). Turkish food legislation. (2nd ed.) Ankara, Turkey: Semih Offset.

Sahu, S., Das, B. K., Mishra, B. K., Pradhan, J., Samal, S. K. & Sarangi, N. (2008). Effect of dietary *Curcuma longa* on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*. *Aquaculture Research*, ***39***: 1720-1730.

Saldamlı, I. & Uygun, U. (2004). Food additives and cancer. Retrieved on July 22, 2020 from http://www.un.org.tr/who/nutrition/gidakatkimadde.htm

Seenapa, M. & Kempton, A. G. (1981). A note on the occurrence of *Bacillus cereus* and other species of Bacillus in Indian spices of export quality. *Journal of Applied Bacteriology*, ***50***: 225-228.

Shamshad, S. I., Zuberi, R. & Qadri, R. B. (1985). Microbiological studies on some commonly used spices in Pakistan. *Pakistan Journal of Scientific and Industrial Research*, ***28***(6): 395-399.

Sharma, A., Padwal-Desai, S. R. & Nair, P. M. (1989). Assessment of microbiological quality of some gamma irradiated Indian spices. *Journal of Food Science*, ***54***(2): 489-490.

Sharma, O. P. (1976). Antioxidant activity of curcumin and related compounds. *Biochemical Pharmacology*, ***25***: 1811–1812.

Shinyoung, H. (2005). Antimicrobial activity of wool fabric treated with curcumin. *Dyes and Pigments,* ***64***: 157e 161.

Sidhu, G. S., Mani, H. & Gaddipati, J. P. (2002). Curcumin differentially regulates TGF-beta 1, its receptors and nitric oxide synthase during impaired wound healing. *Biofactors*, ***16***: 29.

Tainter, D. R. (1992). *Spices and seasonings* pp. 2410-2418. In Hui, Y. H. (Ed.), Encyclopedia of Food Science and Technology. New York, USA: John Wiley & Sons, Inc.

TGKY. (2010). *Turkish Food Codex Regulation*, T.C. Official Newspaper. Number: 23172: 1-220.

Toprak, İ., Şentürk, S., Yüksel, B., Özer, H., Çakır, B. & Bideci, A. (2002). Gıda Katkı Maddeleri. Ankara T.C. Sağlık Bakanlığı Hacettepe Üniv. Temel Sağlık Hizmetleri Beslenme ve Diyetetik Genel Müdürlüğü Bölümü. (*In Turkish*)

Topsoy, H., Demirer, A. & Bozkurt, M. (1991). A research on quantitative determination of synthetic food colours used in some candies and beverage powders. *Turkish Bulletin of Hygiene and Experimental Biology*, ***48***(1): 21-38.

Tripathi, M., Khanna, S. K. & Das, M. (2007). Surveillance on use of synthetic colours in eatables vis a vis prevention of food adulteration act of India. *Food Control*, ***18***: 211-219.

Vanichkul, K., Areechon, N., Kongkathip, N., Strisapoom, P. & Chuchird, N. (2010). Immunological and bactericidal effects of turmeric (*Curcuma longa* Linn.) extract in Pacific white shrimps (*Litopenaeus vannamei* Boone). *Kasetsart Journal Natural Science*, ***44***: 850-858.

Varlık, C., Erkan, Ö., Mol, S. & Baygar, T. (2004). *Seafood processing technology*. İstanbul, Turkey: İstanbul University Publications. 491 p.

Wilson, L. A. (1993). *Spices and flavouring crops* (pp. 4282-4286). In Macrae, R., Robinson, R. K. & Sadler, M. J. (Eds.), Encyclopedia of Food Science, Food Technology and Nutrition. London, UK: Academic Press Limited.

Yentür, G., Ekşi, A. & Bayhan, A. (1996). Investigation of the amount of synthetic dye in cake decorations and some candies provided from Ankara markets. *Ankara University Journal of Veterinary Medicine Faculty*, ***43***: 479-484.

Yentür, G., Erdoğan, Ş., Er, B., Demirhan, B. & Öktem, A. B. (2009). Investigation of synthetic dye levels in some foodstuffs offered for consumption in the Ankara region. *Food Journal*, ***20***(3): 1-5.

Yurttagül, M. & Ayaz, A. (2008). *Additives: Wrongs and truths*. Ankara, Turkey: Hacettepe University Faculty of Health Sciences Press. 30 p.

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RESEARCH ARTICLE

Hierarchical flow among four shipping markets: An integrated approach for capesize shipping markets

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Second-hand market

ABSTRACT

Although there are theoretical researches on the interactions between four shipping sub-markets, empirical studies examining four to cover are rare. Also, market interactions may differ in positive or negative environments. Accordingly, the purpose of this study is to examine the hierarchical structure among the 4 sub-maritime markets by providing a methodological proposition. This study integrates the Interpretive Structural Modelling (ISM) method with the asymmetric causality analysis and examines the hierarchical structure between the four markets in terms of positive and negative shocks through the Capesize markets. The dataset used in the study covers the period between 07.01.2013 - 18.07.2019 and consists of 342 weekly observations. The results show that the hierarchical flow of positive and negative shocks differs in the Capesize market. In terms of positive shocks, the demolition market stands out as the starting point of the shocks, while in terms of negative shocks, freight and new building markets come to the fore. Our study reveals hierarchical structures for maritime markets by testing their relationships empirically and contributes to the maritime literature where the freight market is theoretically considered a pioneer. A new methodical perspective is thought to be presented to the maritime economics literature.

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Introduction

The maritime market is defined as having a derived demand structure (Branch, 2007), which can be explained as being directly affected by changes in demand for commodities and little control over the demand (McConville, 1999). Due to this structure, it is directly affected by the developments in the world economy (Stopford, 2009; Başer and Açık, 2019). Accelerations and slowdowns in economic activities are felt directly in the maritime market. For these reasons, the dry bulk freight market is defined as the leading indicator for the current situation of the world economy (Geman, 2009; UN, 2009; Tarı and İnce, 2019) and future economic activities since it carries the raw materials for production activities (Lawson, 2008;

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Langdana, 2009; Şahin et al., 2018). It can be stated that the maritime market consists of four sub-markets; freight market, sale & purchase market, newbuilding market, and demolition market (Beenstock and Vergottis, 1993; Stopford, 2009). These markets are in a very close relationship with each other. The freight and sell & purchase markets are more dynamic than the others. Newbuilding and demolition markets, on the other hand, contribute to the formation of supply-demand balance by adjusting the carrying capacity in the market (Jugović et al., 2015). However, this balance is very difficult to form since there are too many independent players in the market. Due to the derived demand structure and the fact that there are too many players, there are continuous cycles in the maritime markets (Metaxas, 1988; Stopford, 2009), which are almost unlike each other. These cycles can also have serious returns and costs for cargo owners and ship owners.

Theoretically, the place where the action starts in the maritime markets is seen as the freight market. When the freight market booms, second-hand ship prices increase as current income and future income expectations for ship owners increase. Also, due to investors who want to increase their carrying capacity preferences by ordering new ships, the congestion of shipyards increases, and new ship prices increase. On the other hand, the number of ships sent to scrapping decreases as even old and obsolescence ships can carry out their commercial activities with sufficient profit in live market conditions. Therefore, the demolition prices offered by the scrap centers to the ships also increase. On the contrary, the drop in the freight market leads to a decrease in second-hand ship demand, causing prices to drop. As new orders decrease, new ship prices also drop, and as the number of ships going for scrapping increases, demolition prices also decrease.

Although the theoretical explanation of this current relationship is clear, there is no study as far as the authors know that empirically demonstrated their relationship by addressing the four markets together. In the literature, the relationships of these markets with each other and with some other variables are mostly examined. In this study, we aimed to combine the relations between the four markets on a single result by providing a methodological contribution. Also, considering that the spread of positive and negative shocks between the markets may be different, we also tried to model the two types separately. In our study, we examined the hierarchical relationship structure between the four markets over the Capesize market due to the data limitation. Firstly, we generated the relationship matrices with the asymmetric causality test developed by Hatemi-J (2012). The main reasons for using this method are that it can present relationships between shocks in four different combinations and detect nonlinear relationships between variables. Then, we formed hierarchical market models using these matrices as input in the ISM method. These models provide an important contribution in terms of empirically revealing the theoretical structure. It also reveals that the interaction between the markets has partially differentiated under positive and negative market conditions.

In the second section, we reviewed the studies on maritime markets and positioned our study by revealing its originality. In the third section, after introducing the methods we used in the research, we examined our data in the fourth section. In the fifth section, after presenting our results, we discussed and evaluated our findings.

Literature Review

The interactions between the maritime sub-markets are certain and therefore, the relationships between these markets are mostly examined in the literature. Also, these markets are exposed to external shocks such as fluctuations in international trade, fuel prices, shipbuilding costs, capital returns, and other factors (Beenstock and Vergottis, 1993). Therefore, studies examining the relations of these markets with some external factors are also included in the literature.

There are two approaches to the formation of freights, they can be described as traditional and modern (Efes et al., 2019). In the traditional approach, freights are formed by the balance between supply and demand (Beenstock and Vergottis, 1989). Freight rises or falls with any side dominating. Freight rates tend to increase if the demand for transportation is higher than transport capacity in the market, but if the transport capacity is higher, freights tend to decrease. Due to the shipbuilding period that takes time between 2-4 years (Tsolakis, 2005), the fact that the supply is inelastic (Koopmans, 1939) also contributes to the increase and variety in the freight cycles. For this reason, the only way to increase the supply in the short run can be provided by increasing the speed (Karakitsos and Varnavides, 2014). In the long run, balance can be achieved as new vessels enter the market (Lun et al., 2010). In the modern approach, the traditional model has been partially developed. In this model, the bargaining game is more prominent. Players can be identified as the owner of the cargo and the owner of the ship. According to current market conditions and future expectations, a player's bargaining power may be higher. The shipowner's bargaining power will be higher when the conditions in the freight market are good, but the cargo owner's power will be higher when the market conditions are bad. According to these conditions, the new freight rate is realized above or below the balance price and forms the new balance (Karakitsos and Varnavides, 2014). According to the general

literature, the fluctuations in the economy are felt firstly in the freight market (Strandenes, 2012), as it is known as the place where the first reflection of the demand for maritime is felt. In a study in which the relationship with the economy was analyzed by Başer and Açık (2019) through GPD, it was found that the increases in GPD caused an increase in freight rates in the dry bulk market, but the situation changed slightly after the global crisis. A similar study was carried out by Efes et al. (2019). The authors modeled the dry bulk freight market by assuming China's foreign trade as demand for shipping and the global dry bulk fleet as supply-side for shipping. As a result, they supported the classical approach by concluding that the increase in trade volume affected the freight positively and the increase in the fleet negatively. Regardless of which approach for freights are used, the freights tend to return to the mean in the long run (Tvedt, 2003).

Entrepreneurs intending to second-hand ship purchases in the maritime market often have two purposes; increasing existing carrying capacity or being an asset player. Asset players hope to make a significant profit by trading at the right time (Kavussanos and Alizadeh, 2002). The factors that determine the second-hand value of a ship may be classified into two groups; vessel specific and market-specific. Vessel specific factors are size, type, age, general conditions, equipment, engine, etc., while market-specific ones are current and future income expectations of the owners (Alizadeh and Nomikos, 2009). Also, inflation may be considered as a market-specific factor (Stopford, 2009). The factors determining the second-hand value in the empirical literature were investigated by Pruyn et al. (2011). As a result, the authors found that the variables such as new building price, order book size, freight rates, fuel prices, age, and ship size were used extensively in second-hand ship price modeling. In the studies conducted in the literature, it was examined by Alizadeh and Nomikos (2003) whether the volume of second-hand sale & purchase transactions had an effect on price volatility in the market. As a result, the authors found that the increase in the second-hand transaction volume caused the volatility in the prices of the ships to decrease. In terms of its relevance, the study conducted by Kavussanos (1997) found that the volatility in the prices of larger ships was higher.

The newbuilding market has an important role in the supply and demand balance by affecting the supply side (Stopford, 2009). If the supply amount in the market remains low and the freights are therefore too high, it offers new carrying capacity to the market, thus increasing the supply and ensuring that the freights reach a low level of equilibrium again. However, since shipbuilding takes a certain time, ordering a new ship is partly risky. As the orders are given according to current market conditions and expectations, the market is partially uncertain when the ordered ship is delivered (Tsolakis, 2005). In a study that empirically tested this situation, Başer and Açık (2018) examined the relationship between freights and ship tonnage delivered. According to their results, there is a positive relationship between the variables with a delay of 2 years. In other words, today's freight rate determines the tonnage delivered two years later and therefore determines the current order amount. As we mentioned earlier, the second-hand ship market can be expected to be more dynamic, as second-hand ships are available on the market and new ships are available after a certain period of orders. Adland and Jia (2015) tested this situation empirically in their study and found that new building prices were less volatile than second-hand ship prices. As an integrative study, factors affecting the volatility of new building prices have been studied by Dai et al. (2015). As a result of their empirical analysis, the authors found that the most essential part of the volatility in the new building prices was caused by freight rates. In another study examining the interaction between freight rate and ship value, the relationship between freight rate, second hand price and newbuilding ship price was examined by Açık and İnce (2019) considering their nonlinear structures. According to the results of the research, freight rate affects both second-hand price and newbuilding price for Capesize type ships. In addition, while an effect on freight rate was determined from second hand ship price, no effect on freight could be detected from the newbuilding price.

The ship demolition market plays a balancing role between supply and demand in the maritime market. When the supply surplus occurs in the market and the freight rates drop, a large number of ships are sent to demolition, and the freight rises again and reaches equilibrium as the supply quantity decreases (Buxton, 1991). Since the freight in the market directly affects ship demolition decisions, a negative relationship was expected to be found between them in the literature. This negative relationship was confirmed by Knapp et al. (2008). The authors found a negative relationship between the probability of the ship being sent for demolition and freight revenues. This was also indicated in the study carried out by Açık and Başer (2017). While the number of ships going for demolition at the high freight level decreases, it increases at the low freight level. In terms of the relation of the demolition price with the freight, when the freight rates increase, it can be expected that the demolition prices increase as the number of ships sent for scrapping decreases. This possible relationship was examined by Açık and Başer (2018) and the authors empirically confirmed the positive relationship between the variables. The authors stated that this relationship may be significant not only in terms of the maritime side but also in terms of demand for

steel. Because high freight rates may be indicative of the active period of the economy, demand for steel may also be high. In this case, the demolition price offered to the ships may be high. Mikelis (2007) also evaluated that this relationship could be positive by statistical analysis. Undoubtedly, the price of demolition should be also related to variables other than maritime, because the steel from demolition is used as input to some sectors, such as the construction sector (Açık and Baran, 2019). Kagkarakis et al. (2016) examined the relationship between ship demolition prices and global scrap prices and found that global scrap prices are decisive for ship demolition prices since their small share in the global steel industry. The direct relationship between ship demolition prices and processed steel prices was analyzed by Tunç and Açık (2019) with panel data analysis method, and a significant causal relationship was found between steel prices and the demolition prices of the major ship demolition countries. To evaluate the studies on the demolition market in general, the market has a structure affected by both the freight market and the steel market, which makes its structure a bit complicated.

Studies mentioned in the literature are not limited to these and many more studies can be added. However, the studies mentioned understanding the general integrity of the subject is thought to be sufficient. As can be seen, the relations of the maritime markets among themselves or with the other external variables have been examined. Although theoretically the direction of the effects is explained as spreading to other markets starting from the freight market, there is no study that empirically demonstrates this by including all markets in a single methodology. In this study, we have divided the interactions as negative and positive and tried to put the interaction between the markets into a hierarchical structure. Thus, we wanted to test current theoretical knowledge empirically and understand whether the structure differs according to interaction type since positive and negative markets can produce different hierarchical relations.

Methodology

In the study, an integrated form of causality and ISM analysis is proposed as a method. With the asymmetric causality test developed by Hatemi-J (2012), causal relationships between 4 markets are examined. From these relationships, two separate matrices are generated with those from positive shocks to positive shocks and from negative shocks to negative shocks. These obtained matrices were used as input tables for ISM analysis and hierarchical structures were tried to be determined among the markets. Separate modeling of positive and negative shocks is to determine whether there is a difference in the spread of positive and negative shocks.

Asymmetric Causality

The asymmetric causality test is used to investigate the causality relationship between shocks in nonlinear time series in four different combinations; (i) from positive to positive, (ii) from positive to negative, (iii) from negative to negative, and (iv) from negative to positive. The method was developed by Hatemi-J (2012) and is a very functional method considering that players in the market can react differently depending on the type of shocks (news) whether it is good or bad information.

The method incorporates the idea underlying Toda and Yamamoto (1995) process and makes analyses by taking into account nonlinear effects (Shahbaz et al., 2017). The logic of the test is similar to the standard Granger (1969) causality, with the difference that the cumulative sums of positive and negative shocks are used to determine asymmetric causality (Tugcu and Topcu, 2018). Thus, it becomes possible to obtain results in four different combinations and to distinguish the causal impact of positive shocks from negative ones (Shahbaz et al., 2017).

Since the method is used in the analysis of nonlinear variables, possible autoregressive conditional heteroscedasticity (ARCH) effects on the variables should be considered (Tugcu et al., 2012). Therefore, Hatemi-J (2012) uses the bootstrap simulation technique to calculate critical values and Mwald statistics, and this provides more accurate critical values due to the leverage corrections (Hatemi-J and Uddin, 2012). Furthermore, the financial series are often far from the normal distribution as they are exposed to many unexpected events and shocks (Bildrici and Turkmen, 2015). The bootstrap simulation technique used in this method eliminates the necessity of normal distribution and provides a great advantage (Hatemi-J, 2012).

As the method follows Toda and Yamamoto (1995) process, the series do not have to be stationary, but the maximum degree of integration (dmax) needs to be determined (Umar and Dahalan, 2016). Unit root tests are used for this determination and if any unit root exists, the method is applied by adding extra lags to the estimated unrestricted VAR model (Hatemi-J and Uddin, 2012). For more detailed information, the study of Hatemi-J (2012) can be viewed.

ISM Methodology

The ISM method forms a hierarchical structure by prioritizing relationships of a complex system (Yudatama et al., 2018), which makes it possible to investigate directions of the relationships among these elements (Sage, 1977). Since the method takes into account the dependencies and driving forces among variables (Luthra et al., 2015), understanding macro-scale relationships becomes possible (Chuang et al., 2013).

In the first step of the method, a Structural Self-Interaction Matrix (SSIM) is formed that defines the relationships between the factors. These relationships are defined in 4 different combinations; There is an effect from factor a to factor b, there is an effect from factor b to factor a, there is bidirectional interaction between factors a and b, and there is no interaction between factors a and b. These relationships are represented by letters in the matrix;

- "V" for the one-way relationship from factor a to factor b;
- "A" for the one-way relationship from factor a to factor b;
- "X" for a bidirectional relationship between factors;
- and "O" for no relationship between factors.

Then, by converting the letters into numbers using the following rules, Initial Reachability Matrix (IRM) is obtained. The aim is to digitize the relations and make them ready for further steps;

- if (a, b) in the SSIM equals to "V", (a, b) equals to 1 and (b, a) equals to 0;
- if (a, b) in the SSIM equals to "A", (a, b) equals to 0 and (b, a) equals to 1;
- if (a, b) in the SSIM equals to "X", (a, b) equals to 1 and (b, a) equals to 1;
- if (a, b) in the SSIM equals to "O", (a, b) equals to 0 and (b, a) equals to 0.

Then, Final Reachability Matrix (FRM) is formed by obtaining "Dependence Power" with the sum of column values and "Driving Power" with the sum of rows for each variable. These values are determined to be used in MICMAC graph analysis to understand the position of variables in the complex system. For the ISM model, which presents the hierarchical structure, the IRM matrix is used. The affected factors by the variable are grouped as "Reachability" and the factors affecting it are grouped as Antecedent for each variable. "Intersection" set is obtained by the intersection of these two groups. The variable with the same "Reachability" and "Intersection" sets is positioned at the first level of the model. For other factors, positioning is completed in this way as well. In the MICMAC graph analysis, the factors are clustered in four different areas, which are "Independent", "Dependent", "Autonomous" and "Linkage". These groupings differ according to "Dependence" and "Driving" powers and make it easier to understand the positions of the variables in the complete system.

Data

The dataset used in the study covers the period between 07.01.2013–18.07.2019 and consists of 342 weekly observations. Representative variables were used for the four shipping markets due to the data access restriction. Freight variable refers to the Capesize route from Saldanha (South Africa) to Beilun (China) and the unit of it is freight paid $ per ton. The second-hand variable refers to the value of the 5 years old Capesize ship and the unit of it is $ million. Newbuilding variable refers to Capesize newbuilding price in China and the unit of it is $ million. Lastly, the demolition variable refers to the Indian dry demolition price and the unit of it is $ per ltd. Freight, second hand, and newbuilding variables are obtained from Bloomberg (2019) while demolition price is obtained from Athenian (2019). Descriptive statistics of the raw and return cases of the data used in the study are presented in Table 1. In the period discussed, signs of the skewness values of the return series indicated that negative shocks were more effective in demolition prices while positive shocks were more effective in the other three markets. The fact that the kurtosis values of the return series of the 3 markets are very high compared to the freight is due to the fewer changes. Since weekly data is used, weekly changes are less in the 3 markets and this causes the return values to be concentrated at 0. Fortunately, our analysis uses the method based on the Toda and Yamamoto (1995) approach and it does not require a condition of stationarity.

Table 1. Descriptive statistics of the variables

	Freight	Second Hand	Newbuilding	Demolition	RFR	RSH	RNB	RDEM
Mean	11.62	33.33	46.88	374.78	0.001	0.000	0.000	-0.000
Median	12.08	33.00	46.00	395.00	0.000	0.000	0.000	0.000
Maximum	22.98	52.00	56.50	465.00	0.326	0.162	0.064	0.083
Minimum	4.00	21.00	40.00	225.00	-0.247	-0.103	-0.056	-0.096
Std. Dev.	3.92	7.40	4.65	63.35	0.07	0.02	0.00	0.02
Skewness	0.27	0.46	0.44	-0.72	0.08	1.50	1.78	-0.41
Kurtosis	2.76	2.97	2.14	2.26	4.74	18.13	25.40	6.39
J-Bera	5.16	12.44	21.65	37.40	43.4	3382.5	7312.6	173.2
Prob.	0.07	0.00	0.00	0.00	0.000	0.00	0.00	0.00
Obs.	342	342	342	342	341	341	341	341

Source: Bloomberg (2019); Athenian (2019).

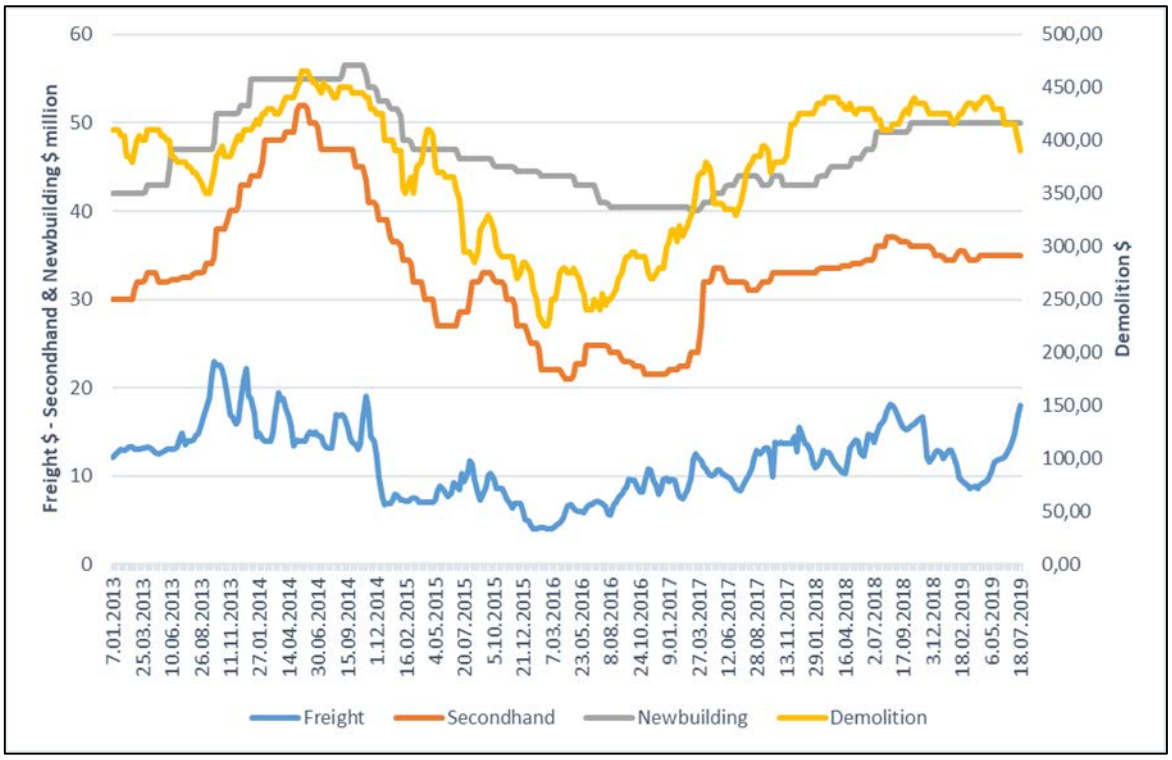

Figure 1. Graphical display of the variables (*Source*: Bloomberg (2019); Athenian (2019))

The visual of the variables used in the study is presented in Figure 1. It can be said that there is a high correlation between the second-hand and demolition values. The new building prices are inherently more stable. The fact that the ship delivery process is partially long and the market is not known when the ship is received causes the prices to be less volatile. Freight also similarly moves around a certain average but with partially high volatility. It can also be said that there are structural breaks both in the value of second-hand ships and in the demolition prices. For this reason, it is important to apply unit root tests that take into account breaks in level and trend. Standard tests may offer a result that they include unit root as they do not take into account possible structural breaks.

Results

In this section, firstly, unit root, and linearity tests, which are necessary for the asymmetric causality test, are applied. According to the results obtained from these tests, asymmetric causality test is applied. Then, two separate ISM models are constructed using the asymmetric causality test results related to the positive-positive and negative-negative analysis.

Unit Root Test Results

To determine the maximum degree of integration in the asymmetric causality analysis, root unit tests with structural breaks were applied to the series and the results are presented in Table 2. One break ADF test (Zivot and Andrews, 1992), one break LM test (Lee and Strazicich, 2013), two break ADF test (Narayan and Popp, 2010), and two break LM test (Lee and Strazicich, 2003) were applied to all series. The maximum lag length was determined as 25 and AICc was used to determine the most suitable lag. According to the results obtained, considering the structural breaks of the variables in the level and level & trend, the null of the unit root hypothesis is rejected with at least one test for all variables used in the analyzes. In this case, the maximum degree of integration value is determined as 0 for all asymmetric analysis combinations.

Linearity Test Results

As the asymmetric causality test used in the study is a nonlinear method, the linearity of the series should be tested. For this reason, all the variables were first converted to the return series, and then the most suitable ARMA models were estimated. After checking the significance and roots of the estimated models, BDS Independence (Brock et al., 1987) and ARCH (Engle, 1982) tests were applied to their residuals. The results of the applied tests were presented in Table 3. The obtained results indicated that the null of the linearity hypothesis was rejected for all variables. In this case, the applicability of the asymmetric causality test is confirmed.

The fact that the structures of the variables are not linear is an indicator that the parameters have changed over time. Unexpected shocks and structural breaks in the global world disrupt the structure of the series. The main reason for this may be the derived demand structure of the maritime markets.

Table 2. Unit root tests with structural breaks for capesize freight market

Test Items	Mod A Freight	Mod C Freight	Mod A S&P	Mod C S&P	Mod A NB	Mod C NB	Mod A Demo	Mod C Demo
One break ADF test (Zivot and Andrews, 1992)								
ADF Stat	-5.35***	-5.37**	-3.59	-3.64	-3.62	-3.29	-3.42	-3.74
Break Date	95	95	94	135	94	94	119	119
Fraction	0.27	0.27	0.27	0.39	0.27	0.27	0.34	0.34
Lag	1	1	7	4	22	22	6	6
One break LM test (Lee and Strazicich, 2013)								
LM Stat	-2.84	-4.35*	-2.02	-2.74	-1.90	-2.25	-1.52	-2.48
Break Date	97	114	221	134	87	129	132	184
Fraction	0.28	0.33	0.64	0.39	0.25	0.37	0.38	0.53
Lag	1	1	4	4	22	22	6	6
Two break ADF test (Narayan and Popp, 2010)								
ADF Stat	-6.01***	-6.35***	-4.68**	-5.72***	-4.58**	-6.05***	-4.79**	-4.03
Break Date	95, 141	95, 187	90, 142	90, 216	94, 172	37, 181	118, 226	128, 289
Fraction	0.27, 0.41	0.27, 0.54	0.26, 0.41	0.26, 0.63	0.27, 0.50	0.10, 0.52	0.34, 0.66	0.37, 0.84
Lag	1	1	7	7	22	22	6	6
Two break LM test (Lee and Strazicich, 2003)								
LM Stat	-3.12	-5.54*	-2.20	-4.62	-2.06	-4.40	-1.93	-3.97
Break Date	97, 101	95, 194	182, 226	90, 221	87, 243	94, 220	109, 220	128, 212
Fraction	0.28, 0.29	0.27, 0.56	0.53, 0.66	0.26, 0.64	0.25, 0.71	0.27, 0.64	0.31, 0.64	0.37, 0.62
Lag	1	1	4	4	22	22	6	6

Note: Mod A refers to break in level, Mod C refers to break in level and trend. H_0 rejected ***99%, **95%, *90%.

Causality Test Results

The asymmetric causality analysis was applied between positive shocks and between negative shocks. The maximum integration degree is determined as 0, the maximum number of lags is selected as 25, and the optimum lag selection criterion is selected as AICc. The causality results among positive shocks are shown in Table 4. According to the results obtained, positive shocks in freights are the cause of positive shocks in second-hand ship value. Positive shocks in second-hand ship value are the cause of positive shocks in new ship value. Finally, positive shocks in demolition prices are the cause of positive shocks in freights.

Results between negative shocks are presented in Table 5. Unlike positive shocks, much more significant relationships were found in negative results. According to the results obtained, negative shocks in freights are the causes of negative shocks in second-hand ship value, new ship value, and demolition prices. Negative shocks in second-hand ship value are the causes of negative shocks in demolition prices. Negative shocks in the value of the new ship are the causes of negative shocks in freights and demolition prices. Finally, negative shocks in demolition prices are the causes of negative shocks in second-hand ship value.

Table 3. Linearity test results

Dimension	Freight	Secondhand	Newbuilding	Demolition
2	6.450***	3.918***	5.219***	4.178***
3	6.550***	2.427**	3.897***	4.481***
4	6.834***	1.719*	3.261***	5.312***
5	6.948***	2.650***	3.744***	5.957***
6	7.047***	3.558***	3.897***	6.915***
ARCH	41.06***	65.01***	5.99**	-5.26**
ARMA (p, q)	(12, 12)	(8, 2)	(11, 9)	(8, 8)
AIC	-2.56	-5.11	-6.78	-4.87

Note: Null of linearity is rejected at ***1%, **5%, *10%.

Table 4. Causality test results related positive shocks

		TO			
		Freight	**Secondhand**	**Newbuilding**	**Demolition**
FROM	**Freight**	X	34.957***	7.097	6.729
	Secondhand	5.417	X	13.245**	1.664
	Newbuilding	0.962	3.964	X	0.380
	Demolition	8.546**	3.042	0.261	X

Note: Null of noncausality is rejected at ***1%, **5%, *10%.

Table 5. Causality test results related negative shocks

		TO			
		Freight	**Secondhand**	**Newbuilding**	**Demolition**
FROM	**Freight**	X	34.957***	7.097	6.729
	Secondhand	5.417	X	13.245**	1.664
	Newbuilding	0.962	3.964	X	0.380
	Demolition	8.546**	3.042	0.261	X

Note: Null of noncausality is rejected at ***1%, **5%, *10%.

After the asymmetric causality analysis, ISM analyzes are applied based on the results obtained. Two separate ISM analyzes are applied for positive and negative shocks in the Capesize markets.

ISM Results

Considering the results obtained from the asymmetric causality test, two separate ISM analyzes were applied for both positive and negative shocks. Thus, it was tried to be determined whether the hierarchical structure of positive and negative interactions changed in different market conditions.

Positive Shocks Related Results

Based on the rules applied in ISM analysis with the matrix presenting the relationship between the positive shocks in Table 4, Structural self-interaction matrix (SSIM) was formed and presented in Table 6.

Table 6. Structural self-interaction matrix (SSIM)

	Freight	Secondhand	Newbuilding	Demolition
Freight		V	X	A
Secondhand			V	X
Newbuilding				X
Demolition				

The initial and final reachability matrix was generated through the letterings in Table 6 and presented in Table 7. This table also includes the driver and dependence powers of the markets. The interpretation of these forces is made clearer in the MICMAC analysis section.

Using the matrix in Table 7, Table 8, which will be used in leveling, was obtained. In this table, all levels are determined so that the markets with the same values in the reachability and intersection columns are at the first level. Accordingly, demolition is at level 4, freight at level 3, second hand at level 2, and newbuilding at level 1. With these level setting values, a visual ISM model was formed.

The ISM model, which examines the spillover of positive shocks in the Capesize market hierarchically, is presented in Figure 2. According to this model, 4 maritime markets are located at 4 different levels. The demolition market located at the bottom is in a position that affects and is not affected by all other markets, which indicates that positive shocks spillover from the demolition market to other markets. The freight market, which is located at level 3, transmits the shocks it receives from the demolition market to other markets through the sale & purchase market. The sale & purchase market transmits the positive shock it receives from the demolition and freight markets to the newbuilding market. The new building market is affected by three other markets, but it cannot affect any other market.

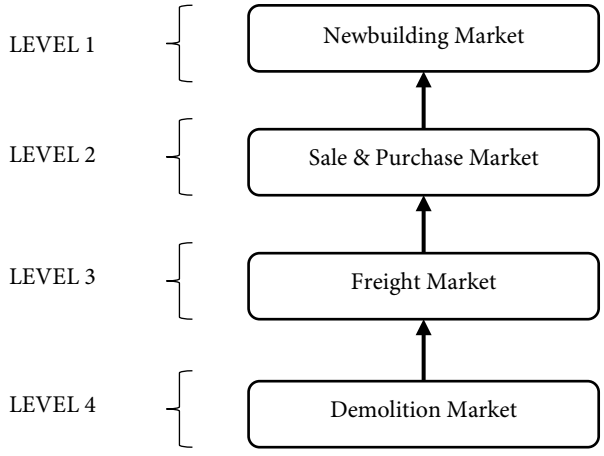

Figure 2. ISM results

Table 7. Initial and final reachability matrix

	Freight	Secondhand	Newbuilding	Demolition	DRIVER
Freight	1	1	0	0	2
Secondhand	0	1	1	0	2
Newbuilding	0	0	1	0	1
Demolition	1	0	0	1	2
DEPENDENCE	2	2	2	1	

Table 8. Use of reachability matrix in level setting

		Reachability	Antecedent	Intersection	Level
1	**Freight**	1, 2	1, 4	1	3
2	**Secondhand**	2, 3	1, 2	2	2
3	**Newbuilding**	3	2, 3	3	1
4	**Demolition**	1, 4	4	4	4

The MICMAC graphic analysis based on the values in Table 8 is presented in Figure 3. This analysis shows how markets are positioned regarding positive shocks. However, since there are few variables (markets) in the analysis, they are located in border regions. Based on the ISM model in Figure 2, it can be said that the Demolition variable is included in the Independent cluster since it is in a position that affects all markets. It can be said that the freight and sale & purchase markets are included in the Linkage cluster, as they transfer the positive shocks they receive to the Newbuilding market. Finally, the Newbuilding market can be said to be in the Dependent cluster, because this market is at the top of the ISM model and does not affect any other market.

Negative Shocks Related Results

Based on the matrix showing the relationship between the negative shocks presented in Table 5, the Structural Self-Interaction Matrix (SSIM) in Table 9 was generated.

The letters in SSIM were digitized according to the ISM methodology, and the Initial and Final Reachability Matrix in Table 10 were obtained. It can be said that the values of Driver and Dependence powers obtained in this matrix vary more than those in positive shocks. In MICMAC graphic analysis, this variety can make it possible to interpret better.

Level determination processes for each market using the values in Table 10 are presented in Table 11. According to this transaction, while second hand and demolition markets are positioned at the 1st level, freight and newbuilding markets are positioned at the 2nd level.

The ISM model formed after the leveling process is presented in Figure 4. According to this model, while the freight and new building markets are positioned at the 2nd level, the sale & purchase and demolition markets are positioned at the 1st level. This shows that negative shocks are reflected from freight and new building markets to other markets. The markets where negative shocks have ended were identified as sale & purchase and demolition markets.

Markets are positioned again on the borders as the number of variables is low in the MICMAC graph analysis. However, when cluster determination is applied with the help of the ISM model, Newbuilding and Freight markets can be said to be in the Independent cluster since these markets are the sources of negative shocks. On the other hand, it can be said that the demolition and sales & purchase markets are in the Dependent cluster since these markets receive negative shocks from other markets.

Discussion

In this study, firstly, the price information of the variables that make up the 4 main maritime markets of Capesize shipping is considered as representative values. For instance, freight refers to Capesize route from Saldanha (South Africa) to Beilun (China), the second-hand variable refers to the value of the 5 years old Capesize ship, the newbuilding variable refers to Capesize newbuilding price in China, and the demolition variable refers to Indian dry demolition price. Therefore, defining the relationship between them may not be very confident, but it is believed that reasonable results have been obtained in these current conditions related to the data limitation.

Table 9. Structural self-interaction matrix (SSIM)

	Freight	Secondhand	Newbuilding	Demolition
Freight		V	X	A
Secondhand			V	X
Newbuilding				X
Demolition				

Table 10. Initial and final reachability matrix

	Freight	Secondhand	Newbuilding	Demolition	DRIVER
Freight	1	1	1	1	4
Secondhand	0	1	0	1	2
Newbuilding	1	0	1	1	3
Demolition	0	1	0	1	2
DEPENDENCE	2	3	2	4	

Table 11. Use of reachability matrix in level setting

		Reachability	Antecedent	Intersection	Level
1	**Freight**	1, 2, 3, 4	1, 3	1, 3	2
2	**Secondhand**	2, 4	1, 2, 4	2, 4	1
3	**Newbuilding**	1, 3, 4	1, 3	1, 3	2
4	**Demolition**	2, 4	1, 2, 3, 4	2, 4	1

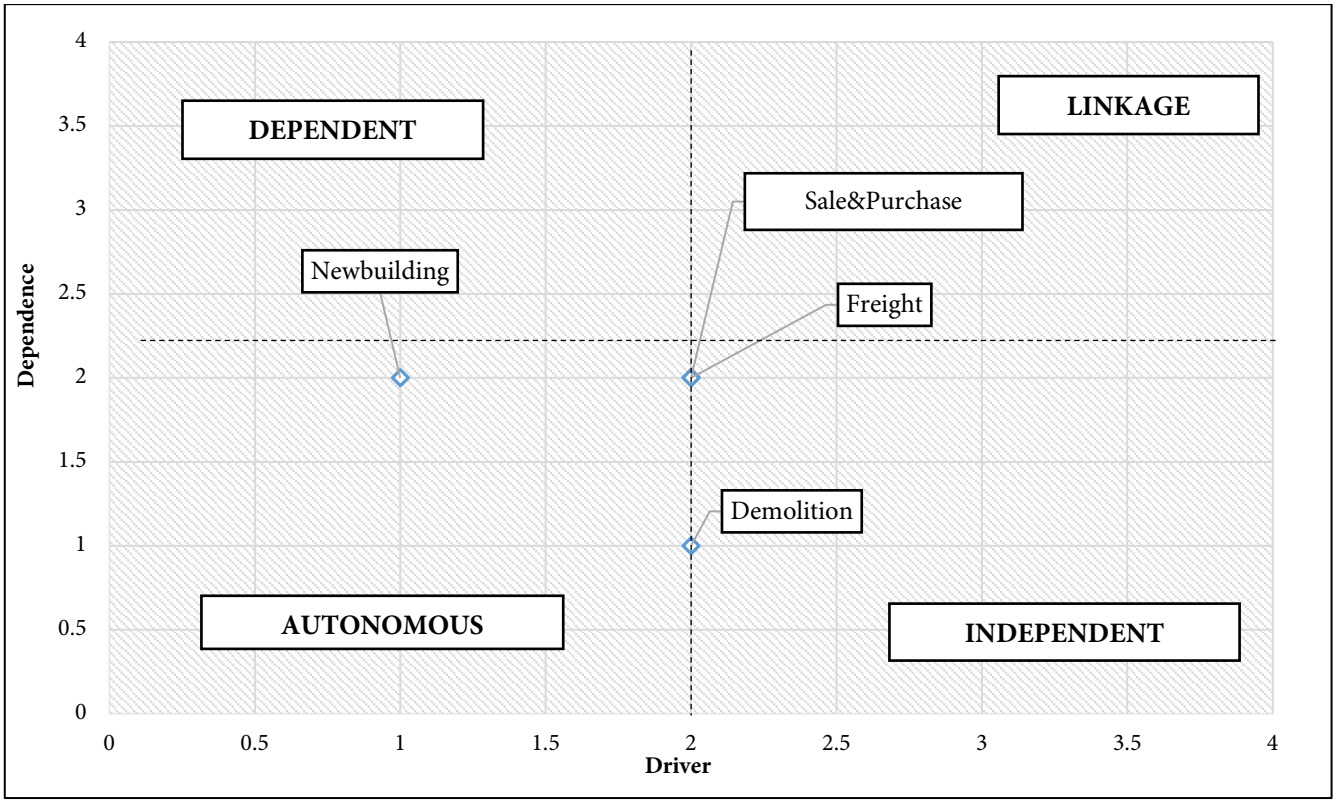

Figure 3. MICMAC analysis

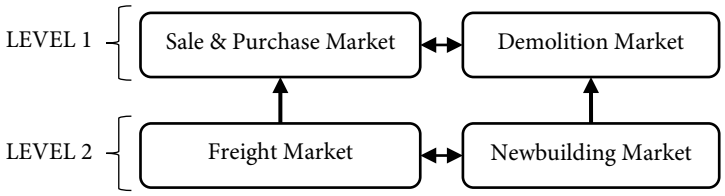

Figure 4. ISM results

The ISM model obtained related to the hierarchical flow of positive shocks is quite compatible with the theoretical structure. The location of markets other than the Demolition market is generally in line with the accepted approach in the literature. The increases in the freight market cause an increase in the value of second-hand ships, as the current income and future income expectations increase (Alizadeh and Nomikos, 2009; Pruyn et al., 2011; Açık and İnce, 2019). Also, as the demand for new ships increases in this buoyant market environment, there is a positive flow from second-hand ship prices to new ship prices. Considering that, the price of the new ship is affected by the volatility in the freight (Dai et al., 2015), and it is less volatile than the price of the second ship (Adland and Jia, 2015), the position of it in the model is reasonable. The position of demolition prices in the model does not seem very reasonable in the maritime market at first glance. However, considering the relationship between demolition prices and economic activities, a reasonable explanation can be made. Because ship demolition prices are closely related to steel prices (Tunç and Açık, 2019) and the increase in steel prices often causes an increase in demolition prices. Also, an increase is observed in demolition prices as the ship going to dismantling decreases in live market conditions. The increase in steel prices is experienced in times when the economy is alive considering the widespread use of steel in the global industries. Therefore, positive shocks from demolition prices can be considered as an indicator of economic recovery. As the demand for maritime transportation with derived demand structure increases as a result of the increase in economic activities, firstly, there is a positive reflection on freights. This positive relationship between demolition price and freight rates was also confirmed empirically in the literature (Açık and Başer, 2018).

On the other hand, the fact that the freight and the new building markets are at the 2nd level in the structure related to negative shocks do not partially comply with the evaluation regarding the positive shocks. Assuming that the demolition market represents the world economy, negative shocks could be expected to start from this market. The fact that freights are at the second level is reasonable. Negative shocks in freights are transferred to the sale & purchase market since it is related to the current and future income expectations (Alizadeh and Nomikos, 2009; Pruyn et al., 2011; Açık and İnce, 2019). Also, it is reasonable to see a decrease in the demolition market due to the falling ship values and the increasing ship amount sent to the scrapping (Knapp et al., 2008; Açık and Başer, 2017).

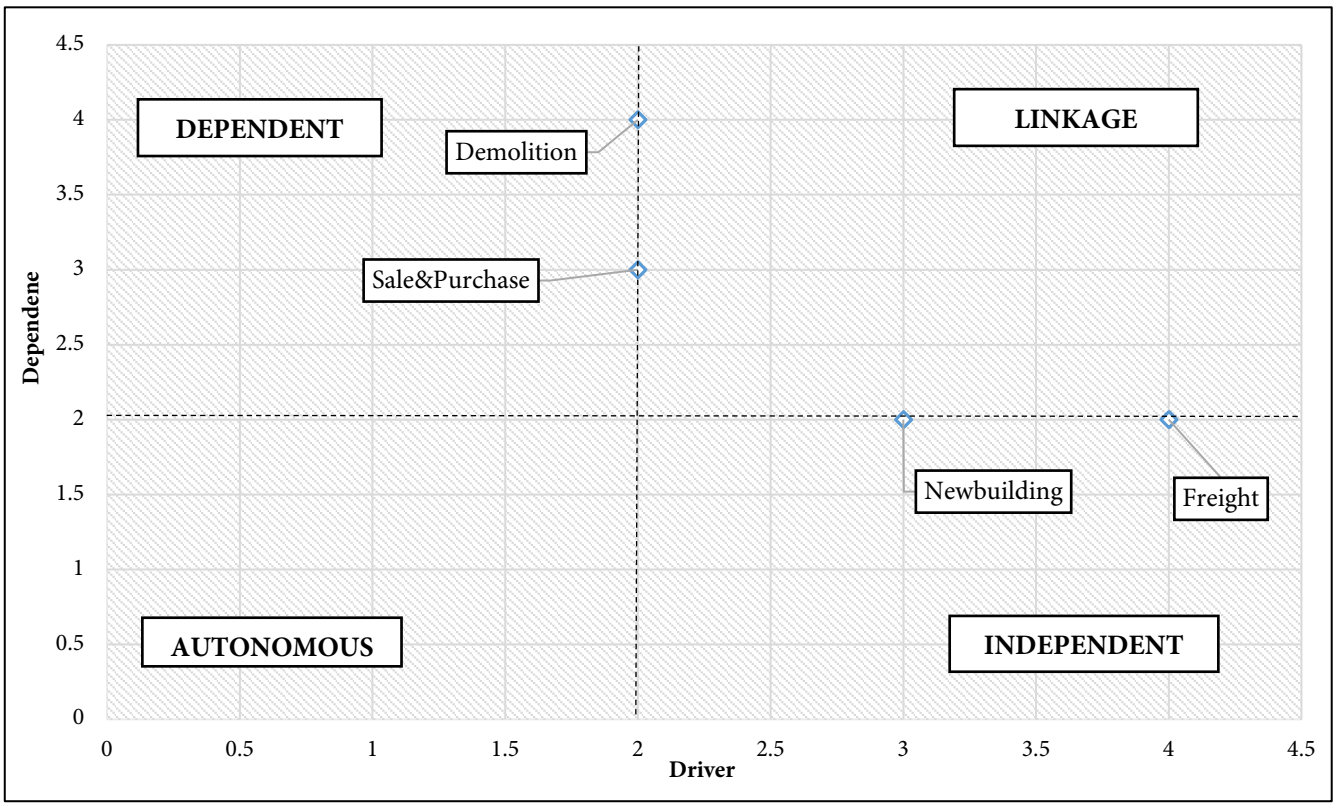

Figure 5. MICMAC analysis

The reason why the new building market is at the 2nd level may be related to the role of sentiment in ship orders because one of the most important factors affecting ship investors' decisions is their sentiment related to the economic environment (Stopford, 2009). Therefore, the declining new order demand may be causing a drop in price, and such negative shocks may be spreading to other markets.Of course, this study is evaluated within the maritime markets. There are also many macro variables that affect the shipping market such as interest rates, commodity prices, exchange rates, etc., and these are the main sources of the shocks. Therefore, analyzes including such variables can provide more comprehensive results.

Conclusion

We aimed to conduct research examining the hierarchical structure of freight, sale & purchase, newbuilding and demolition markets, which are accepted as the 4 sub-markets of the maritime market, in a single model. There are studies in the literature that examine the relationships between these markets and some other external variables. However, as far as the authors know, there is no study examining a hierarchical structure by considering all of them in a single model. In this respect, it is considered that this research applied to the Capesize market has made an original contribution. In our study, we also provided a methodological contribution by integrating the two models and by examining the relationships in two separate structures, positive and negative. The ISM model makes it possible to determine the hierarchical structure and priority order of the elements in a complex system. The asymmetric causality test is a method that examines nonlinear causality relationships through shocks that variables contain. Based on the classical maritime market view, we wanted to demonstrate empirically that shocks coming to the maritime market spread to the freight market first and then to other markets. We determined that the models that emerged as a result of the research differed according to the positive and negative shocks and the hierarchies of the structures changed. We have linked the reason for the demolition market to come to the fore in the positive model, not just to be dependent on the shipping market and to be closely related to economic activities.

As a limitation of the study, it can be stated that the Capesize type is used and limited the scope of the research. The results may be more generalizable if analysis can be applied for other types of ships or if common value indices can be developed for all ships. Also, contrary to the classical view that examines the maritime markets in themselves, other important macro variables, such as interest rates, exchange rates, commodity prices, can be added to the models based on the modern view that relates it to other macro variables.

Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Açık, A. & Baran, E. (2019). The reflection of ship demolition prices to construction costs in Turkey. *Marine Science and Technology Bulletin*, ***8***(1): 23-29. https://doi.org/10.33714/masteb.547103

Açık, A. & Başer, S. Ö. (2018). The relationship between freight rates and demolition prices. *Uluslararası Ticaret ve Ekonomi Araştırmaları Dergisi*, ***2***(1): 16-32. https://doi.org/10.30784/epfad.363721

Açık, A. & Başer, S. Ö. (2017). The relationship between freight revenues and vessel disposal decisions. *Journal of Research in Economics, Politics & Finance*, ***2***(2): 96-112. https://doi.org/10.30784/epfad.363721

Açık, A. & İnce, M. R. (2019). Income and asset value relationship: A nonlinear approach to Capesize shipping market (pp. 35-43). In Koc, S., Yilmaz Genc, S., Benli, V.H. (Eds.), *Economic Issues: Global and Local Perspectives*. London: Cambridge International Academics. 196p.

Adland, R. & Jia, H. (2015). Shipping market integration: The case of sticky new building prices. *Maritime Economics & Logistics*, ***17***(4): 389-398. https://doi.org/10.1057/mel.2014.35

Alizadeh, A. H. & Nomikos, N. K. (2003). The price-volume relationship in the sale and purchase market for dry bulk vessels. *Maritime Policy & Management*, ***30***(4): 321-337. https://doi.org/10.1080/0308883032000145627

Alizadeh, A. & Nomikos, N. (2009). *Shipping Derivatives and Risk Management*. London: Palgrave. 527p.

Athenian, S. A. (2019). Weekly demolition report. Retrieved on August 5, 2019 from https://www.atheniansa.gr.

Başer, S. Ö. & Açık, A. (2019). The effects of global economic growth on dry bulk freight rates. *Uluslararası Ticaret ve Ekonomi Araştırmaları Dergisi*, ***3***(1): 1-17. https://doi.org/10.30711/utead.507566

Başer, S. Ö. & Açık, A. (2018). The response of shipbuilding activities to freight market. *International Journal of Economics and Administrative Sciences*, ***4***(1): 120-136. https://doi.org/10.29131/uiibd.415303

Beenstock, M. & Vergottis, A. (1993). *Econometric Modelling of World Shipping*. Netherlands: Springer. 254p.

Beenstock, M. & Vergottis, A. (1989). An econometric model of the world market for dry cargo freight and shipping. *Applied Economics*, ***21***(3): 339-356. https://doi.org/10.1080/758522551

Bildirici, M. E. & Turkmen, C. (2015). Nonlinear causality between oil and precious metals. *Resources Policy*, ***46***(2): 202-211. https://doi.org/10.1016/j.resourpol.2015.09.002

Bloomberg (2019). Freight, Second hand, Newbuilding Values Retrieved on August 7 from https://www.bloomberg.com/professional

Branch, A. E. (2007). *Elements of Shipping* (8th ed.) New York: Routledge. 528p.

Brock, W., Dechect, W. & Scheinkman, J. (1987). *A test for independence based on the correlation dimension*. *Working Paper*, Department of Economics, University of Wisconsin, Madison.

Buxton, I. L. (1991). The market for ship demolition. *Maritime Policy & Management*, ***18***(2): 105–112. https://doi.org/10.1080/03088839100000034

Chuang, H-M., Lin, C-K., Chen, D-R. & Chen, Y. S. (2013). Evolving MCDM applications using hybrid expert-based ISM and DEMATEL models: An example of sustainable ecotourism. *The Scientific World Journal*, ***2013***: 1-18. https://doi.org/10.1155/2013/751728

Dai, L., Hu, H., Chen, F. & Zheng, J. (2015). The dynamics between new building ship price volatility and freight volatility in dry bulk shipping market. *International Journal of Shipping and Transport Logistics*, ***7***(4): 393-406. https://doi.org/10.1504/IJSTL.2015.069666

Efes, K. Ö., Başer, S. Ö. & Açık, A. (2019). Supply-demand interaction in the formation of freight rates: China's trade volume as demand side in the dry bulk market. *Pomorstvo*, ***33***(1): 46-55. https://doi.org/10.31217/p.33.1.5

Engle, R. (1982). ARCH with estimates of variance of United Kingdom inflation. *Econometrica*, ***50***(4): 987-1007. https://doi.org/10.2307/1912773

Geman, H. (2009). *Risk Management in Commodity Markets: From Shipping to Agriculturals and Energy* (Vol. 445), England: John Wiley & Sons. 320p.

Hatemi-J, A. (2012). Asymmetric causality tests with an application. Empirical Economics, ***43***(1): 447-456. https://doi.org/10.1007/s00181-011-0484-x

Hatemi-J, A. & Uddin, G. S. (2012). Is the causal nexus of energy utilization and economic growth asymmetric in the US?. *Economic Systems*, ***36***(3): 461-469. https://doi.org/10.1016/j.ecosys.2011.10.005

Jugović, A., Komadina, N. & Hadžić, A. (2015). Factors influencing the formation of freight rates on maritime shipping markets. *Scientific Journal of Maritime Research*, ***29***: 23–29.

Kagkarakis, N. D., Merikas, A. G. & Merika, A. (2016). Modelling and forecasting the demolition market in shipping. *Maritime Policy & Management*, ***43***(8): 1021-1035. https://doi.org/10.1080/03088839.2016.1185181

Karakitsos, E. & Varnavides, L. (2014). *Maritime Economics: A Macroeconomic Approach*. London: Springer. 386p.

Kavussanos, M. G. (1997). The dynamics of time-varying volatilities in different size second-hand ship prices of the dry-cargo sector. *Applied Economics*, ***29***(4): 433-443. https://doi.org/10.1080/000368497326930

Kavussanos, M. G. & Alizadeh, A. H. (2002). Efficient pricing of ships in the dry bulk sector of the shipping industry. *Maritime Policy & Management*, ***29***(3): 303-330. https://doi.org/10.1080/03088830210132588

Knapp, S., Kumar, S. N. & Remijn, A. B. (2008). Econometric analysis of the ship demolition market. *Maritime Policy & Management*, ***32***(6): 1023-1036. https://doi.org/10.1080/03088839.2016.1185181

Koopmans, T. C. (1939). *Tanker Freight Rates and Tankship Building: An Analysis of Cyclical Fluctuations*. Holland: Haarlem.

Langdana, F. K. (2009). *Macroeconomic Policy: Demystifying Monetary and Fiscal Policy* (2nd Ed.). US: Springer. 279p.

Lawson, M. (2008). If Not Now, When? Three Actions the G20 Must Take Now to Protect the Word's Poor from The Economic Crisis and Build a New Political and Economic Governance System. London: Oxfam.

Lee, J. & Strazicich, M. C. (2003). Minimum Lagrange Multiplier unit root test with two structural breaks. *Review of Economics and Statistics*, ***85***(4): 1082-1089. https://doi.org/10.1162/003465303772815961

Lee, J. & Strazicich, M. C. (2013). Minimum LM unit root test with one structural break. *Economics Bulletin*, ***33***(4): 2483-2492.

Lun, Y. V., Lai, K. H. & Cheng, T. E. (2010). *Shipping and Logistics Management*. London: Springer. 231p.

Luthra, S., Garg, D. & Haleem, A. (2015). An analysis of interactions among critical success factors to implement green supply chain management towards sustainability: An Indian perspective. *Resources Policy*, ***46***(1): 37-50. https://doi.org/10.1016/j.resourpol.2014.12.006

McConville J. (1999). *Economics of Maritime Transport, Theory and Practice*. London: Whiterby. 394p.

Metaxas, V. (1988). *Principles of Maritime Economics*. Athens: Papazisis.

Mikelis, N. E. (2007). A statistical overview of ship recycling. *Proceedings of the International Symposium on Maritime Safety, Security & Environmental Protection*, Athens, Greece.

Narayan, P. K. & Popp, S. (2010). A new unit root test with two structural breaks in level and slope at unknown time. *Journal of Applied Statistics*, ***37***: 1425-1438. https://doi.org/10.1080/02664760903039883

Pruyn, J. F. J., Van de Voorde, E., & Meersman, H. (2011). Second hand vessel value estimation in maritime economics: A review of the past 20 years and the proposal of an elementary method. *Maritime Economics & Logistics*, ***13***(2): 213-236. https://doi.org/10.1057/mel.2011.6

Sage, A. P. (1977). *Interpretive Structural Modeling: Methodology for Large-scale Systems*. New York: McGraw-Hill. 445p.

Şahin, B., Gürgen, S., Ünver, B. & Altin, I., (2018). Forecasting Baltic Dry Index by using an artificial neural network approach. *Turkish Journal of Electrical Engineering & Computer Sciences*, ***26***(3): 1673-1684. https://doi.org/10.3906/elk-1706-155

Shahbaz, M., van Hoang, T. H., Mahalik, M. K. & Roubaud, D. (2017). Energy consumption, financial development and economic growth in India: New evidence from a nonlinear and asymmetric analysis. *Energy Economics*, ***63***: 199-212. https://doi.org/10.1016/j.eneco.2017.01.023

Stopford, M. (2009). *Maritime Economics* (3rd ed.). New York: Routledge. 815p.

Strandenes, S. P. (2012). Maritime freight markets (pp. 107-120). In Walley, T.K. (Ed.), *The Blackwell Companion to Maritime Economics*. UK: Wiley-Blackwell. 735p.

Tarı, R. and İnce, M. R. (2019). Analysis of global trade volume within the scope of maritime transport market: Markov regime switching model. *Kocaeli Üniversitesi Sosyal Bilimler Dergisi*, ***37***: 1-20. https://doi.org/10.35343/kosbed.581404

Toda, H. Y. & Yamamoto, T. (1995). Statistical inference in Vector Autoregressions with Possibly Integrated Processes. *Journal of Econometrics*, ***66***: 225–250. https://doi.org/10.1016/0304-4076(94)01616-8

Tsolakis, S. (2005). Econometric analysis of bulk shipping markets: Implications for investment strategies and financial decision-making. Ph.D. Thesis. Erasmus University, Rotterdam.

Tugcu, C. T. & Topcu, M. (2018). Total, renewable and non-renewable energy consumption and economic growth: revisiting the issue with an asymmetric point of view. *Energy*, ***152***: 64–74. https://doi.org/10.1016/j.energy.2018.03.128

Tugcu, C. T., Ozturk, I. & Aslan, A. (2012). Renewable and non-renewable energy consumption and economic growth relationship revisited: evidence from G7 countries. *Energy Economics*, ***34***(6): 1942–1950. https://doi.org/10.1016/j.eneco.2012.08.021

Tunç, M. & Açık, A. (2019). The impact of steel price on ship demolition prices: Evidence from heterogeneous panel of developing countries. *Sosyoekonomi*, ***27***(42): 227-240. https://doi.org/10.17233/sosyoekonomi.2019.04.12

Tvedt, J. (2003). A new perspective on price dynamics of the dry bulk market. *Maritime Policy & Management*, ***30***(3): 221-230. https://doi.org/10.1080/0308883032000133413

Umar, M. & Dahalan, J. (2016). An application of asymmetric Toda-Yamamoto causality on exchange rate-inflation differentials in emerging economies. *International Journal of Economics and Financial Issues*, ***6***(2): 420–426.

UN. (2009). United Nations. World Economic Situation and Prospects 2009 (World Economic and Social Survey. Supplement). New York. 33p.

Yudatama, U., Hidayanto, A. N. & Nazief, B. A. A. (2018). Approach using interpretive structural model (ISM) to determine key sub-factors at factors: Benefits, risk reductions, opportunities and obstacles in awareness IT Governance. *Journal of Theoretical and Applied Information Technology*, ***96***(16): 5537-5549.

Zivot, E. & Andrews, D. W. K. (1992). Further evidence on the Great Crash, the oil-price shock, and the unit-root hypothesis. *Journal of Business & Economic Statistics*, ***20***(1): 25-44. https://doi.org/10.1080/07350015.1992.10509904

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RESEARCH ARTICLE

Length-weight relationship, sex ratio and condition factor of *Merlangius merlangus* (Linnaeus, 1758) from the Sea of Marmara, Turkey

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ABSTRACT

In this study, some biological properties of *Merlangius merlangus* (Linnaeus, 1758) were examined. In this context, relationship of length-weight, distribution of length-frequency, sex ratios and condition factors were examined. A total of 303 individuals were taken by random sampling from fishing vessels (beam trawl) in the Sea of Marmara. Minimum length and weight values of all sample individuals was found 10.0 cm and 6.6 g, maximum length 41.2 cm and 535 g was found. The average length was found 17.15±0.49 cm and the average weight was 47.03±7.57 g. Length-weight relationship for all samples as $W=0.0044TL^{3.1777}$ regression coefficient $r^2=0.98$ was determined. Growth type, positive allometric was determined for all samples and both sexes ($b>3$; $P<0.05$). The sex ratio (F:M) was calculated as 1:1.27. The mean values of condition factor (K) were calculated as 0.729±0.089, 0.733±0.091 and 0.737±0.081 for male, female and combined sex respectively. There are no significant differences between male and female ($P>0.05$).

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Introduction

Merlangius merlangus (Linnaeus, 1758), belongs to the Gadidae family and it is demersal fish species (Hureau, 1986). *M. merlangus* is one of the economically important fish species in Turkey (Sağlam and Sağlam, 2012). There are many studies on this species such as biology, population characteristics, growth parameters, diet, mortality rates and biomass in Black Sea and Aegean Sea (Samsun and Erkoyuncu, 1998; Özaydın and Taşkavak, 2006; Kalaycı et al., 2007; Ak et al., 2009; Samsun, 2010; Kasapoğlu and Düzgüneş, 2014; Mazlum and Bilgin, 2014; Samsun and Akyol, 2017; Samsun et al., 2017; Türker and Bal, 2018; Taylan et al., 2018; Aksu, 2020). However, there are a few studies on growth and biological characteristics of *M. merlangus* in the Sea of Marmara (Göksungur, 2004; Bök et al., 2011; Demirel and Dalkara, 2012).

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It is necessary to carry out such studies continuously monitoring study for fisheries management and fisheries biology the conservation of economically important species aquatic ecosystems. The population parameters such as length-weight relationship, length frequency, condition factors and biological parameters are important data sources for fisheries and fisheries management. Especially length and weight parameters gives information about the growth type of fish, whether growth is isometric or allometric (Ricker, 1975).

In this study, relationship of length-weight, distribution of length-frequency, condition factor and sex ratio of whiting were determined. It is expected that the data of the study may be source for both the fisheries of the region and fisheries science.

Material and Methods

A total of 303 samples were sampled by randomly from fishing vessels (beam trawl) and commercial fisheries in fishing season (between September and April, 2019) in the Sea of Marmara. The beam trawl had 5 m width and 50 cm mouth opening; with a cod end 32 mm mesh size. Samples, total length (cm) with 1 mm precision, weight (g) with 0.01 g accuracy recorded and macroscopically sex (female and male) was determined. $W=aL^b$ equation was used to determine the length-weight relationship (LWR) of fishes (Ricker, 1975). Where, *W* indicates the weight of the fish in g, *L* the total length in cm, *a* the condition of the fish, and *b* to the growth type of the fish. Student t test was used to determine the growth type (if; b=3, isometric; b<3, negative allometric; b>3, positive allometric) (Ricker, 1975). Fulton's condition factor was calculated (Froese, 2006).

$$K = \left(\frac{W}{L^3}\right) \times 100$$

According to the formula given above; W indicates fish weight (g), L indicates total fish length (cm).

Results

Length-Weight Relationships

The length-weight relationship was calculated according to all of the samples examined and gender groups, and it was found that the regression coefficient between the relationships was high. Growth types for each gender group and all samples were calculated and growth was found to be positive allometric for all. The results of the examples are given in Table 1.

Distribution of Length-Frequency

For all individuals, the minimum length 10 cm and 6.6 g. The maximum length 41.2 cm, maximum weight of 535 g was determined. The minimum, maximum, average and standard error values of the lengths and weights of the samples are given in Table 2.

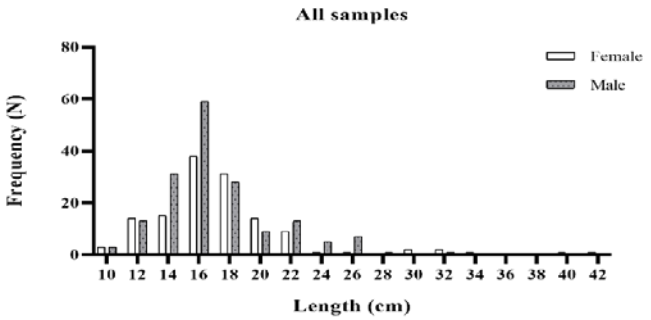

Figure 1. Distribution of length-frequency of *Merlangius merlangus*

Table 1. Parameters of length-weight relationship and growth type of *Merlangius merlangus*

Sex	N	a	b	±95% CI of b	SE(b)	r^2	Growth Type	P
F	133	0.0039	3.2281	3.152-3.303	0.038	0.9821	+ Allometric	<0.05
M	170	0.0052	3.1168	3.045-3.188	0.036	0.9776	+ Allometric	<0.05
F+M	303	0.0044	3.1777	3.122-3.224	0.026	0.9796	+ Allometric	<0.05

Note: N: number of sampling; F: female; M: male; a: intercept; b: slope of the relationship; r^2: coefficient of determination; SE(b): standard error of b; CI: confidence interval.

Table 2. Parameters of length and weight of *Merlangius merlangus*

Sex	N	TL (cm)		TW (g)	
		(Min-Max)	Mean±SE	(Min-Max)	Mean±SE
F	133	10.0-41.2	17.46±5.16	6.7-535	53.95±38.61
M	170	10.0-32.6	16.83±0.49	6.6-272.36	40.70±7.57
F+M	303	10.0-41.2	17.15±0.49	6.6-535	47.03±7.57

Note: N: number of sampling; F: female; M: male; TL: total length, cm; TW: total weight, g; SE: standard error.

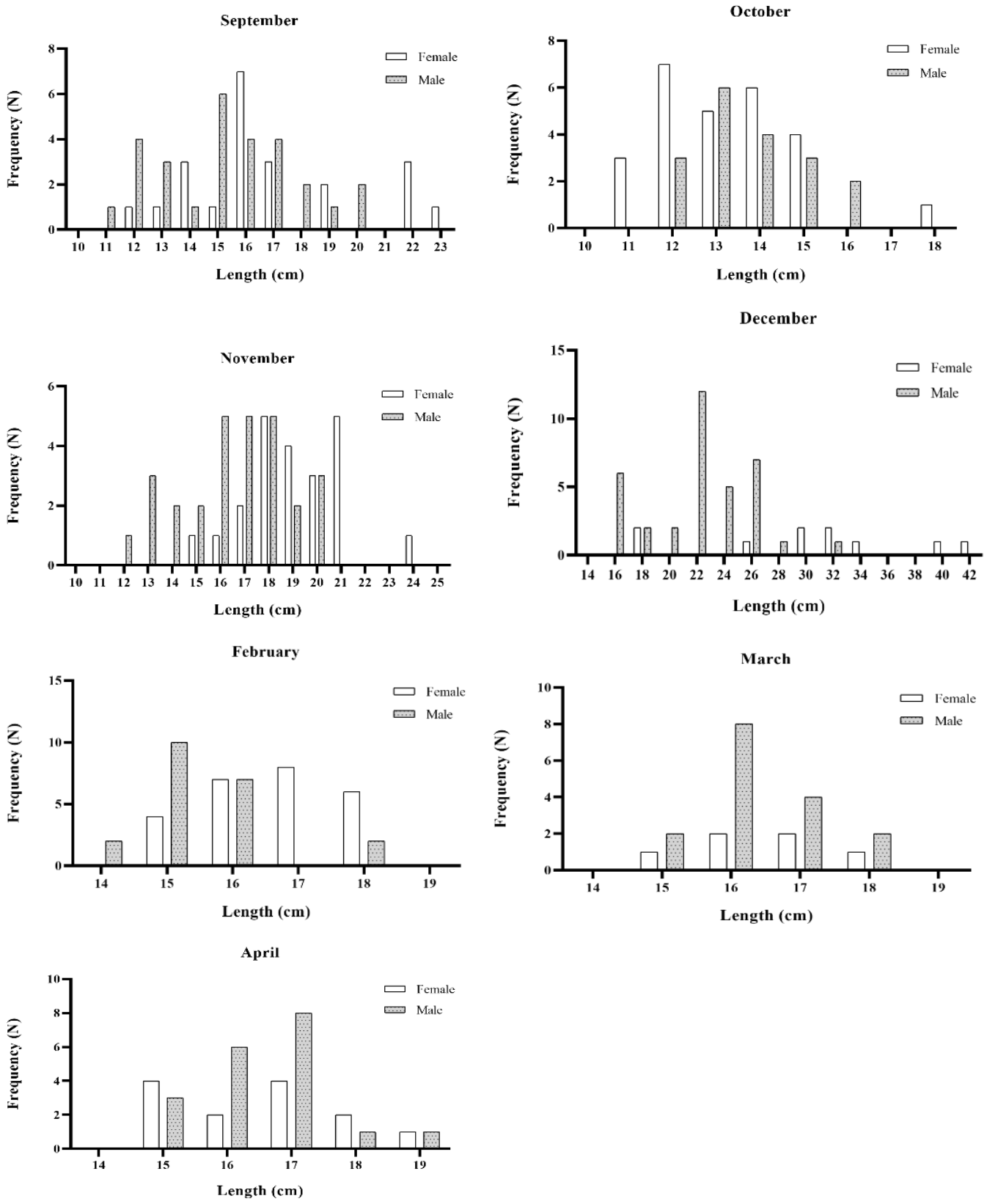

Figure 2. Distribution of length-frequency in catch seasons of *Merlangius merlangus*

It has been determined that the size distribution of the samples is generally between 14 and 18 cm. In this study, 68% of all samples are over 18 cm (Figure 1).

The length-frequency all samples and distributions according to the months were calculated and graphics were drawn (Figure 2).

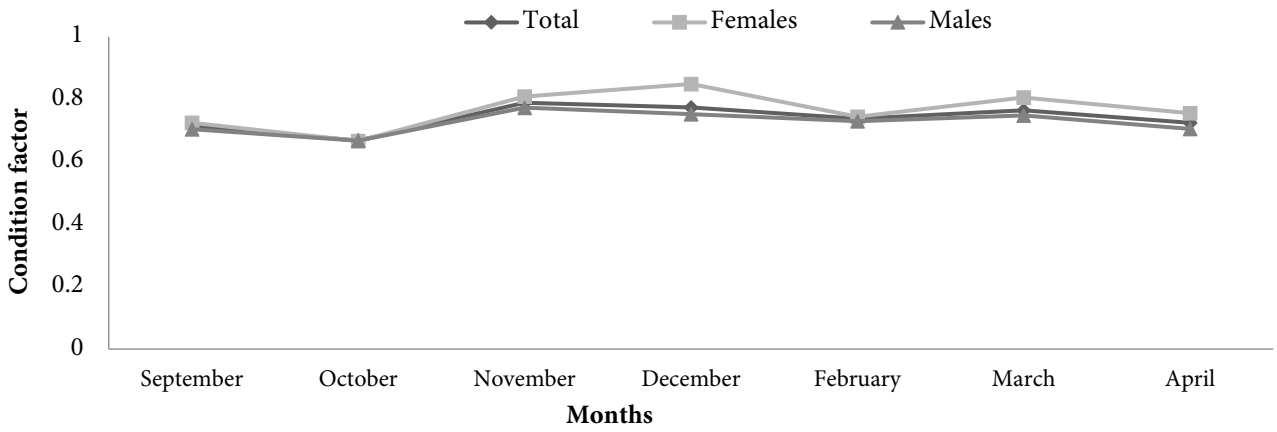

Figure 3. Condition factor of *Merlangius merlangus* in the Sea of Marmara

Table 3. Comparison parameters of length-weight relationship, growth type, sex ratio and condition factor with previous studies

Study area	N	Length (cm) (min-max)	Weight (g) (min-max)	a	b	r^2	Growth type	Sex ratio	Condition factor (K)	References
MBS	7716	3.2-25.6		0.005	3.07	0.96	A⁻			Aksu, 2020
SEBS	480	11.8-21.9	11.1-21.9							Balık and Öztaş, 2019
WBS	4003	6-25.9	1.60-135.54	0.004	3.25	0.96	A⁺	1:1.85		Yıldız and Karakulak, 2019
WBS	318	7.8-22.7	2.67-76.28	0.006	3.01	0.96	I			Türker and Bal, 2018
EBS	70	12.6-23.3	15.59-95.72	0.007	3.02	0.94				Taylan et al., 2018
CBS	1891	7.5-23.4			2.90					Samsun et al., 2017
CBS	1495	8.8-22.8	5.3-83.2	0.011	2.86	0.92	A⁻			Samsun and Akyol, 2017
EBS	140	10-27	9-118	0.013	2.77	0.91	A⁻			Çalık and Erdoğan Sağlam, 2017
BS	2292	5.9-22.2		0.005	3.15	0.92				Kasapoğlu and Düzgüneş, 2014
SEBS	598	10.6-27.4						1:1.52		Mazlum and Bilgin, 2014
SEBS	1952	11.6-30.7						1:1.35		Bilgin et al., 2012
MS	234	10.6-24.5		0.012	2.83	0.93	A⁻			Demirel and Dalkara, 2012
SEBS	1884	10.3-21	6.4-67.2	0.006	3.04	0.88	I	1:1.38		Sağlam and Sağlam, 2012
MS	166			0.004	3.14		A⁺			Bök et al., 2011
MBS	2238	8.4-31.5	3.35-259	0.004	3.20	0.97	A⁺	1:1.15		Samsun, 2010
EBS	943	6.7-29.5	2.15-241.2	0.004	3.16	0.98	A⁺	1:1.95		Ak et al., 2009
MBS	904	7.7-22.7	2.99-79.79	0.006	3.02	0.96	I			Kalaycı et al., 2007
EAS	100	16.0-31.7	30.27-229.37		2.94	0.96				Özaydın and Taşkavak, 2006
MBS		8.4-31.5		0.004	3.20			1:1.15		Samsun, 2005
MS	920	7-25	5.2-84.6	0.005	3.14			1:1.55	0.746	Göksungur, 2004
EBS	1730	11-30.40	8.23-283.80					1:1.84		Çiloğlu et al., 2001
MBS	1302	9.0-24.0	5.7-118.7	0.003	3.24	0.94	A⁺	1:1.17	0.740	Samsun and Erkoyuncu, 1998
MBS					3.19				0.740	Samsun et al., 1994
MS	303	10.0-41.2	6.6-535	0.004	3.17	0.97	A⁺	1:1.27	0.737	Present study

Note: MS: Marmara Sea; BS: Black Sea; MBS: Middle Black Sea; WBS: Western Black Sea; EAS: Eastern Aegean Sea; CBS: Central Black Sea; SEBS: South-Eastern Black Sea; EBS: Eastern Black Sea.

Sex Ratio

A total of 303 specimens of *Merlangius merlangus* consisting of 133 females (10.0–41.2cm; 17.46±5.16 TL) and 170 males (10.0–32.6 cm; 16.83±0.49 TL) were examined from the Sea of Marmara. The sex ratio of male: female=1:1.27 was significantly different from 1:1 ($P<0.05$).

Condition Factors

The mean highest condition factor was recorded 0.849 in December for females, 0.773 in September for males. The mean low condition factor was recorded 0.668 in October for females, 0.667 in October for males. The mean values of condition factor (K) were calculated as 0.729±0.089, 0.733±0.091 and 0.737±0.081 for male, female and combined sex respectively (Figure 3). There are no significant differences between male and female ($P>0.05$).

Discussion

Length-weight relationship (LWR) is very important data sources for fisheries. It has many applications in fishery management, ecological studies such as estimating the condition, feeding and spawning (Gonçalves et al., 1997; Stergiou and Moutopoulos, 2001). Although, there are many study on whiting especially Black Sea and Aegean Sea, there is a few information on growth and biological characteristics of *Merlangius merlangus* in the Sea of Marmara (Göksungur, 2004; Bök et al., 2011; Demirel and Dalkara, 2012).

The growth pattern (b) typically varies between 2.0 and 3.5 (Froese and Pauly, 2010).Values of b changeable in different population of same fish species. The growth pattern (b) depends on temperature, salinity, food, conditional of environmental conditions (predation) sex and stage of maturity (Ricker, 1973; Freitas et al., 2017). In this study, the growth pattern (b) of all samples positive allometric ($b>3$; $P<0.05$) was found. It is similar to the Bök et al., (2011). However, Demirel and Dalkara, (2012) reported that they found the b value of this species to be negative allometric ($b<3$; $P<0.05$). It has been determined that this aspect is not similar to our study results.

In previous studies, growth pattern of b values respectively as five positive allometric (Samsun and Erkoyuncu, 1998; Ak et al., 2009; Samsun, 2010; Bök et al., 2011; Yıldız and Karakulak, 2019), four negative allometric (Demirel and Dalkara, 2012; Samsun and Akyol, 2017; Çalık and Erdoğan Sağlam, 2017; Aksu, 2020) and tree isometric (Kalaycı et al., 2007; Sağlam and Sağlam, 2012; Türker and Bal, 2018) were determined (Table 3).

The growth pattern b value was not significantly ($b=3$; $P>0.05$) differences to the 3 (Kalaycı et al., 2007; Sağlam and Sağlam, 2012; Türker and Bal, 2018).

Growth pattern (b), range from 2.77 (Çalık and Erdoğan Sağlam, 2017)-3.24 (Samsun and Erkoyuncu, 1998). It's estimated that the reason for the difference in b value between stocks in different regions and unsuitable environmental conditions due to overfishing.

The coefficient of determination (r^2) in previous study range from 0.88-0.98 and highly significant ($P<0.05$) (Samsun and Erkoyuncu, 1998; Göksungur, 2004; Özaydın and Taşkavak, 2006; Kalaycı et al., 2007; Ak et al., 2009; Samsun, 2010; Bök et al., 2011; Sağlam and Sağlam, 2012; Demirel and Dalkara, 2012; Bilgin et al., 2012; Mazlum and Bilgin, 2014; Kasapoğlu and Düzgüneş, 2014; Çalık and Erdoğan Sağlam, 2017; Samsun and Akyol, 2017; Samsun et al., 2017; Taylan et al., 2018; Türker and Bal, 2018; Aksu, 2020). The coefficient of determination (r^2) very high in present study also ($P<0.05$).

The result about sex ratio in present study is also similar by Erkoyuncu, (1998) and Bilgin et al., (2012) in the Black Sea. But different from the F:M ratio found by Ak et al., (2009) as 1.00:1.95. Condition factor in previous studies, Samsun et al. (1994) 0.740, Samsun and Erkoyuncu (1998) 0.740, and Göksungur (2004) 0.746 values similar to in present study (0.737). These minor differences are thought to be due to changes in the ecosystem. The parameters obtained from LWR, sex ratio and condition factor for previous study is showed in Table 3.

Conclusion

In present study, it has been determined that the size distribution of the samples is generally between 14 cm and 18 cm. The absence of specimens larger than 18 cm indicates a prey pressure on the species. The regular monitoring studies are essential to understand the dynamics of exploited whiting stocks under the pressure of environmental changes in the Sea of Marmara ecosystem. Therefore, whiting fishery needs new regulation measures and management plans for the sustainable fisheries. It is expected that the data of the study may be source for both the fisheries of the region and fisheries science.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval

For this type of study, formal consent is not required.

References

Ak, O., Kutlu, S. & Aydın, İ. (2009). Length-weight relationship for 16 fish species from the Eastern Black Sea, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, ***9***(1): 125-126.

Aksu, H. (2020). Estimation of some growth parameters using length frequency data of whiting (*Merlangius merlangus euxinus*, Nordmann 1840), by trawl fishing in Black Sea (Sinop, Turkey). *Journal of New Results in Science*, ***9***(2): 39-45.

Balık, İ. & Öztaş, M. (2019). Comparison of length-weight relationships for whiting, *Merlangius merlangus* (Linnaeus, 1758) caught from three different areas of the south-eastern Black Sea. *Su Ürünleri Dergisi*, ***36***(1): 57-63. https://doi.org/10.12714/egejfas.2019.36.1.07

Bilgin, S., Bal, H. & Taşçı, B. (2012). Length based growth estimates and reproduction biology of whiting, *Merlangius merlangus euxinus* (Nordman, 1840) in the southeast Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, ***12***(4): 871-881. https://doi.org/10.4194/1303-2712-v12_4_15

Bök, T. D., Göktürk, D., Kahraman, A. E., Alicli, T. Z. & Acun, T. (2011). Length-weight relationships of 34 fish species from the Sea of Marmara, Turkey. *Journal of Animal and Veterinary Advances*, ***10***(23): 3037-3042.

Çalık, S. & Erdoğan Sağlam, N. (2017). Length-weight relationships of demersal fish species caught by bottom trawl from Eastern Black Sea (Turkey). *Cahiers de Biologie Marine*, ***58***: 485-490.

Çiloğlu, E., Şahin, C., Zengin, M. & Genç, Y. (2001). Determination of some population parameters and reproduction period of whiting (*Merlangius merlangus euxinus* Nordmann, 1840) on the Trabzon-Yomra coast in the eastern Black Sea. *Turkish Journal of Veterinary and Animal Sciences*, ***25***(6): 831-837.

Demirel, N. & Dalkara, E. M. (2012). Weight-length relationships of 28 fish species in the Sea of Marmara. *Turkish Journal of Zoology*, ***36***(6): 785-791.

Erkoyuncu, İ., Erdem, M., Samsun, O., Özdamar, E. & Kaya, Y. (1994). A Research on the determination of meat yields, chemical composition and weight-length relationship of some fish species caught in the Black Sea. *İstanbul University Journal of Aquatic Products*, ***8***(1-2): 181-191 (In Turkish).

Freitas, T. M. S., Souza, J. B. S, Prudente, B. S. & Montag, L. F. A. (2017). Length-weight relationship in ten fish species from the Nhamundá River, the Amazon Basin, Brazil. *Acta Amazonica*, ***47***(1): 75-78. https://doi.org/10.1590/1809-4392201601272

Froese, R. (2006). Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*, ***22***(4): 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x

Froese, R. & Pauly, D. (2010). Fishbase World Wide Web Electronic Publication. Retrieved on September 09, 2018 from http://www.fishbase.org

Göksungur, E. G. (2004). Marmara denizi mezgit (*Merlangius merlangus euxinus* nordmann, 1840) balığının biyolojisi. Ph.D. Thesis. Marmara University, İstanbul, Turkey.

Gonçalves, J. M. S., Bentes, L., Lino, P. G., Ribeiro, J., Canario, A. V. & Erzini, K. (1997). Weight-length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. *Fisheries Research*, ***30***(3): 253-256. https://doi.org/10.1016/S0165-7836(96)00569-3

Hureau, J. C. (1986). Scorpaenidae. *Fishes of the North-eastern Atlantic and the Mediterranean*, ***3***: 1211-1229.

Kalaycı, F., Samsun, N., Bilgin, S. & Samsun, O. (2007). Length-weight relationship of 10 fish species caught by bottom trawl and midwater trawl from the middle Black Sea, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, ***7***: 33-36.

Kasapoğlu, N. & Düzgüneş, E. (2014). Length-weight relationships of marine species caught by five gears from the Black Sea. *Mediterranean Marine Science*, ***15***(1): 95-100. https://doi.org/10.12681/mms.463

Mazlum, R. E. & Bilgin, S. (2014). Age, growth, reproduction and diet of the whiting, *Merlangius merlangus euxinus* (Nordmann, 1840), in the southeastern Black Sea. *Cahiers de Biologie Marine*, ***55***:463-474.

Özaydin, O. & Taşkavak, E. (2006). Length-weight relationships for 47 fish species from Izmir Bay (eastern Aegean Sea, Turkey). *Acta Adriatica: International Journal of Marine Sciences*, ***47***(2): 211-216.

Ricker, W. E. (1973). Linear regressions in fishery research. *Journal of the fisheries board of Canada*, ***30***(3): 409-434. https://doi.org/10.1139/f73-072

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, ***191***: 1-382.

Sağlam, N. E. & Sağlam, C. (2012). Population parameters of whiting (*Merlangius merlangus euxinus* L., 1758) in the South-Eastern Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, ***12***(4): 831-839. https://doi.org/10.4194/1303-2712-v12_4_11

Samsun, N. & Erkoyuncu, İ. (1998). Investigation of some parameters related to fishery biology of whiting (*Gadus merlangus euxinus* N. 1840) caught with bottom trawls in Sinop Region (Black Sea). *Ege Journal of Fisheries Aquatic Sciences*, ***15***(1-2): 19-31.

Samsun, O. & Akyol, O. (2017). Exploitation rate of whiting, *Merlangius merlangus* (Linnaeus, 1758) in the Central Black Sea, Turkey. *Türk Denizcilik ve Deniz Bilimleri Dergisi*, ***3***(1): 20-26.

Samsun, O., Akyol, O., Ceyhan, T. & Erdem, Y. (2017). Length-weight relationships for 11 fish species from the Central Black Sea, Turkey. *Su Ürünleri Dergisi*, ***34***(4): 455-458. https://doi.org/10.12714/egejfas.2017.34.4.13

Samsun, O., Özdamar, E. & Aral, O. (1994). Fisheries biology of whiting (*Gadus merlangus euxinus*. Nord. 1840) sampled by bottom trawls in the Central Black Sea. *Ege Üniversitesi Fen Fakültesi Dergisi*, ***16***(1): 1003–1011.

Samsun, S. (2005). Mezgit balığının (*Gadus merlangus euxinus* Nordmann, 1840) bazı üreme ve beslenme özellikleri üzerine bir araştırma. MSc. Thesis. Ondokuz Mayıs University, Samsun, Turkey.

Samsun, S. (2010). 2001-2003 Av sezonunda orta Karadeniz'deki mezgit balığının (*Merlangius merlangus* Linnaeus, 1758) bazı populasyon parametrelerinin belirlenmesi. *Fırat University Journal of Science*, ***22***(1): 47-57.

Stergiou, K. I. & Moutopoulos, D. K. (2001). A review of length-weight relationships of fishes from Greek marine waters. *Naga, the ICLARM Quarterly*, ***24***: 23-39.

Taylan, B., Gürkan, S., Taskavak, E., & Uncumusaoglu, A. A. (2018). A preliminary study of fecundity of whiting, *Merlangius merlangus euxinus* (Linnaeus, 1758) in coast of Tirebolu (Eastern Black Sea). *Turkish Journal of Agriculture-Food Science and Technology*, ***6***(3): 322-325. https://doi.org/10.24925/turjaf.v6i3.322-325.1700

Türker, D. & Bal, H. (2018). Length–weight relationships of 13 fish species from the western Black Sea (Zonguldak-Amasra), Turkey. *Journal of the Black Sea/Mediterranean Environment*, ***24***(2): 115-127.

Yıldız, T. & Karakulak, F. S. (2019). Age, growth and mortality of whiting (*Merlangius merlangus Linnaeus*, 1758) from the western Black Sea, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, ***19***(9): 793-804. https://doi.org/10.4194/1303-2712-v19_9_08

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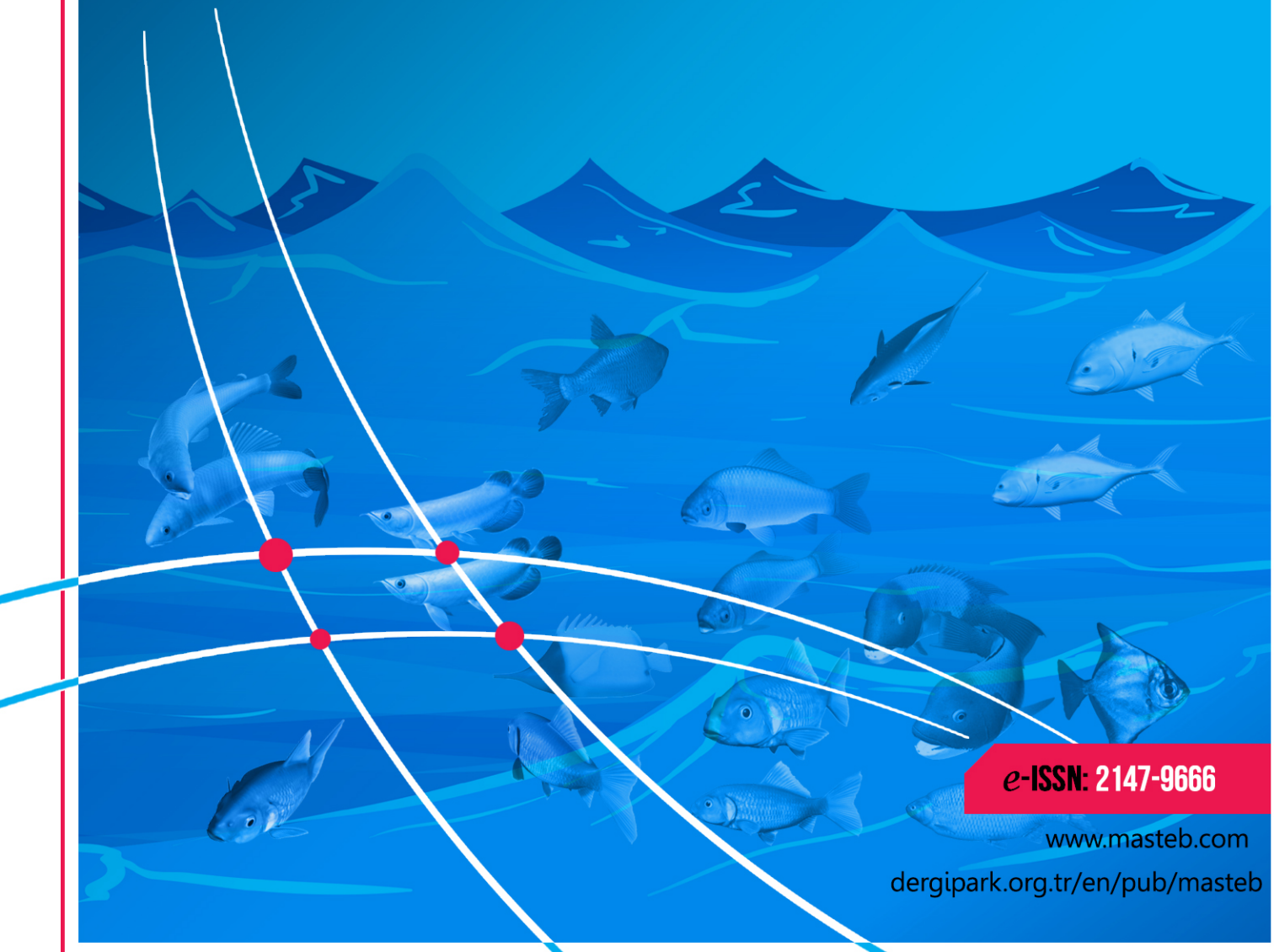

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