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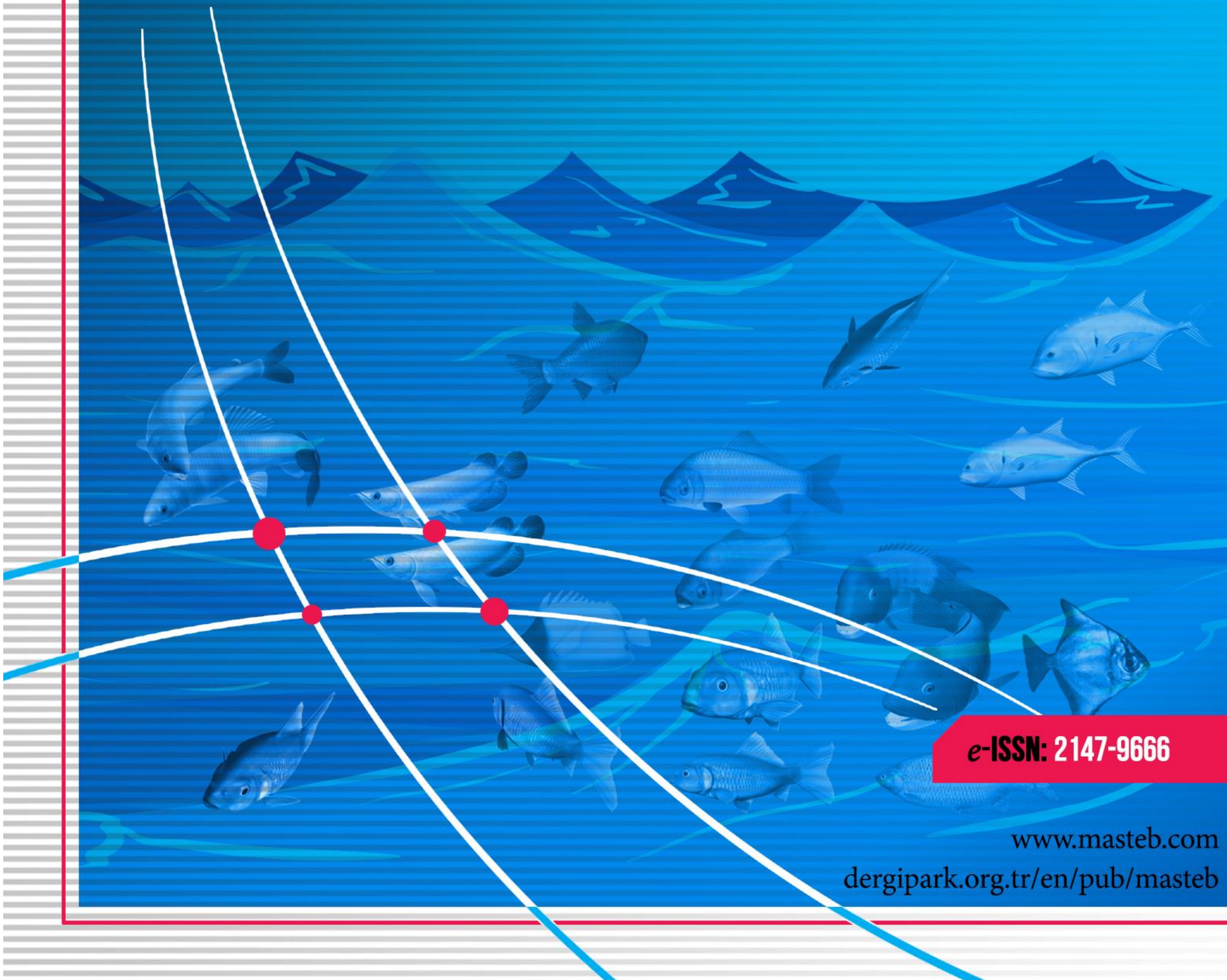

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Marine Science and Technology Bulletin

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Marine Science and Technology Bulletin

RESEARCH ARTICLE

An adapted slipping process to exclude jellyfish in the Sea of Marmara purse seine fishery

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ABSTRACT

Excluding the jellyfish from the bunt-end is a common slipping process used in the Sea of Marmara purse seine fishery. For this aim, a sheet of netting piece, larger mesh size and thicker diameter, is rigged on the bunt-end of the purse seine net. The jellyfish mass on the netting piece are slipped by rolling over the headline (floating line) after partially hauling or drying-up the net while it is still in the water. In this study, the catch amount of this slipping was roughly estimated with the introduction of the slipping process only used by the purse seiners in the Sea of Marmara. There were eight successful purse seine operations conducted between 8 and 11 September 2018 in depth ranged 77 to 677 m. The percentage of landed species versus to jellyfish varied between 23% and 85%. The mean landed anchovy amount is 4379 (±3756.6) kg for per operation. The mean slipped amount of jellyfish is 3812.5 (±2404.4) kg. However, both anchovy (99.8%) and jellyfish (96.3%) are the vast majority species that landed and slipped, respectively. In the operations totally 100 boxes of anchovy (1180 kg) unintentionally was slipped with the jellyfish. In addition, two sharks with larger size were slipped to the sea as alive over the floating line of the net. Although slipping practised rarely in Turkey, all the purse seiner in the Sea of Marmara have to use the adapted slipped process to get rid of jellyfish. However, there are no records and scientific findings regarding slipped amount of the jellyfish. For this reason, this study is important to presented preliminary results regarding amount of the jellyfish. In conclusion, this study is extended completely the Sea of Marmara practised to understand the dimensions of jellyfish amount and slipping process.

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Introduction

Purse seining is energy efficient, environmentally friendly fish capture methodology generally targeting large and small pelagic fish shoals (Handegard et al., 2017) with sonar detection, light sources or Fish Aggregation Devices (FAD). Purse seining solely accounts for about 30% of the world's total fisheries catch (Watson et al., 2006) and approximately three out of four domestic catch of Turkey (TurkStat, 2018). The fishing operations is performed in the Sea of Marmara by 122 purse seiners registered to the various ports and the amount of landings according to the record of the 2017 fishing season varies between 20000-25000 tons (TurkStat, 2018). Anchovy (*Engraulis encrasicolus*) is dominated fish species landed with 8340 tons. It is followed by European pilchard (*Sardina pilchardus*) with 5685 tons, two kind of horse mackerels (*Trachurus trachurus, T. mediterraneus*) with 4447 tons, Atlantic bonito (*Sarda sarda*) with 1103 tons, bluefish (*Pomatomus saltatrix*) with 720 tons, mackerel (*Scomber scombrus*) with 287 tons, grey mullet species with 239 tons, chub mackerel (*Scomber japonicus*) with 147 tons and garfish (*Belone belone*) with 93 tons. Almost all of these landed species are captured by the purse seiners.

The Sea of Marmara, connected to the Black Sea and the Aegean Sea by the Straits of Istanbul and Dardanelles, is an inland sea that forming a transition zone and one of the most important migratory routes between the Black Sea and the Aegean Sea for many commercially valuable species (Yıldız and Karakulak, 2016). Additionally, due to the high level of nutrients and plankton abundance, it is reproduction and growth area for many species (Yüksek, 2016).

In recent years, the warning signs of ecological deterioration such as algal blooms, fishery collapse, massive mucilage events, and jellyfish blooms have increased significantly in the Sea of Marmara (İşinibilir and Yılmaz, 2016). Overfishing as a major anthropogenic impact that causes increasing jellyfish blooms through removing jellyfish predators and competitors (Daskalov et al., 2007). Latest studies also showed that quantities of jellyfish have been increasing in the Sea of Marmara in the last decades and *Aurelia aurita* is the most important jellyfish species for the basin with yearlong occurrence and prolonged blooms with higher biomass (İşinibilir and Yılmaz, 2016).

Despite advances in sonar technology and highly experienced fishermen, it is currently difficult to determine a reliable quantity and a size-range for the fish before the catch has been densely crowded at the end of the haul (Breen et al., 2012). Purse seines, particularly for small species, are generally considered to be a non-selective fishing gear once a target shoal has been encircled, primarily because of the small mesh sizes used in the main body of the net, typically lower 20 mm (Marçalo et al., 2019). Therefore, the release of unwanted catch (UWC) generally happens by "slipping" all or part of the UWC out of the net while it is still in the water or by "discarding", when the catch is taken aboard and any unwanted components are removed and returned to the sea alive or dead (Marçalo et al., 2019).

Releasing or slipping is not a new strategy to remove unwanted catch from the purse seine net. Slipping of the entire or portion of the catch from the purse-seine is a common practice method due to a variety of economic (catch quality, market price/demand) and regulatory (quotas, sizes, protected species) drivers in European Atlantic waters (Marçalo et al., 2019). Due to aforementioned reasons, there has been a common practice of discarding unwanted catch by *rolling* the fish over the headline of purse seine net in British mackerel fishery (Lockwood et al., 1983), in western Australian Sardinops (*Sardinops sagax*) fishery (Mitchell et al., 2002), in Norwegian mackerel (Huse and Vold, 2010) and herring (*Clupea harengus*) (Tenningen et al., 2012) fisheries, in Spanish anchovy fishery and Portuguese sardine fishery (Marçalo et al., 2019). In these processes, entire or part of the catch is released by rolling the fish over the headline (floating line) of the net after partially hauling or drying-up the net. However, a modified slipping procedure is developed by Marçalo et al. (2018) enable the fish to escape through an opening (escape window) created by adding weights to the float line. In this procedure, the net was bunted (manually hauled) and 4±5 sets of 10 kg weights were put along the headline to form an escape opening. In the slipping, sardines freely swimming out of the net through the opening.

When the slipping not performed in good conditions, it can cause highly significant mortality in released fish. Unacceptability high rates of mortality resulted by the conventional slipping for mackerel (Lockwood et al., 1983; Huse and Vold, 2010), herring (Tenningen et al., 2012), sardine (Stratoudakis and Marçalo, 2002; Marçalo et al., 2006; Marçalo et al., 2010) and Sardinops (Mitchell et al., 2002). However, various modified slipping procedures tested by Huse and Vold (2010) and Marçola et al. (2018) significantly reduced the highly significant mortality.

Excluding a substantial amount of jellyfish is a common slipping process in the Sea of Marmara purse seine fishery (Tosunoğlu and Kasapoğlu, 2019). Experienced skippers of the purse seiners have developed a different slipping process to exclude jellyfish in the Sea of Marmara for a long time. For this aim, a sheet of netting piece, larger mesh size, and thicker diameter, rigged on the bunt-end of the net. First of all, jellyfishes are intensified in the bunt of the net, just like fish and bycatch with the crew hauling the net aboard the vessel by manually. When reached the netting piece, the crew haul only the netting so that fishes pass the larger mesh while jellyfish retained on the net. At the latest stage, the jellyfish on the netting piece is slipped by rolling over the headline (floating line) of the net after partially hauling or drying-up the net while it is still in the water (Figure 1). After the slipping finalized, the netting sheet is opened from side-end and the fish pump is submerged to concentrated fish in the bunt-end section for the transfer of the fish to the deck of vessel. The main purpose of the study is to introduce the slipping process only used by the Sea of Marmara purse seine fishery. In addition, it is roughly to estimate about the amount of slipped jellyfish.

Figure 1. Jellyfish slipping over the headline from the bunt-end section of a purse seine net in the Sea of Marmara

Material and Methods

In the Sea of Marmara purse seine fishery, a shoal of fish is commonly aggregated to artificial light or rarely detected by the sonar. The season of fishing with light is restricted between 15 September and 31 October. Once, the skipper evaluates the fish composition, shoal size, and chances of capturing the aggregation, and then the purse seine vessel sets the net around the aggregated fish to quickly encircle them within the purse seine net. When the shape of net becomes a circular, purse seine vessel and skiff come back together and the gear is closed by hauling the purse line running through the rings at the bottom of the net (pursing). Then, until the volume of the net becomes smaller, the net is pulled out of the water and stacked back on the deck of fishing boat with the aid of the hydraulic power block and the crew (hauling). The concentrated fish (dense mass) in the section of net which is hauled last (bunt-end) in is transferred by a fish pump to the sieve on the deck of the vessel. This final stage of hauling (crowding in the bunt) is often done manually (i.e. with the crew hauling the net aboard the vessel by hand). After the catch is inspected, if the decision is made to harvest it, it is then transferred it into the boat. If the catch volume is lower, a larger scoop called kital or netting sheet is used by driven of a winch otherwise fish pump is engaged.

Table 1. Details of the purse seine operations in the Sea of Marmara

Date	Operation	Vessel	Coordinates	Depth (m)	Shoal depth (m)	Light duration (min)	Wind	Net length (m)	Net depth (m)
8-9.10.2018	1	A	40°44'073"N 29°17'237"E	366	10-40	20.30-12.50 (260)	North 2-4	840	146
	2	A	40°44'668"N 29°17'486"E	201	10-30	01.15-03.15 (120)	North 2-4	840	146
	3	A	40°41'134"N 29°16'215"E	397	10-20	05.15-05.45 (30)	North 1-2	840	146
9-10.10.2018	1	B	40°53'142"N 28°40'848"E	677	10-40	22.00-01.00 (180)	North 2-4	600 (720)	164
	2	B	40°52'543"N 28°41'062"E	183	10-20	04.00-05.15 (75)	North 2-4	600 (720)	164
	1	C	40°47'238"N 29°09'917"E	220	10-20	22.30-00.30 (120)	North 3-5	600 (800)	164
	2	C	40°46'164"N 29°10'273"E	329	10-20	01.15-03.15 (120)	North 2-4	600 (800)	164
10-11.10.2018	1	B	40°56'575"N 28°44'802"E	92	10-20	01.10-03.10 (120)	North 2-4	600 (720)	164
	2	B	40°55'691"N 28°43'936"E	77	10-30	04.30-06.30 (120)	North 2-4	600 (720)	164

There were eight successfully purse seine operation realized during the study period in the Sea of Marmara (Figure 2). However, one operation was abandoned due to aggregation of the fish shoal under the light vessel was not enough amount to catch it. Stages of operation in the first paragraph, details regarding the purse seine operations and vessels characteristics used are given in Table 1 and Table 2, respectively.

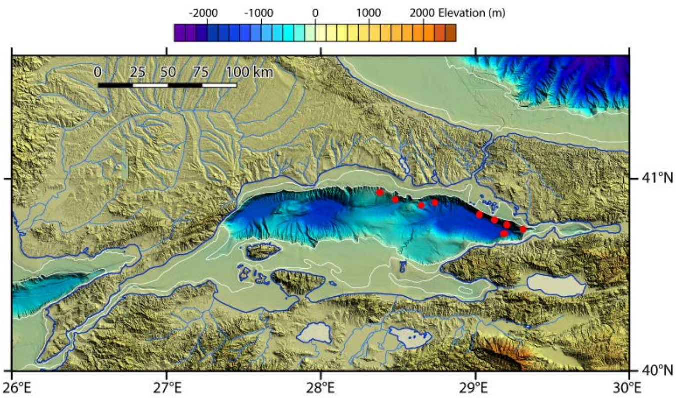

Figure 2. Purse seine operation locations realized in the Sea of Marmara

The operating depths were greater than the depth of purse seine net in seven operations whereas only two were shallower (Table 1). Captured shoals generally detected at 10-20 m depth ranges in water column by echo-sounder. During the study period, the moon was in the dark phase. For this reason, the duration of the artificial light was changed between 30 and 180 min generally 120 min. Four operations were realized by the purse seiner B and her light vessel Bb, three operations by A and Aa and two operations C and Cc (Table 1 and Table 2). The full lengths of the nets in many operations were not used depending on the size of the shoals aggregated under the light vessel.

On the contrary to traditional using in the Sea of Marmara, a slipping method was adapted in purse seine fishing targeted anchovy, horse mackerels and bluefish to release jellyfish by scrolling a special netting sheet from the bunt-end section of the net which called *pelte* net in the Turkish fishermen. Details of the netting piece (drawn in green) rigged over the bunt-end were given without scale for only anchovy purse seine in figure 3. The *pelte* net consists of a 45×45 m square netting piece. The mesh size and thickness of the net is 68 mm and 210d/72 no, respectively. Thanks to *pelte* net, larger sizes of commercial fish, vulnerable species (shark and rays) and cetacean can pass through the meshes of the net and easily slipped by the net and roll over the floating line of the purse seine net. All the sides of net except left are rigged strictly to the bunt-end. Left side is always open, because the net is opened from this side and aggregated fish in the bunt-end is transferred by a fish pump on the deck of the purse seiner, easily.

The mesh size of the purse seine nets was 15 mm because the size of the targeted fish anchovy was a small pelagic. To estimate total catch of anchovy, styrofoam fish boxes filled with the fishes were converted to kg (each of 15 kg). In addition, the amount of jellyfish estimated visually confirmed by experienced skipper and crew just like Huse and Vold (2010). Difference between landed and slipped fish amounts was assessed using a Student's t-test.

Table 2. Technical characteristics of the purse seiners and light vessels

	Main Vessel	Light Vessel	Main Vessel	Light Vessel	Main Vessel	Light Vessel
	A	*Aa*	*B*	*Bb*	*C*	*Cc*
LOA (m)	39.6	10.7	23.9	12.7	36.0	11.2
Width (m)	8.0	2.9	11.0	4.6	9.2	8.4
Draft (m)	3.1	1.1	2.7	1.4	2.7	1.0
Gross tonnage (GRT)	213	7.17	202	14.08	189	9.5
Main engine (kW)	581.88	126.87	448.51	104.44	601.00	100.7
Auxiliary engine (kW)	487.14				434.17	
Generator (kW)	406.57	44.70	340.30	20.00	402.84	20.00
Port of Registry	Istanbul	Yalova	Gemlik	Gemlik	Yalova	Yalova
Building Year	1989	1987	2018	2006	1993	1988

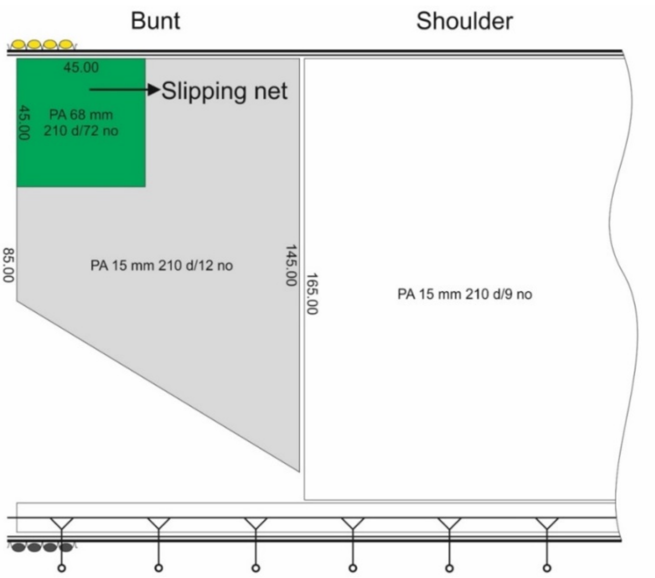

Figure 3. Slipping netting piece (in green square) rigged on bunt-end of the purse seine net

Results

The percentage of landed species versus to jellyfish varied between %23 and %85 and mean percentage of landed fish is nearly same like slipped jellyfish. There was no statistical difference between amount of landed and slipped fish ($p=0.790$). The maximum landed amount of fish is 10755.8 kg, whereas slipped jellyfish is 7800.0 kg. The percentage of jellyfish amount is not same for all the operations (Table 3).

Table 3. Catch amounts and percentages of landed and slipped fishes in the Sea of Marmara purse seine fishery

	Catch Amount (kg)			Percent (%)	
Haul	*Landed*	*Slipped*	*Total*	*Landed*	*Slipped*
1	1519.9	5021.5	6541.4	23	77
2	1655.6	4510.6	6166.2	27	73
3	9804.8	3150.0	12954.8	76	24
4	3400.4	600.0	4000.4	85	15
5	2311.1	7800.0	10111.1	23	77
6	10755.8	6103.3	16859.1	64	36
7	1470.0	1200.0	2670.0	55	45
8	4200.2	3301.2	7501.4	56	44
Total	35117.7	31686.6	66804.2	53	47

Anchovy is the most important landed fish species for the Sea of Marmara purse seine fishery in this period. The mean landed amount is 4379 (±3756.6) kg for per operation. The mean slipped amount of jellyfish is 3812.5 (±2404.4) kg. However, both anchovy and jellyfish are the vast majority species that landed and slipped, respectively (Table 4).

While anchovy constituted 99.8% of the landed species, slipped jellyfish percentage was 96.3% (Figure 4). In the beginning of fishing season anchovy is the only dominated fish species that targeted by the purse seiners. Very little amount (86 kg) of horse mackerel, Atlantic bonito, garfish, sprat, sardine, bluefish etc. caught as by-catch (Table 5). Approximately 100 boxes of anchovy (1180 kg) also slipped with the jellyfish releasing operations. In the operations larger size two shark individuals and bonito individuals were seen over the netting sheet. While the bonito individuals collected by a scoop net over the netting piece, the sharks were slipped to the sea over the floating line of the net.

Table 4. Descriptive statistics of the landed (anchovy) and slipped (jellyfish) fishes in the Sea of Marmara purse seine fishery

Statistics	Anchovy	Landed	Jellyfish	Slipped	Total
Mean	4379.0	4389.7	3812.5	3960.8	8350.5
Standard error	1328.2	1332.4	850.1	853.2	1672.5
Standard deviation	3756.6	3768.7	2404.4	2413.2	4730.6
Range	9230	9286	7000	7200	14189
Minimum	1470	1470	500	600	2670
Maximum	10700	10756	7500	7800	16859
Total	35032	35118	30500	31687	66804

Table 5. Landing and slipped amounts of fish species caught by purse seiners in the Sea of Marmara

Landed		Slipped	
Species	*Amounts (kg)*	*Species*	*Amounts (kg)*
Anchovy	35032.0	Jellyfish	30500.0
Horse mackerel	72.1	Anchovy	1180.0
Atlantic bonito	5.5	Atlantic bonito	4.3
Garfish	2.9	Shark	2.3
Sprat	1.8		
Sardine	1.7		
Bluefish	1.0		
Chub mackerel	0.3		
Round sardine	0.2		
Golden mullet	0.2		
Whiting	0.1		
Seahorse	0.02		
Total	35117.7	Total	31686.6

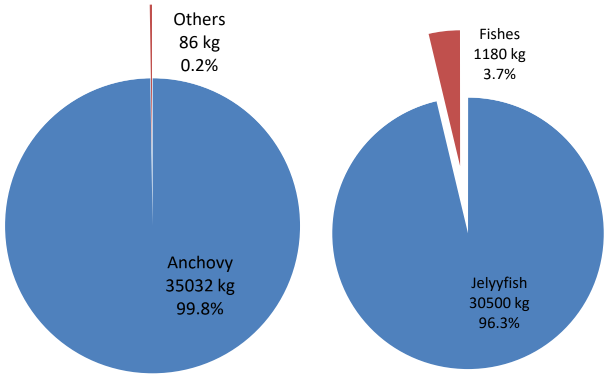

Figure 4. Landed (a) and slipped (b) amounts and percentages of fishes

Discussion

In this study, a different slipping procedure to exclude jellyfish from the captured commercial fish in the bunt-end of the purse seine net was investigated for the first time in the Sea of Marmara. The purpose of the slipping process is totally different from conventional slipping procedure used by North Atlantic countries e.g. Norway, Portugal and Spain (Marçalo et al., 2019). In an ordinary or modified slipping procedure, the crowded fishes in the bunt-end of a purse seine net are released over the headline of the net for the market (quality) and regulation (size and quota) reasons. However, in this procedure that used only in the Sea of Marmara as far as we know, the unwanted jellyfish excluded by roll over headline of the bunt end of the purse seine net by a special netting which has a larger mesh size and thicker diameter. All the purse seiners occupy only in the Sea of Marmara have to use the special netting for operational and handling reasons. Because, it is impossible that commercial catch does not separate from jellyfish when the mixed fish and jellyfish directly goes pump to the deck by purse seiner.

In the excluding operations, a little amount of commercial catch (anchovy) released over the headline of the purse seine nets. However, this amount is very small when compared to the total jellyfish amount (approximately 1/30). In the meanwhile, larger sizes commercial species such as Atlantic bonito and unwanted species shark were seen over the netting piece before slipping practise started. Excluding of jellyfish started after individuals of bonitos were picked up by a scoop in a short time (2 min). Sharks were seen alive during the slipping process. Although this process thought to be useful for the survival of the larger vulnerable species, the complete pulling up and ultimately slipping over the headline causes more physical injury and reduces the probability of survival of slipped small size pelagic fish (Marçalo et al., 2018). Since survival of the jellyfish and little amount of anchovy after excluded from bunt-end of the purse seine net is not known, this issue should be examined by scientific methods. Slipping practises are rarely applied by the Aegean purse seiner in Turkey for regulation and market reasons. However, there is no information regarding slipping amount and the survival rate of the species caught by the purse seiner.

Survival ratio of slipped species can be affected by different factors such as holding time and catch density in the bunt, size of the fish and condition factor, scale loss and water temperature (Marçalo et al., 2018). In the bunt, the catch can become highly crowded, and can be fatally harmed by oxygen depletion, exhaustion and physical injury from contact with the net and catch (Tenningen et al., 2012). The mortality is directly related to their treatment within the bunt-end and increase with density and crowding duration (Lockwood et al., 1983; Tenningen et al., 2012; Marçalo et al., 2010). In the slipping process, before the catch does not become too crowded, survival can be higher. If the whole catch is to be released, the bunt end/wing is opened and several purse rings are released (Marçalo et al., 2019). However, if only a portion of the catch is to be released, opening must be adjusted carefully. According to European Union regulations, slipping of mackerel and herring is completed before 80%/90% of the net has been hauled (the point of retrieval) in NW waters and the North Sea (Marçalo et al., 2019).

Conclusion

Although slipping practised rarely in Turkey, all the purse seiner in the Sea of Marmara have to use the adapted slipped process to get rid of jellyfish. However, there are no records and scientific findings regarding slipped amount of the jellyfish. For this reason, this study is important to presented preliminary results regarding amount of the jellyfish. In conclusion, this study is extended completely the Sea of Marmara practised to understand the dimensions of jellyfish amount and slipping process.

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Compliance with Ethical Standards

Authors' Contributions

Authors contributed equally to this work.

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.

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SHORT COMMUNICATION

Maximum length report of *Gonostoma denudatum* Rafinesque, 1810 in the Eastern Mediterranean Sea

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ABSTRACT

The present paper reports the maximum length of the rare seen deep-sea fish bristlemouth *Gonostoma denudatum* Rafinesque, 1810 in the Mediterranean. One specimen of *G. denudatum* was caught using a trawler from the deep seas of Northern Cyprus waters on June 22th 2019. The total length (TL) of the specimen was 165 mm, the standard length (SL) was 149.5 mm, and the total weight was 16.2 g. Till today, this sample shown to this species maximum length (TL), which was recorded for the Mediterranean as well as in Eastern basin waters.

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Introduction

The bristlemouth *Gonostoma denudatum* Rafinesque, 1810, is a species mainly found in Atlantic tropical and subtropical waters. Its distribution extends until Eastern Atlantic Ocean, Mediterranean Sea, and off southern Africa spreading from the Atlantic slopes and western Atlantic (Schaefer et al., 1986).

G. denudatum is a deep mesopelagic species usually associate with continental and island slopes. The species exhibits diel vertical migrations for juveniles and adults in 400-700 m by daytime and 100-200 m by nighttime (Badcock, 1984). It was commonly feeding on Euphausiids and copepods (Costa et al., 1991; Froese and Pauly, 2019).

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The first record of *G. denudatum* from the Turkish waters was reported from Iskenderun coast in 2014 (Bilecenoglu et al., 2014). The species was recorded in the ichthyoplankton samples belonging to *Gonostoma* genus from Cyprus waters in 1998 (Çoker and Cihangir, 2015) and Mersin, Erdemli, in 2018 (Bayhan and Ergüden, 2019). Later, the species was reported from the eastern Mediterranean Cyprus coast in 2019 (Akbora et al., 2020).

Up today, the sampling reported by the present paper shows the maximum length recorded for *G. denudatum* in the Mediterranean as well as in Eastern Mediterranean waters. By this way, this study aims to contribute to the knowledge of the bristlemouth, especially regarding size and distribution in the North Cyprus waters.

Material and Methods

On June 22th 2019, one specimen of the bristlemouth *G. denudatum* with a total length of 165.0 mm was caught by a trawler in the deep seas of Northern Cyprus, Eastern Mediterranean (Fig 1), (34.466 N, 36.0075 E) at a depth of 550 m. The sample was measured and stored in the Museum of Systematic, Faculty of Fisheries, Mersin University (catalogue number MEUFC-19-11-128). All values agree with the literature (Ahlstrom et al., 1984; Quéro et al., 1990).

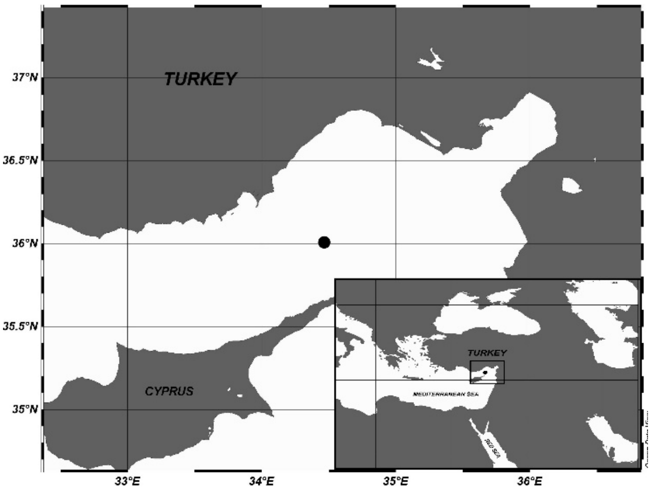

Figure 1. Catch location of *G. denudatum* caught off the Coast of Northern Cyprus Sea

Results

The total length of the specimen is 165 mm, it has a 149.5 mm of standard length, and total weight is 16.2 g. Body moderately elongated. Standard length proportions of head length (HL), pre-dorsal length, pre-anal length, pre-pectoral length, and maximum body depth is 21.5%, 62.8%, 64.2%, 22.7%, and 16.7%. Head length proportions of eye diameter, snout length, and pre-orbital length is 18%, 14.3%, and 27.1%. Meristic counts of dorsal fin rays 14, pectoral fin rays 12; pelvic fin rays 8, anal-fin rays 28 and gill rakers 16 (on first arch). These values are included within the ranks proposed by Badcock (1984) and Schaefer et al. (1986) for *G. denudatum*. The body color of the fresh specimen is transparent and light brown. The fish's head and cheek were naked and bluish. Photophores appears silvery gray, and the caudal peduncle is light brown (Fig 2). The geographical comparison data are shown in Table 1.

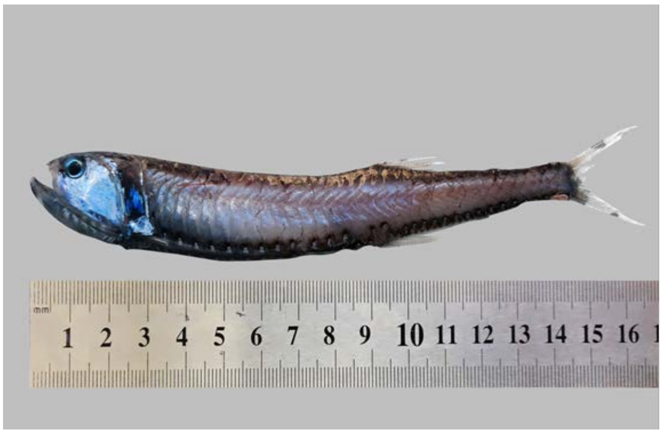

Figure 2. *G. denudatum* (149.5 mm SL) (Photo: Deniz AYAS)

Discussion

In fishery researches, information about the maximum length-weight data is crucial for calculating theoretical parameters (Borges, 2001). In the literature, the maximum total length of *G. denudatum* reported was 140 mm standard length in the eastern Atlantic (Quéro et al., 1990), 118 mm in Eastern Mediterranean, Iskenderun Bay (Bilecenoglu et al., 2014), 117.5 mm in the Northeastern Mediterranean, Turkey (Bayhan and Ergüden, 2019) and 122.5 mm in the Eastern Mediterranean, N. Cyprus (Akbora et al., 2020). The size of the present specimen was the maximum length ever registered not only for the Eastern Mediterranean and but also for the other Mediterranean regions. Moreover, the present study also reported the maximum length for the species (149.5 mm, SL), namely bigger than the maximum published standard length, 140 mm (Quéro et al., 1990) in FishBase. In Mediterranean waters, the maximum lengths of this species in the other previous studies were shown in Table 1.

Although *G. denudatum* is widespread throughout the Eastern Central Atlantic, the existence of this species has been identified in the Mediterranean waters in recent years. According to Çoker and Akyol (2015), Cyprus waters are subject to significant currents flowing across the eastern Mediterranean Basin, where the Atlantic current entering the Mediterranean Sea through the Straits of Gibraltar sweeps along the North African shores eastwards. This species most likely possibility for the present finding is that it comes with by active migration.

Table 1. The reported maximum length of bristlemouth, *G. denudatum* from different locations

Authors	Location	Sample Time	Sample Size	Sampling Gear	Depth (m)	Total Length, TL (mm)	Standard Length, SL (mm)
Battaglia et al. (2010)	Mediterranean Sea (Strait of Messina), Italy	2007-2009	1	Trawl	80-300	-	131.2
Bilecenoglu et al. (2014)	Eastern Mediterranean, Turkey	2002-2014	1	Trawl	200	-	118.0
Çoker and Cihangir (2015)	Northern Cyprus	July 1998	Larvae*	Bottom Trawl	300-1200	-	-
Bayhan and Ergüden (2019)	North-Eastern Mediterranean, Turkey	July 2019	1	Bottom Trawl	595	130.7	117.5
Akbora et al. (2020)	Eastern Mediterranean, N. Cyprus	May 2018	1	Bottom Trawl	420-640	128.0	122.0
This study	Northern Cyprus	June 2019	1	Bottom Trawl	550	165.0	149.5

Note: *; These larvae values are given for frequency of appearance as 4.38

Up to date, no species-specific conservation measures take place for *G. denudatum* in the Mediterranean. Ragonese et al. (2001) stated that *G. denudatum* is taken as by-catch during red shrimp targeted deep-water bottom trawls.

The present data record informs the maximum length of *G. denudatum* along the Eastern Mediterranean Sea, including the Cyprus waters. To the authors' knowledge, this study is a new reference for the size of *G. denudatum* species for the whole Mediterranean.

Conclusion

In recent years, the number of non-indigenous immigrant fish species to the Eastern Mediterranean has rapidly increased (Bilecenoglu et al., 2014; Bayhan and Ergüden, 2019; Ergüden et al., 2019). Further longer-term monitoring surveys should be carried out, particularly in deep-sea investigations to support the fisheries development and management.

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Compliance with Ethical Standards

Authors' Contributions

DA and DE is interested in searching and studied the fish species, including the subject of the current study and designed the final study and HDA is checked and re-revised the final edit of this paper.

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.

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RESEARCH ARTICLE

The length-weight relationship and condition factor of yellowspotted puffer *Torquigener flavimaculosus* Hardy & Randall, 1983 in the Eastern Mediterranean (Yumurtalık Bight, Turkey)

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ABSTRACT

The length-weight relationships (LWRs) of fish are helpful in fisheries science. A total of 504 yellowspotted puffer (*Torquigener flavimaculosus*) specimens (257 female and 247 male) were caught from the Yumurtalık coast of Turkey (Eastern Mediterranean Sea) between 2018 and 2019 by using a trammel net set with a depth range between 5 and 42 m. The sex ratio was found 1:1.08 for males and females, respectively. The length-weight relationship was described for combined both sexes by the following formula $W = 0.00130 \times L^{3.094}$ ($r^2 = 0.918$) with a positive allometric growth. Fulton condition factor (CF) was calculated as 1.713 ± 0.145 for combined sexes. This study presents the first results available for the length-weight relationships and condition factor of *T. flavimaculosus* according to both sexes in the Yumurtalık coast of Turkey.

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Introduction

The length-weight relationships (LWRs) are an important indicator in fisheries management and conservation (Froese, 2006). In addition, condition factors are also important parameters for the evaluation of fish stocks (Erguden and Turan, 2017; Erguden et al., 2018).

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The yellowspotted puffer, *Torquigener flavimaculosus* Hardy & Randall, 1983, is a reef-associated species, found at depths ranging from 3 to 57 meters (Randall, 1995), commonly feeding on marine invertebrates and reaching a maximum total length of 16.0 cm (Sabour et al., 2014). The species is distributed in the western Indian Ocean, from East Africa to Seychelles, in the Red Sea and Persian Gulf (Randall, 1995). It is also found in the Mediterranean Sea, introduced via the Suez Canal (Golani et al., 2002) being one of the seven non-native puffers (belongs to the Tetraodontidae family). *T. flavimaculosus* has been also recorded from the Turkish coasts of the Levantine Sea and the Aegean Sea (Bilecenoglu, 2005; Erguden and Gurlek, 2010; Bilecenoglu et al., 2014).

The knowledge of length-weight relationships is important in fisheries science while length-weight relationships are essential to better understand the ecology and life characteristics of fish species. Fulton's condition factor (CF) is widely used in fisheries and fish biology studies (Erguden and Turan, 2017; Erguden et al., 2017; Aydın and Sözer, 2019). LWRs are used by fisheries scientists to convert growth in length equations to growth in weight for stock assessment models (Morato et al., 2001; Stergiou and Moutopoulos, 2001), and also to estimate biomass from the length-frequency distribution (Petrakis and Stergiou, 1995; Erguden et al., 2009; Erguden et al., 2011).

To date, length-weight relationship studies have been reported very scarcely for the pufferfish species (Erguden et al., 2015; Bilge et al., 2017; Ayas et al., 2019). Besides, LWRs and condition factors according to sexes for *T. flavimaculosus* have not been reported from the Eastern Mediterranean. Thus, the present study provides the first detailed information on the length-weight relationships and condition factor of *T. flavimaculosus* on the Eastern Mediterranean coast of Turkey (Yumurtalık Bight).

Material and Methods

For this study, 504 specimens (257 female and 247 male) of *T. flavimaculosus* were caught by using a trammel net (22 mm (bar length) inner panel mesh size and 110 mm outer panel mesh size) between September 2018 and November 2019 from Yumurtalık Bight, Turkey (Fig 1). The sampling areas are characterized by sandy-muddy substrates and depths between 5-42 meters. After captured, all samples were transported immediately to the laboratory in an ice bag and then frozen (−20°C) for preventing deterioration. Species identification was based on Golani et al. (2002). Total length (nearest 0.1 cm) and total weight (nearest 0.01 g) were recorded for its specimen while the sex of each specimen was determined by examining the gonads macroscopically.

The length-weight relationship of the fish was calculated by applying the exponential regression equation $W = aTL^b$, (Ricker, 1975). This equation can also be expressed in logarithmic form: $\log W = \log a + b \log L$, where W is the weight (g), L is the total length (cm), a is the intercept and b is the slope of the linear regression. Parameters a and b were calculated by least-squares regression, as was the coefficient of determination (r^2). 95% confidence limits (CI) of b was also estimated (Pauly, 1993). The relationships among the variables were identified using the regression analysis by ANOVA. The student's t-test was used to test for the difference of the parameter "b" from the theoretical value of 3 (Sokal and Rohlf, 1969). The sex ratio was checked with a Chi-Square test for equality to determine whether the ratio differ from 1:1.

Fulton's coefficient of condition factor (K) was calculated by $K=100 \times W/L^3$, where 'L' is total length (cm) and 'W' is the weight (g) (Le Cren, 1951; Sparre and Venema, 1992). The condition factor was estimated for each sexual category (males, females and individuals with undetermined sex) and for the total samples.

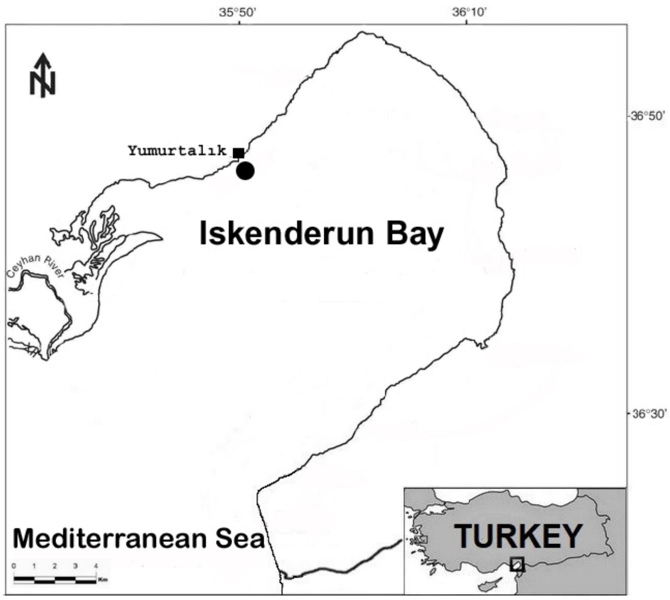

Figure 1. The black circle indicates the sampling area

Results

In the present study, 257 female and 247 male *T. flavimaculosus* specimens were investigated. The total sex ratio for female and male samples (M:F) was 1.00:1.04. The difference between the sex ratios was not found to be statistically significant (X^2= 0.535, P > 0.05).

The total length values of females, males, and combined individuals ranged from 6.8-13.7 cm, 5.7-13.1 cm, and 5.7-13.7 cm respectively (Table 1). Descriptive statistics of the total length and weights were offered in Table 1 for females, males, and combined individuals.

Table 1. Mean and standard deviation, maximum, minimum for length (L) and weight (W) parameters of each sex of *T. flavimaculosus*

Sex	N	Total Length (TL) (Min-Max)	TL Mean±SD	Total Weight (W) (Min-Max)	W Mean±SD
Female	257	6.80-13.70	9.250±0.853	4.18-46.35	14.06±4.780
Male	247	5.70-13.10	9.213±0.862	3.56-38.50	13.72±4.340
Combined	504	5.70-13.70	9.235±0.857	3.56-46.35	13.89±4.560

Table 2. Length-weight relationships of *T. flavimaculosus* from Yumurtalık coast, Turkey

N	Sex	a	b	r^2	SE of b	95% CI of b	P	Growth Type
257	Female	0.00110	3.175	0.918	0.059	3.059-3.292	>0.05	Allometric (+)
247	Male	0.00160	3.022	0.919	0.058	2.908-3.135	>0.05	Isometric
504	Combined	0.00130	3.094	0.918	0.041	3.013-3.175	>0.05	Allometric (+)

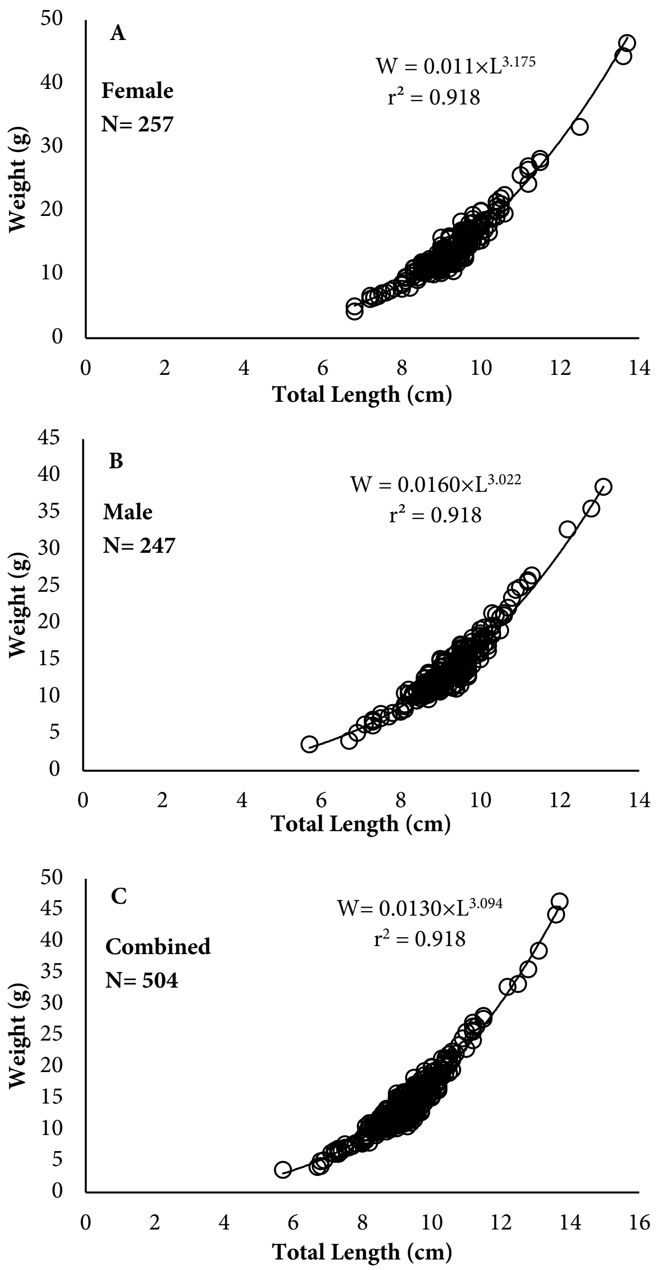

Figure 2. Length-weight relationship of female (**A**), male (**B**) and combined sexes **(C)** for *T. flavimaculosus* from the Yumurtalık coast (Turkey)

The length-weight relationships are given in Table 2 for females, males, and sexes combined, providing sample size (N), level of equation parameters, "a" and "b" as well as the 95% confidence limits for both parameters and the coefficient of determination (r^2).

The values of the exponent b provide information indicating the type of growth when the isometric growth b is 3.0. When the value of b is higher than 3 (b> 3.0), the weight represents positive allometric growth; a lower value (b< 3.0) shows negative allometric growth. Our data suggested that *T. flavimaculosus* showed positive allometric growth for combined sexes (b=3.094) and females (b=3.175). Besides, it shows isometry for males (b=3.022). The value of "b" is slightly different than 3 for the female and combined and is not significantly different than 3 for male p> 0.05 (Table 2).

The length-weight relationship of *T. flavimaculosus* was calculated as $W=0.0011TL^{3.175}$ (r^2=0.918) for females, $W=0.0016TL^{3.022}$ (r^2=0.918) for males and $W=0.0013TL^{3.094}$ (r^2=0.918) for combined (Fig 2). Fulton's condition factor (CF) was 1.720±0.147 for females, 1.707±0.144 for males and 1.713±0.145 for combined. Condition factor values also showed significant variations (P<0.001) for female and male individuals of *T. flavimaculosus*.

Discussion

In this study, we report the length-weight relationships of *T. flavimaculosus* in the Mediterranean, based on 504 specimens collected from the Yumurtalık coast. The sex ratio was found 1:1.04 for males and females (49.0% male and 51.0% female). Çek-Yalnız et al. (2017) reported that the sex ratio of *T. flavimaculosus* was 62.5% female and 37.5% male collected from the Iskenderun Bay, Turkey. Ramadan and Magdy (2019) similarly reported the male:female sex ratio (M/F) as 1.00:1.08 for the species population from the Gulf of Suez (Egypt). According to Mendonca et al. (2006), this small difference may be related to the variance in the availability of both sexes for the fishery, and their feeding behavior. Besides, this difference may be also associated with the spatial segregation of both sexes and environmental factors. Because different environmental factors can cause spatial segregation of the sexes.

The exponent b often has a value close to three but varies between 2.5 and 3.5 (Froese, 2006). The relationship between length and weight for this species indicates that growth shows positive allometry (b = 3.094) for combined sexes. Erguden et al. (2015) reported negative growth (b=2.970) in their study conducted in Iskenderun Bay. Similarly, Bilge et al. (2017) stated negative allometric growth (b=2.836) from the Mugla coast. However, Ayas et al. (2019) reported positive allometric growth (3.326) from Mersin Bay. These small differences could be the result of sample size, fishing equipment, season, fishing pressure and reproduction season (Petrakis and Stergiou, 1995). Besides, these differences depend on environmental or regional changes.

According to our knowledge of fish biology, condition factor (CF) is an expression to assess the condition of the fish and used to allow for comparisons of species growth between different regions. According to Le Cren (1951), CF values greater than 1 indicated the good condition of the fish whereas a value < 1 is indicative of the reverse nature. In this study, the minimum condition factor value was estimated at 1.707, and maximum as 1.720 for *T. flavimaculosus*. The present results revealed that both males and females indicate that both of the sexes are in good condition for *T. flavimaculosus*.

Although length-weight relationships in fish are affected by many factors including habitat, area, seasonal effect, gonad maturity, sex, diet and stomach fullness, health and preservation techniques (Tesch, 1971), all these are not considered in this study.

Conclusion

The present study is the first study focusing on the length-weight relationship and condition factor for *T. flavimaculosus* in the southern Mediterranean. Besides, this study revealed that both male and female have a uniform CF value, which indicates that both the sexes are in quite well condition for the optimum growth of this fish species. The results obtained from this study will be useful to researchers and fisheries biologists in the Mediterranean, for better understanding of the invasion ecology of the species.

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Compliance with Ethical Standards

Authors' Contributions

Author SAE designed the study, DE and DA wrote the first draft of the manuscript, SAE and DE performed and managed statistical analyses. All 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.

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RESEARCH ARTICLE

Purification of glucose 6-phosphate dehydrogenase from *Capoeta umbla* gill and liver tissues and inhibition effects of some metal ions on enzyme activity

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ABSTRACT

In this study, the *in vitro* effects of some metal ions (Ag^{+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+}) on freshwater fish *Capoeta umbla* liver and gill glucose 6-phosphate dehydrogenase (G6PD) have been investigated. For this purpose, *C. umbla* liver and gill G6PD enzymes were purified, with a specific activity of 31.52 and 22.83 EU/mg protein, 3353.2 and 736.5 fold in a yield of 19.28% and 23.15%, respectively. In order to control the enzyme purification SDS-PAGE showed a single band for the enzyme. In addition, *in vitro* effects of metal ions on enzyme activity were researched. As a result, Ag^{+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} inhibited fish liver G6PD; Ag^{+}, Cd^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+} inhibited fish gill G6PD. Besides, it was found that the most effective inhibitor of G6PD enzyme within metal ions used was Ag^{+}. Our results also demonstrate that these metals might be dangerous at low micromolar concentrations for fish G6PD enzyme.

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Introduction

Glucose 6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ oxidoreductase EC 1.1.1.49; G6PD) is a key and critical enzyme that is the oxidative compound of the pentose phosphate metabolic pathway (Beutler, 2008). The important physiological mission of G6PD is the production of NADPH and ribose 5-phosphate. Ribose-5-phosphate is involved in making DNA and RNA. NADPH is a coenzyme participating in the synthesis of a number of organic molecules such as proteins, nucleic acids and membrane lipids (Srivastava and Beutler, 1970; Hopa et al., 2015). NADPH is also used as a substrate by the glutathione pathway and cytochrome P450 enzymes, which protect cells against oxidative stress and harmful chemicals (Riganti et al., 2012).

The pollution of aquatic environment by contaminants especially metals has become a matter of great concern over the last few decades not only because of the threat to fresh water supplies but also with the damage caused to the aquatic life (Koca et al., 2005). Metals reach the aquatic resources as a consequence of industrial, agricultural, sewage disposal, soil leaching and rainfall, thus aquatic organisms including fishes are exposed to a significant amount of these pollutants in aquatic environments (Heath, 1987; Atli et al., 2006). Several authors reported that heavy metals cause toxicity in aquatic environments and the heavy metal damage is an important factor in many pathological and toxicological processes. It has been reported that metals can change enzymatic activities by binding the functional groups, comprising the carbonyl, carboxyl and sulfhydryl or by substituting of the metal associated with the enzyme (Sivaperumal et al., 2006; Kaya et al., 2013; Kucuk and Gulcin, 2016). The toxicological effects of metals are usually enzyme inhibition and denaturation (Ekinci and Beydemir, 2010). Generally metal inhibition of enzyme is based on metal binding to the protein. Therefore, human metabolism is affected by metal toxicity. Over consumption of fish causes a variety of diseases such as cancer, diabetes, Alzheimer and Parkinson diseases (Jomova and Valko, 2011; Kirici et al., 2017a; Hunsaker and Franz, 2019).

Liver is an important and specific tissue for storing of metals. In the studies conducted on various species of fish metal bioaccumulation has been found higher in the liver, when the compared to other organs (Rainbow and White, 1990). The gill is the first tissue contacting with the contaminants in the water. Due to its large surface area and the small diffusion distance between the water and blood, the gills are primarily affected by contaminants such as metals. In general, the gill cells respond rapidly to various chemicals to overcome physiological impairment or tissue damage, and chemicals may have a negative effect on the overall gill function, enhancing fish susceptibility to toxic compounds and potentially leading to fish mortality (Cerqueira and Fernandes, 2002; Demir et al., 2016).

It is well known that almost all metals show their effects on various enzymes in the metabolism. Particularly, some enzymes are target for this substances such as carbonic anhydrase, glutathione reductase, G6PD and paraoxonase (Ekinci et al., 2007; Alici et al., 2008; Ekinci and Beydemir, 2009; Şentürk et al., 2011; Söyüt et al., 2012; Türkeş et al., 2015; Kirici et al., 2016a).

Also, G6PD-metal interactions have been investigated on a variety of fishes *in vitro* for a long time (Cankaya et al., 2011; Hu et al., 2013; Comakli et al., 2015; Kirici et al., 2016a). In our study, we investigated the toxicological effects of some metal ions, including Ag^+, Cd^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+} on the G6PD enzyme purified from the liver and gill of freshwater fish *Capoeta umbla* using the affinity chromatography method under *in vitro* conditions.

Material and Methods

Chemicals

$Pb(NO_3)_2$, $3CdSO_4.8H_2O$, $FeCl_3$, $NiCl_2.6H_2O$, $AgNO_3$, $CuSO_4{\cdot}5H_2O$, 2',5'-ADP Sepharose 4B was purchased from Pharmacia. $NADP^+$, glucose 6-phosphate, protein assay reagent and chemicals for electrophoresis were purchased from Sigma-Aldrich (Taufkirchen, Germany). All other chemicals used were of analytical grade and were purchased from Merck (Darmstadt, Germany)

Fish

Capoeta umbla (healthy, adult fish-weighing 150-250 g) were caught from Murat River (Turkey, Bingol). Fish samples were brought in accordance with the cold chain rules (+4°C). The fish were decapitated and their livers and gills were extracted and stored at -80°C. *C. umbla*, Transcaucasian barb, inhabits Euphrates-Tigris River Systems. It is also known as "lake fish or stream fish" locally and it is the most commercially valued fish for the local people around Murat River (Çoban et al., 2013).

G6PD activity assay

Enzyme activity was performed at 37°C in the spectrophotometer in accordance with the Beutler method (Beutler, 1971).

Preparation of homogenate

The frozen liver and gill tissues were thawed and cut into small pieces by using a scalpel. Liver and gill simples (10 g) were washed three times with 0.9% sodium chloride solution. These samples were homogenized gently for about 45 sec. and suspended in standard homogenizator buffer, containing 50 mM KH_2PO_4, 1 mM Phenylmethylsulfonyl fluoride (PMSF), 1 mM Ethylenediaminetetraacetic acid (EDTA) and 1 mM Dithiothreitol (DTT). The homogenates were centrifuged for 2 h at 13.500 rpm. The supernatant was collected and kept for analysis (Kirici et al., 2016b).

Ammonium sulfate precipitation

The ammonium sulfate precipitation experiments were performed according to our previous studies (Kirici et al., 2016a; Kirici et al., 2016b; Kirici et al., 2017a). We determined that the ammonium sulfate was precipitated at 40–80% for gill and 40–80% for liver in our present study. The precipitate was obtained after centrifugation at 13500×rpm for 15 min (+4°C) and redissolved in 50 mM KH_2PO_4 buffer (pH 7.2).

Dialysis

Dissolved precipitate as mentioned above was dialyzed at two hours in Dialysis Buffer (65 mM K-phosphate + 50 mM K-acetate) (pH 7.0) (Kirici et al., 2016a).

Affinity chromatography

2', 5'-ADP Sepharose 4B affinity column (10 cm×1 cm) was prepared according to our previous studies (Kirici et al., 2016a; Kirici et al., 2016b; Kirici et al., 2017a). The column material was equilibrated with buffer (0.1 M K-acetate + 0.1 M K-phosphate, pH 6.0) by means of a peristaltic pump (flow rate: 50 ml/h). After the dialyzed enzyme solution was loaded on the column and the flow rate was regulated to 20 ml/h. The column was respectively washed with 25 ml of 0.1 M K-acetate + 0.1 M K-phosphate (pH 6.0) and 25 ml of 0.1 M K-acetate + 0.1 M K-phosphate (pH 7.85). Eventually, the enzyme was eluted with a solution of 80 mM K-phosphate + 80 mM KC1 + 0.5 mM NADP+ + 10 mM EDTA (pH 7.8). The enzyme activity was measured, and the activity-containing tubes were collected together (Ninfali et al., 1990).

Protein determination

Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method for liver and gill G6PD enzymes (Bradford, 1976).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The control of enzyme purity was carried out using Laemmli's procedure in 3% and 8% acrylamide concentrations for running and stacking gel, respectively (Laemmli, 1970). Samples were applied as 15 µL to the electrophoresis. Gel was stained with Coomassie Brilliant Blue R-250.

In vitro inhibition studies

In order to determine the effects of the metal ions (Ag^+, Cd^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+}) on fish liver and gill G6PD, different concentrations of metal ions were added to the reaction medium. The enzyme activity was measured and an experiment in the absence of inhibitor was used as control (100% activity). The IC_{50} values were obtained from activity (%) vs. metal ion concentration plots. In order to determine K_i constants in the media with inhibitor, the substrate (G6P) concentrations were 0.03, 0.06, 0.09, 0.15 and 0.27 mM. Inhibitor solutions were added to the reaction medium, resulting in 3 different fixed concentrations of inhibitors in 1 mL of total reaction volume. Lineweaver–Burk graphs were drawn by using 1/V vs. 1/[S] values and K_i constant were calculated from these graphs. Regression analysis graphs were drawn for IC_{50} using inhibition % values by a statistical package (SPSS 17) (Student t-test; n = 3).

Results

G6PD which is an important and a key enzyme for the pentose phosphate metabolic pathway, was purified from *Capoeta umbla* liver and gill by simple chromatographic method and the inhibitory effects of some metal ions (Ag^+, Cd^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+}) were examined on the enzyme activities in the present study.

G6PD was purified by using preparation of the hemolysate, ammonium sulfate fractionation (30–80% for liver; 40–80% for gill) and 2 ', 5'-ADP Sepharose 4B affinity chromatography (Table 1 and Figure 1). The overall purification, fish gill G6PD was obtained with a yield of 23.15% a specific activity of 22.83 EU/mg proteins, and this enzyme was purified approximately 736.5 fold (Table 1); fish liver G6PD was obtained with a yield of 19.28% a specific activity of 31.52 EU/mg proteins, and this enzyme was purified approximately 3353.19 fold (Table 1).

In order to show inhibitory effects, while the most suitable parameter is the K_i constant some researchers use the IC_{50} value (Kirici et al., 2016c; Kucuk and Gulcin, 2016; Kirici et al., 2017b; Caglayan et al., 2019). K_i constant was calculated from Lineweaver-Burk graphs and IC_{50} value was calculated from the regression graphs. Therefore, in this study, both the K_i and IC_{50}

parameters of these metal ions for G6PD were determined. The effects of metal ions on G6PD enzyme from *C. umbla* gill and liver were determined as millimolar levels.

Table 2 and Figure 2 shows the *in vitro* effects and inhibition types of metal ions Ag^{+}, Cd^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+} on gill G6PD activity. IC_{50} values of Ag^{+}, Cd^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+} were determined as 0.035, 0.376, 0.755, 1.487 and 0.096 mM; K_i constants were determined as 0.019, 0.318, 0.402, 0.152 and 0.036 mM for gill G6PD, respectively. Ag^{+} and Cd^{2+} showed noncompetitive inhibition, while Fe^{2+} and Pb^{2+} inhibited the enzyme in a competitive manner and Ni^{2+} inhibited the enzyme in an uncompetitive manner.

Table 1. Purification scheme of G6PD from *C. umbla* gill and liver

Tissue	Purification step	Activity (U/mL)	Protein (mg/mL)	Total volume (ml)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification factor	Yield (%)
Gill	Hemolysate	0.286	9.13	30	8.58	273.9	0.031	1	100
	Ammonium sulfate precipitation	0.613	19.56	6	3.678	117.6	0.0313	1.01	42.87
	2’, 5’-ADP Sepharose 4B affinity chromatography	0.662	0.029	3	1.986	0.087	22.83	736.5	23.15
Liver	Hemolysate	0.535	57.07	33	17.655	1883.3	0.0094	1	100
	Ammonium sulfate precipitation	0.747	55.76	8	5.976	446.08	0.013	1.38	33.85
	2’, 5’-ADP Sepharose 4B affinity chromatography	0.851	0.43	4	3.404	0.108	31.52	3353.2	19.28

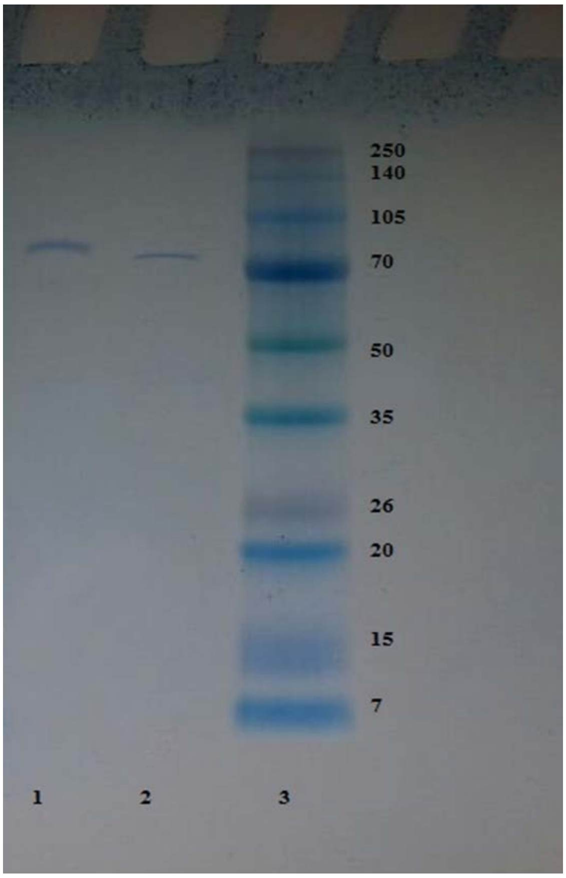

Figure 1. SDS-PAGE photograph (Lane 1: *C. umbla* liver G6PD; Lane 2: *C. umbla* gill G6PD; Lane 3: Standard proteins)

Table 3 and Figure 3 show the *in vitro* effects and inhibition types of metal ions Ag^{+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} on liver G6PD activity. IC_{50} values of Ag^{+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} were determined as 0.00038, 0.423, 8.887, 1.819 and 0.118 mM; K_i constants were determined as 0.00014, 0.335, 4.287, 1.828 and 0.327 mM for liver G6PD, respectively. Ag^{+} and Cd^{2+} showed competitive inhibition, while Cu^{2+}, Ni^{2+} and Pb^{2+} inhibited the enzyme in a competitive manner.

Table 2. IC_{50} values, K_i constants and inhibition types of some metal ions G6PD obtained from *C. umbla* gill

Metal ions	IC_{50} (mM)	K_i (mM)	Inhibition type
Ag^{+}	0.035	0.019 ± 0.00308	Noncompetitive
Cd^{2+}	0.376	0.318 ± 0.00612	Noncompetitive
Fe^{2+}	0.755	0.402 ± 0.102	Competitive
Ni^{2+}	1.487	0.152 ± 0.048	Uncompetitive
Pb^{2+}	0.096	0.036 ± 0.0099	Competitive

Table 3. IC_{50} values, K_i constants and inhibition types of some metal ions G6PD obtained from *C. umbla* liver

Metal ions	IC_{50} (mM)	K_i (mM)	Inhibition type
Ag^{+}	0.000377	0.00014 ± 0.000042	Competitive
Cd^{2+}	0.423	0.335 ± 0.167	Competitive
Cu^{2+}	8.887	4.287 ± 0.197	Noncompetitive
Ni^{2+}	1.819	1.828 ± 1.142	Noncompetitive
Pb^{2+}	0.118	0.327 ± 0.054	Noncompetitive

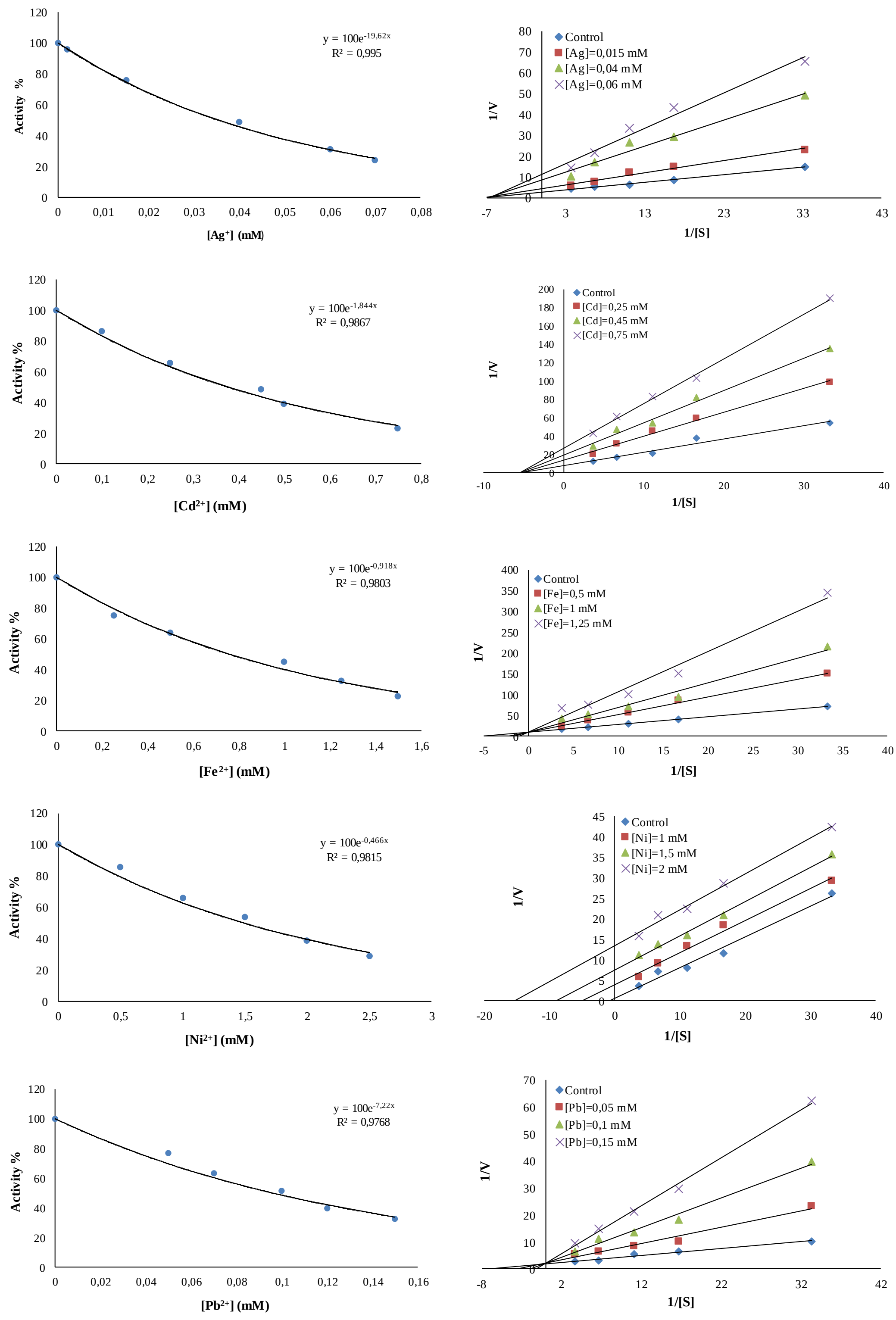

Figure 2. IC_{50} and K_i graphics for *C. umbla* gill G6PD

Discussion

G6PD is widespread in all tissues and blood cells, catalyzing the conversion of glucose 6-phosphate to 6-phosphogluconolactone in the presence of $NADP^+$. The conversion of glucose-6-phosphate to 6-phsophogluconolactone is important in the generation of NADPH, an important coenzyme and reducing agent in lipid and nucleic acid synthesis (Patrinostro et al., 2013). G6PD inhibition within chemical compounds directly affects the increase of reactive oxygen species reducing the amount of intracellular NADPH and GSH, involve to protect cells against reactive oxygen species (Riganti et al., 2012; Hopa et al., 2015).

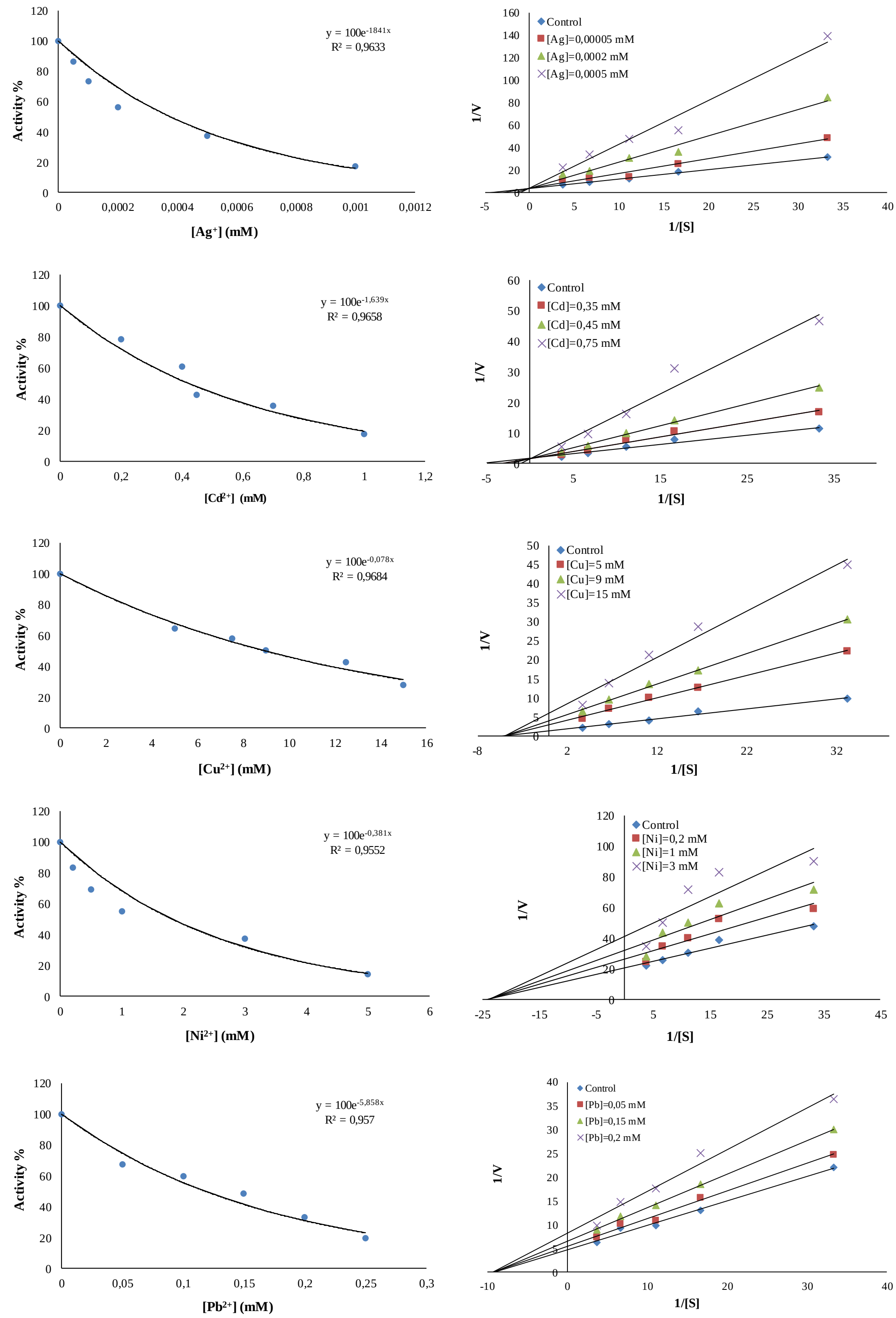

Figure 3. IC_{50} and K_i graphics for *C. umbla* liver G6PD

Some metals such as iron, zinc, copper, calcium, function in the active site of the enzymes as ligands (Alim and Beydemir, 2012). Many enzymes contain Zn^{2+} with in their structures, such as carbonic anhydrase and sorbitol dehydrogenase. Moreover, some enzymes, such as paraoxonase contain multiple metal ions in their three-dimensional structure. The toxicological effects of metals are usually enzyme inhibition and denaturation (Ekinci and Beydemir, 2010; Caglayan et al., 2019). Generally, the mechanism underlying the metal inhibition of the enzyme is based on metal binding to the protein. Metals particularly, bind sulfhydryl groups in proteins and produce mercaptan (Jomova and Valko, 2011; Hunsaker and Franz, 2019).

In this study, the G6PD enzyme was purified from the liver and gill of the *C. umbla* and the toxicological effects of metal ions on the activity of this enzyme were calculated. This metaloenzyme is quite common among all organisms. G6PD was first isolated from human erythrocytes by Yoshida and Huang (Yoshida and Huang, 1986). Up to now, G6PD enzymes have been purified from many various sources (Bautista et al., 1988; Özer et al., 2002; Yilmaz et al., 2002; Beydemir et al., 2003; Türkoğlu et al., 2003; Şentürk et al., 2009; Adem and Ciftci, 2012; Comakli et al., 2015; Adem and Ciftci, 2016).

Many chemicals effect the metabolism via changing normal enzyme activity, particularly inhibition of a specific enzyme and the effects can be dramatic and systemic (Adem and Ciftci, 2012). The effects of different chemical substances, drugs and metal ions on G6PD enzyme have been investigated in many *in vitro* studies, performed with various organisms. For example; Şentürk et al. (2009) investigated the *in vitro* effects of deltamethrin, cypermethrin and propoxur on rainbow trout erythrocyte G6PD. They found that deltamethrin and cypermethrin showed noncompetitive inhibition while propoxur inhibited the enzyme in an uncompetitive manner. Hu et al. (2013) purified G6PD from grass carp (*Ctenopharyngodon idella*) hepatopancreas and determined the inhibition effects of Zn, Mn, Al, Cu and Cd on G6PD activity in *in vitro*. Their results showed that Zn, Al, and Cd showed competitive inhibition, while Cu inhibited the G6PD in a noncompetitive inhibition manner. Besides, the effects of furosemide, digoxin and dopamine on G6PD enzyme, which was purified from rat heart by Adem and Ciftci (2016), were investigated. Their result showed Dopamine inhibited the activity of these enzyme as competitive, whereas furosemide and digoxin inhibited the activity of the enzyme as noncompetitive.

Conclusion

Consequently, accumulation of metals in fish may be considered as an important warning signal for fish health and human consumption. It is a good way to understand the toxic potential of compounds *in vitro* studies. Therefore, we investigated *in vitro* the inhibitory effect on the *C. umbla* liver and gill G6PD enzyme activities of some metal ions. According to these results, Ag^{+} and Cd^{+2} bind to the active sites of the fish liver G6PD enzyme; other metal ions are connected to other regions outside the active region. Besides, Fe^{+2} and Pb^{+2} bind to the active sites of the fish gill G6PD enzyme; other metal ions are connected to other regions outside the active region. IC_{50} values and K_i constants showed that Ag^{+} had the highest inhibitory effect on fish liver and gill G6PD activities.

Compliance with Ethical Standards

Authors' Contributions

MuK and MaK performed the research, analyzed the data and helped to draft the manuscript; MuK, MaK, MA and ŞB conceived and designed the work and wrote the manuscript. All authors contributed to 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.

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RESEARCH ARTICLE

Determining the relative efficiency of container terminals in Turkey using fuzzy data envelopment analysis

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ABSTRACT

In this study, fuzzy data envelopment analysis (FDEA) was conducted to determine the relative efficiency of the container terminals operating in Turkey. For this purpose, the data from the container terminals were converted into fuzzy values using the "set of α cuts approach" and the upper and lower limit values for each terminal were determined. The lower and upper limit efficiency values were calculated using the input-oriented CCR-FDEA method. The minimax regret approach was used to sort and compare the relative efficiency of terminals. According to the resulting efficiency scores, one container terminal was effective at all α cutting levels. It was calculated that the four container terminals had the lowest values at all α cutting levels regarding the maximum efficiency loss values of other container terminals.

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Introduction

Maritime transport, acknowledged as the lowest-cost option among transportation models (Keskin, 2011), is the most preferred means for transportation, allowing large volumes of cargo to be transported at once and minimizing the loss of property, as well as being safe and environmentally friendly (Ateş and Esmer, 2013). Maritime transport comprises three main components, which are the vessels, the cargo, and the ports (Ateş, 2010). Ports constitute vital connection points of the international trade chain (Bray et al., 2014). Maritime transport is now rapidly moving towards containerization. Container transportation comprised 102 million tons in 1980 and reached 1834 million tons in 2017 (UNCTAD, 2018). Therefore, competition in the market has gradually increased and ports must seek ways to become more modernized, rapid,

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and efficient in order to gain a larger share of the market (Oğuz, 2018). Container terminals need to be highly productive and must work efficiently in an environment in which the developments in world trade are rapid and competition is intense (Wang et al., 2017). Productive and efficient operation of terminals is one of the most important factors that increase the productivity of maritime transport (Ateş, 2010).

Productivity, which may be defined as the division of produced output values into the values used as input, is one of the most important performance indicators for companies, economies, and processes (Krajewski et al., 2015). Efficiency, on the other hand, is the ability to deliver a product or service using minimal resources (Tangen, 2005). It would not be reasonable to expect an increase in productivity values without also increasing efficiency values; however, a company with low productivity values could be efficient. Therefore, efficiency could be acknowledged as an element of productivity (Kök, 1991). When the literature is reviewed, it is seen that the concepts of efficiency and productivity have been used interchangeably and there is no consensus on these terms (Zengin and Taşdöven, 2015).

In this study, calculations are performed using fuzzy data envelopment analysis (FDEA) to determine the relative efficiencies of ports in Turkey with container terminals. Following the information on maritime transport and efficiency provided in the first section, a literature review will be given in Section 2. In the third section, the efficiency measurements of 22 container terminals are presented after providing information on FDEA and the "set of α cuts approach." In the final section, the findings are interpreted together with discussions on the operations required for terminal ports to be more productive.

Literature Review

It is seen that different methods are used in the literature on performance measurement in ports. These methods are; The calculation of the load handling efficiency at the docks (Bendall and Stent, 1987; Tabernacle, 1995; Ashar, 1997), the measurement of the efficiency of a single factor (De Monie, 1987), the performance measurements for comparing the port's optimal handling capacity with the handling in a given time period (Talley, 1988), the estimation of the port cost function (Neufville and Tsunokawa, 1981), the calculation of the total efficiency factor of a port (Kim and Sachish, 1986) and the establishment of the port performance and efficiency model with multiple regression analysis (Tongzon, 1995). The most commonly used techniques are Regression Analysis, Cost Benefit and Cost Effectiveness Analysis, Stochastic frontier analysis, Simulation, Data Envelopment Analysis-DEA) and Malmquist Analysis. Among these methods, simulation method and data envelopment analysis methods are used extensively in port efficiency and performance measurements (Esmer, 2009).

Data envelopment analysis plays an important role in determining the relative efficiencies of ports. The first study in the literature on port efficiency was conducted in 1993 by Roll and Hayuth (1993). This study was a theoretical assessment of efficiency rather than implementation. Later, Tongzon (1995), Poitras et al. (1996), and Martinez-Budria et al. (1999) tried to determine port efficiencies using the DEA method. The number of studies on port efficiency has increased since 2000. Tongzon (2001), in a study that applied DEA CCR (Charnes, Cooper, and Rhodes) and BCC (Banker, Charnes, and Cooper) models, calculated that the Rotterdam, Yokohama, Melbourne, and Osaka ports were more efficient than other ports. Valentine and Gray (2001) compared the efficiencies of 31 ports in North America and Europe using the DEA-CCR method. Itoh (2002) used DEA to measure the efficiencies of Japanese container terminals between 1990 and 1999. That study revealed that DEA was an efficient method in measuring port efficiencies, and it was indicated that the Tokyo and Nagoya ports were efficient. Wang et al. (2003) used input-oriented CCR and BCC methods in their study aimed at measuring the efficiencies of 57 ports around the world. Barros (2003), based on efficiency measurements of container terminals in Portugal using DEA, asserted that revising port rules would positively affect port efficiency. Turner et al. (2004) investigated ports in the USA and Canada using the DEA method. They argued that larger ports were more efficient. Barros and Athanassiou (2004) conducted a study on input and output using DEA to measure the efficiencies of Greek and Portuguese ports. The results of that study emphasized that privatization could be a good solution to increase the productivity of ports. Al-Eraqi et al. (2008) scrutinized the efficiencies of 22 large container terminals in the Middle East and East Africa, and they claimed that physically larger ports were more efficient. Bichou (2013) performed an efficiency measurement of 420 container terminals. The results indicated that larger-scale ports, modernized and equipped with automation systems, were more efficient. Yuen et al. (2013) conducted efficiency measurements of 21 container terminals in China between 2003 and 2007. Based on the findings obtained via DEA, 3 terminals were efficient in those years. Schøyen and Odeck (2013) measured the efficiencies of Norwegian container terminals between 2002 and 2008 using the DEA method. The results of that study indicated that Norwegian ports had higher efficiency than other ports, and the authors argued that the ports needed to be physically larger to be more efficient. Rajasekar and Deo (2014) used the DEA method to determine the efficiencies of 8 ports in India between 1993 and 2011. According to the efficiency scores obtained, the physically larger ports were more efficient. It was also found that ports that were not efficient could become efficient with infra- and superstructure modernizations. Al-Mawsheki and Shah (2017) measured the efficiencies of container terminals operating in the Middle East.

Turning to studies in Turkey, Baysal et al. (2004) tried to reveal the efficiencies of 7 ports operated by the Turkish State Railways. For this purpose, input- and output-oriented DEA models were implemented under fixed-income and variable-yield scale hypotheses, and the efficiency values for the ports were determined. Bayar (2005) used the DEA method in his study to measure the efficiency of container terminals in Turkey and suggested proposals for the potential improvement of unproductive terminals. Ateş (2010) applied the DEA method to the 2005-2009 data of 13 private or state-operated container terminals and found that the İzmir and MIP (Mersin International Port) ports were relatively the most efficient ports in the 5-year period. Ateş and Esmer (2011) determined the efficiency statuses of 15 Turkish container terminals using input- and output-oriented CCR and BCC models with 2010 data. DEA was used in Çağlar's (2012) study to determine the productivity of private ports in Turkey, and it was argued that relative analysis methods were not sufficient on their own to determine the potentials of port management. Ateş et al. (2013) examined the change in the efficiencies of 9 container terminals (Novorossiysk, Odessa, Varna, Batumi, Burgaz, Poti, Ilyichevsk, Constanta, and Trabzon), 5 of which were located in countries with coasts on the Black Sea and that were included in the European-Caucasian-Asian transport corridor (TRACECA) in the 2008-2009 period, as well as Russia, which was not included in the program. The efficiency values were calculated using DEA.

Ateş and Esmer (2013) investigated the efficiency change in 13 container terminals operating in Turkey before and after the 2009 global economic crisis. Relative efficiency values were measured using DEA and the changes in the efficiency values in a certain period were measured with the Malmquist total factor productivity (TFP) index. Ateş and Esmer (2014) evaluated the productivity of Turkish container terminals using free disposal hull (FDH) analysis and DEA. In a 2014 study by Güner et al. (2014) the operational efficiency levels of ports were examined by assessing the data pertaining to five ports privatized in 1997. Using DEA and the Malmquist TFP, 14 years of efficiency values were measured comparatively for 5 ports. Akgül et al. (2015) determined the efficiencies of container terminals using DEA. In a 2017 study by Acer and Timor (2017), the DEA method was implemented to determine the efficiencies of 20 container terminals. They made a comparison for redetermining the efficiencies of 16 similar ports using cluster analysis. Gökçek and Şenol (2018) determined the efficiencies of 28 container terminals in 9 Mediterranean countries using 2016 data. In that study, which was conducted using CCR and BCC input-oriented models, it was found that 4 ports were efficient with both methods.

Searching the literature for studies conducted to determine port efficiency using FDEA, several such works can be identified. Chen (2007) measured the efficiencies of the 20 terminals that had handled the most containers in 2004 in the Asian region using classical DEA and FDEA methods. A CCR model was used in that study and it was found that 10 terminals were efficient according to the results obtained from classical DEA. In calculations with FDEA, it was found that the segregation ability was more powerful. The study concluded that the efficiency scores obtained by classical DEA and FDEA were similar. Bray et al. (2015), who implemented the FDEA method using Tongzon's (2001) data, determined the efficiencies of 16 international container terminals using a CCR input-oriented model with six input and four output variables. The same values were used in both models; only the delay time value was expressed differently at three different levels (20%, 30%, and 40%) using triangular fuzzy numbers. At the end of the calculations, it was concluded that the results obtained at the 30% level were acceptable for making a comparison with the classical DEA method. Although the efficiency values obtained by the two models were close to each other, it was found that 12 terminals were efficient and 4 terminals were inefficient, and one particular terminal was inefficient in FDEA while it was efficient in classical DEA. Wang et al. (2017) determined the efficiencies of 12 container terminals situated in Taiwan and its periphery using fuzzy DEA. Evaluations for 6 input and 7 output variables were performed verbally, and these values were transformed into fuzzy numbers. In that study, conducted with a CCR model that was both input- and output-oriented, 2 terminals were found to be partially efficient and the rest were not efficient. In another study by Wang and Han (2018), input- and output-oriented FDEA models were set up to calculate the efficiencies of container terminals in Taiwan and the countries surrounding it. In both models, 2 terminals were found efficient while the other terminals were relatively not efficient.

As seen from this brief review, studies conducted using classical DEA to measure port efficiency are popular in the literature. On the other hand, the number of studies conducted using FDEA is small. The present study may therefore provide a helpful contribution to the literature, as studies on ports using FDEA are insufficient while studies on other topics have increased gradually.

Material and Methods

In this study, the FDEA method was used to calculate the relative efficiency of the container terminals operating in Turkey. The first study on DEA was conducted by Farrell (1957), in which the efficiencies of units were measured by linear programming using a single input variable and multiple output variables. The CCR model, named for the initials of Charnes, Cooper, and Rhodes (1978) was developed, in which multiple inputs and outputs were used as variables. With this method, measurements of general technical productivities were performed under constant returns to scale. The BCC model, named for the initials of Banker, Charnes, and Cooper (1984), was also proposed. This model enables the calculation of the scale and technical productivity separately under variable returns to scale. In the following years, DEA was improved by methods including multiplicative, non-oriented, and additive approaches (Aladağ et al., 2018).

Today, different FDEA models are being developed and implemented in many areas (industry, health, transportation, etc.). In this study, the data pertaining to container terminals were transformed into fuzzy values using Zimmermann's (2011) set of α cuts approach and the relative lower and upper limit efficiency values of the terminals were determined with FDEA using the EMS 1.3 software package with regard to the model proposed by Wang et al. (2005). For each α cut level of the obtained efficiency values, the lowest values of maximum efficiency loss (MEL) were calculated with the minimax regret approach (Wang et al., 2005), and the calculation and comparison of the relative efficiencies of the terminals were conducted. For this purpose, the following steps were followed:

i. Determining decision-making units,
ii. Determining input and output variables,
iii. Determining lower and upper limit values of variables using the set of α cuts approach,
iv. Calculating efficiency values of decision units with the FDEA method,
v. Ranking efficiency values using the minimax regret approach.

Determination of Decision-Making Units (DMUs)

It is important that the DMUs used in the implementation have a similar configuration and that the observation set be homogeneous for the FDEA results to be statistically significant. The exclusion of DMUs at the extremes is preferred (Şenol et al., 2019). In this context, only the terminals that handle 10,000 TEU or more were considered in this study, and it was ensured that the DMUs were homogeneous. The 22 container terminals included in the study are presented in Table 1.

Table 1. Container terminals included in the study

No.	Container Terminals	No.	Container Terminals
1	Assanport (CP1)	12	Mersin International Port (CP12)
2	Çelebi Bandırma (CP2)	13	Nemport (CP13)
3	Asyaport (CP3)	14	Roda Port (CP14)
4	Ege Gübre (CP4)	15	Samsunport (CP15)
5	Evyap (CP5)	16	Yılport (CP16)
6	Gemport (CP6)	17	Alsancak Port (CP17)
7	Kumport (CP7)	18	TCDD Haydarpaşa (CP18)
8	Alport (CP8)	19	Mardaş (CP19)
9	Limakport (CP9)	20	Borusan (CP20)
10	Limaş (CP10)	21	DP World Yarımca (CP21)
11	Marport (CP11)	22	Port Akdeniz (CP22)

In the DEA method, it is required that the number of DMUs be m+s+1 or 2*(m+s), where m is the number of inputs and s is the number of outputs, for the reliability of the research (Boussofiane et al., 1991). In this study, 4 input variables and 1 output variable were used. The number of DMUs was 22. Therefore, both conditions were met for the number of DMUs.

Determination of Input and Output Variables

It is imperative that the input and output variables be selected attentively and that they be reliable for DEA, which is a data-oriented efficiency measurement method (Göktolga and Artut, 2014). In DEA models, the number of input and output variables should be high to obtain better discrimination. However, the input and output variables to be used should be operative for all forms of decision-making (Boussofiane et al., 1991). The input and output variables that are most preferred in the literature and that play an important role in determining the efficiencies of container terminals are used in this study as presented in Table 2.

Table 2. Input and output variables used in the study

	Inputs		Output
X_1	Terminal's dock length (m)	Y_1	Number of containers handled (TEU)
X_2	Terminal area (m²)		
X_3	Maximum draft (m)		
X_4	Container handling capacity (TEU)		

In this study, the efficiencies of the terminals were measured by the model prepared with 4 input variables and 1 output variable. The data used in the study were gathered from the information provided on the websites of TÜRKLİM (Port Operators Association of Turkey), the Ministry of Transportation and Infrastructure, and the ports. The descriptive statistics for input and output variables are presented in Table 3.

Table 3. Descriptive statistics for input and output variables

Input and output variables	Mean	Standard error	Standard deviation	Minimum	Maximum
Terminal's dock length	1666.182	197.5207	926.4544	405	3413
Terminal area	424543.5	65681.35	308072.8	90000	1200000
Maximum draft	17.22273	1.387341	6.507207	10	36
Handling capacity	931590.9	165133.6	774545	150000	2600000
Number of containers handled in 2015	369221.5	96742.22	453761.2	2077	1576611
Number of containers handled in 2016	395558.9	98376.9	461428.5	11463	1844015
Number of containers handled in 2017	440978.5	100685.8	472258.4	11419	1709047

Determination of Lower and Upper Limit Values of Variables

Although data obtained from different sources are acknowledged as exact data, these data may vary due to incompleteness, errors, and alterations. These differences cause controversy regarding whether or not the data pertaining to the ports in Turkey are reliable. DEA is a technique that is extremely sensitive to data errors, and minute errors in gathering these data could lead to different efficiency scores being obtained.

In this study, it is considered that using FDEA would yield more reliable results to prevent the aforementioned errors. In the FDEA method, data are categorized into many classes such as limited, ordered, proportional, exact value known, or value unknown (Ebrahimi et al., 2018). If the lower and upper limit values of the data can be calculated, limited data may be obtained. Therefore, this study ensured the formation of fuzzy data by determining the lower, central, and upper limit values for the input and output values by a triangular membership function. Later, the fuzzy data were transformed into offset value data using Zimmermann's (2011) set of α cuts approach. The determination of the lower and upper limits, depending on the constancy of the variable data to be used in the study, could be performed in two ways.

Determination of Lower and Upper Limit Values of Non-Fixed Data

As the number of containers handled (Y1), which is used as the output variable in determining the efficiencies of container terminals, shows variation for each year, it is a non-fixed variable. In order to transform these data into offset data, the 2015-2017 data pertaining to this variable are determined as the lower, central, and upper limits using the triangular membership function and fuzzy data are obtained. Later, lower and upper limit values are calculated at five different α levels using Zimmermann's (2011) set of α cuts approach.

$$a^{-}_{\propto}=a+\propto(m-a) \quad (1)$$

$$b^{+}_{\propto}=b-\propto(Fb-m) \quad (2)$$

Here:

$a^{-}_{\propto}$ = lower limit value of the variable at α cut level,

$b^{+}_{\propto}$ = upper limit value of the variable at α cut level,

a = lower limit value of the variable,

b = upper limit value of the variable,

m = central value of the variable.

For instance, the number of handled containers (Y_1) in 2015, 2016, and 2017 in the Marport (CP11) container terminal is 1576611, 1844015, and 1709047, respectively. These values are written as fuzzy triangular numbers as [1576611; 1709047; 1844015]. According to these data, the lower ($a^{-}_{\propto}$) and upper ($b^{+}_{\propto}$) limit values of variable Y_1 at the α = 0.25 cut level for the CP11 container terminal are calculated using Eq. 1 and Eq. 2 as follows:

$$a^{-}_{\propto}=a+\propto(m-a) = 1576611+0.25(1709047-1576611) = 1609720$$

$$b^{+}_{\propto}=b-\propto(b-m) = 1844015-0.25(1844015-1709047) = 1810273$$

When the same calculations are performed for other α cut levels, the lower and upper limit values in Table 4 are obtained for the variable of the number of handled containers (Y_1) for the CP11 container terminal.

Table 4. Lower and upper limit values of the Y_1 variable for the Marport container terminal according to α levels

α cut level	Lower limit value	Upper limit value
0.00	1576611	1844015
0.25	1609720	1810273
0.50	1642829	1776531
0.75	1675938	1742789
1.00	1709047	1709047

Table 5. Lower and upper limit values of the X_4 variable for the Marport container terminal according to α levels

α cut level	Lower limit value	Upper limit value
0.00	2234866.4	2565133.6
0.25	2276149.8	2523850.2
0.50	2317433.2	2482566.8
0.75	2358716.6	2441283.4
1.00	2400000.0	2400000.0

Determination of Lower and Upper Limit Values of Fixed Data

Although the terminal's dock length (m), terminal area (m^2), maximum draft (m), and container handling capacity (TEU), which were used as input variables in this study, change over the years, the amount of this change may be ignored since it is minor enough to be deemed unimportant, especially for the period in which the efficiency analysis is being conducted. Therefore, these variables are considered as fixed data. These fixed data are transformed into fuzzy data using the following equations (Güneş, 2006):

$$a = m - S_h \tag{3}$$

$$b = m + S_h \tag{4}$$

Here, S_h is the standard error value. These values are calculated as lower and upper limits for five different α values using Eq. 1 and Eq. 2. Accordingly, the lower and upper limits for the container handling capacity (X_4) of the Marport (CP11) container terminal are calculated using Eq. 3 and Eq. 4 as follows:

$$a = m - S_h = 2400000 - 165133.6 = 2234866.4$$

$$b = m + S_h = 2400000 + 165133.6 = 2565133.6$$

These values could be expressed as triangular numbers as [2234866.4; 2400000; 2565133.6]. The lower ($a_\propto^-$) and upper ($b_\propto^+$) limits at the α = 0.25 cut level of the X_4 variable (offset value data) for the CP11 container terminal are then calculated as below using Zimmermann's (2011) set of α cuts approach:

$$a_\propto^- = a + \propto (m - a) = 2234866.4 + 0.25\,(2400000 - 2234866.4)$$

$$= 2276149.8$$

$$b_\propto^+ = b - \propto (b - m) = 2565133.6 - 0.25\,(2565133.6 - 2400000)$$

$$= 2523850.2$$

When the same calculations are made for other α cut levels, the lower and upper limits of the container handling capacity (X_4) for the CP11 container terminal, presented in Table 5, are obtained.

Calculation of Efficiency Values of Decision-Making Units with FDEA Method

There are four main approaches in the literature for conducting efficiency measurements using FDEA: the tolerance approach, possibility approach, fuzzy ranking approach, and α cuts approach. Besides these four primary groups, there are also some other approaches that could be considered within different categories (Hatami-Marbini et al., 2011; Emrouznejad et al., 2008). In this study, the FDEA method, which is based on the input-oriented CCR model following the method proposed by Wang et al. (2005) and which enables the lower and upper efficiency limits of the terminals to be obtained at five different α levels, was applied using the sets of α cuts approach. The CCR model calculates the inputs at minimum and the outputs at maximum. In addition, if the control over the inputs is low (or absent), an output-oriented model should be set up, and if the control over the outputs is low, an input-oriented model should be used (Dinc and Haynes, 1999). Since there is limited control over outputs in container handling terminals, an input-oriented model was preferred in this study. In this respect, the equations regarding the set of α cuts approach will be as follows:

Upper limit efficiency value:

$$Max\ (\theta_{j0})_\alpha^U = \sum_{r=1}^{s} u_r (y_{rj0})_\alpha^U \tag{5}$$

$$\sum_{i=1}^{m} v_i (x_{ij0})_\alpha^L = 1 \tag{6}$$

$$\sum_{r=1}^{s} u_r y_{rj}^U - \sum_{i=1}^{m} v_i x_{ij}^L \le 0 \quad j = 1, \dots, n \tag{7}$$

$$u_r, v_i \ge \varepsilon, \qquad \forall r, i.$$

Upper limit efficiency value:

$$Max\ (\theta_{j0})_\alpha^L = \sum_{r=1}^{s} u_r (y_{rj0})_\alpha^L \tag{8}$$

$$\sum_{i=1}^{m} v_i (x_{ij0})_\alpha^U = 1 \tag{9}$$

$$\sum_{r=1}^{s} u_r y_{rj}^U - \sum_{i=1}^{m} v_i x_{ij}^L \le 0 \quad j = 1, \dots, n \tag{10}$$

$$u_r, v_i \ge \varepsilon, \qquad \forall r, i.$$

Here:

θ_{j0}^{U} is the upper limit efficiency value of the terminals to be analyzed,

θ_{j0}^{L} is the upper limit efficiency value of the terminals to be analyzed,

n is the number of terminals,

i is the number of inputs (i = 1, 2, …, m),

r is the number of outputs (r = 1, 2, .., s),

$y_j = \{y_{1j}, y_{2j}, \dots, y_{rj}, \dots y_{sj}\}$ is the rth output value for the jth terminal,

$x_j = \{x_{1j}, x_{2j}, \dots, x_{ij}, \dots x_{sj}\}$ is the ith input value for the jth terminal,

y_{rj} is the output vector for the jth terminal,

x_{ij} is the input vector for the jth terminal,

v_i is the input weights,

u_r is the output weights,

L is the lower limit value for the terminal,

U is the upper limit value for the terminal.

The set $[\theta_{j0}^{L}, \theta_{j0}^{U}]$ formed by the lower limit efficiency value $(\theta_{j0})_{\alpha}^{L}$ and upper limit efficiency value $(\theta_{j0})_{\alpha}^{U}$, calculated at different α cut levels to obtain fuzzy efficiency, is the relative best efficiency interval of the relevant DMU. The upper limit value of outputs and the lower limit value of inputs are used in calculating the upper limit efficiency value. In calculating the lower limit efficiency value, on the other hand, the lower limit values of the outputs and the upper limit values of the inputs are used (Güneş, 2006). The DMUs with calculated efficiency values equal to 1.00 are considered as efficient, while the DMUs with calculated efficiency values smaller than 1.00 are inefficient DMUs. The lower and upper efficiency values calculated at five different α cut levels using Eq. 7 and Eq. 8 are presented in Table 6.

Table 6. Upper and lower limit efficiency values of the container terminals

DMU	α=0.00		α=0.25		α=0.50		α=0.75		α=1.00	
	U	L	U	L	U	L	U	L	U	L
CP1	1.000	0.354	1.000	0.421	1.000	0.502	0.898	0.603	0.735	0.735
CP2	0.126	0.036	0.111	0.043	0.099	0.052	0.088	0.062	0.074	0.074
CP3	1.000	0.133	0.923	0.275	0.846	0.416	0.773	0.559	0.703	0.703
CP4	0.791	0.687	0.840	0.736	0.908	0.790	0.951	0.852	0.922	0.922
CP5	1.000	0.589	1.000	0.684	1.000	0.782	1.000	0.883	0.988	0.988
CP6	0.593	0.631	0.649	0.661	0.720	0.695	0.772	0.732	0.772	0.772
CP7	0.717	0.468	0.714	0.530	0.710	0.590	0.707	0.648	0.704	0.704
CP8	0.191	0.036	0.113	0.040	0.079	0.044	0.066	0.049	0.056	0.056
CP9	0.353	0.192	0.352	0.229	0.352	0.266	0.348	0.304	0.342	0.342
CP10	0.128	0.052	0.110	0.056	0.099	0.061	0.086	0.067	0.076	0.076
CP11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CP12	0.933	0.969	0.929	0.955	0.925	0.942	0.921	0.930	0.918	0.918
CP13	0.914	0.615	0.879	0.643	0.841	0.676	0.802	0.713	0.757	0.757
CP14	1.000	0.439	1.000	0.490	1.000	0.560	1.000	0.660	0.817	0.817
CP15	0.458	0.204	0.403	0.221	0.377	0.242	0.353	0.270	0.306	0.306
CP16	0.580	0.518	0.587	0.525	0.593	0.533	0.578	0.542	0.552	0.552
CP17	0.916	0.871	0.908	0.873	0.901	0.877	0.895	0.882	0.888	0.888
CP18	0.249	0.169	0.246	0.184	0.242	0.199	0.240	0.216	0.235	0.235
CP19	0.530	0.431	0.531	0.450	0.532	0.470	0.524	0.491	0.513	0.513
CP20	0.912	0.646	0.917	0.684	0.926	0.729	0.893	0.783	0.847	0.847
CP21	0.463	0.002	0.361	0.016	0.260	0.029	0.158	0.043	0.056	0.056
CP22	0.583	0.420	0.559	0.436	0.540	0.454	0.523	0.475	0.501	0.501

Ranking of Efficiency Values by Minimax Regret Approach

As the efficiency value obtained by FDEA is considered as an interval, a simple and easily applicable method is required for the evaluation of these efficiencies. There are several approaches for the evaluation of interval numbers. However, the great majority of these methods are not powerful in discriminating interval numbers with the same centers but different widths. The minimax regret approach, developed by Wang et al. (2005) and frequently used in the literature, could be used to rank and compare the efficiency intervals of DMUs even if these DMUs are concentric and have different widths. According to the minimax regret approach:

Let $A_i = [a_i^L, a_i^U] = (m(A_i), w(A_i))$ $(i = 1, \ldots, n)$ be the efficiency intervals of n DMUs. The midpoints of the DMUs are:

$$m(A_i) = \frac{1}{2}(a_i^U + a_i^L) \tag{11}$$

and the widths of the DMUs are:

$$w(A_i) = \frac{1}{2}(a_i^U - a_i^L) \tag{12}$$

Let $A_i = [a_i^L, a_i^U]$ be selected as the best efficiency interval without exceptions, and let $b = \left[\max_{j\neq i}\{a_j^U\}\right]$. In this case:

a) if $a_i^L < b$, then the decision maker would experience efficiency loss and feel regret. The MEL is:

$$max(r_i) = b - a_i^L = \max_{j\neq i}\{a_j^U\} - a_i^L \tag{13}$$

b) if $a_i^L \geq b$, the decision maker would not experience efficiency loss or feel regret. In this case, the regret of the decision maker is defined as zero, i.e. $r_i = 0$. When these two cases are considered, the following is obtained:

$$max(r_i) = \max\left[\max_{j\neq i}(a_j^U) - a_i^L, 0\right] \tag{14}$$

Therefore, the minimax regret criterion would select the best efficiency interval, which fulfils the condition below:

$$\min_i\{max(r_i)\} = \min_i\left\{max\left[\max_{j\neq i}(a_j^U) - a_i^L, 0\right]\right\} \tag{15}$$

Based on the analysis above, with the aim of comparing and ranking the productivity intervals, let $A_i = [a_i^L, a_i^U] = (m(A_i), w(A_i))$ $(i = 1, \ldots, n)$ be an efficiency interval set. The MEL for each efficiency interval A_i could be calculated as follows:

$$R(A_i) = max\left[\max_{j\neq i}(a_j^U) - a_i^L, 0\right] \tag{16}$$

$$= max\left[\max_{j\neq i}\{m(A_j) + w(A_j)\} - (m(A_i) - w(A_i)), 0\right],$$

$$i = 1, \ldots, n$$

The minimum efficiency interval is the most attractive efficiency interval. The MELs, which are relevant numbers, are calculated according to the maximum efficiency among all other efficiency losses; however, these could not be used directly to rank them. The following elimination steps are proposed to produce a ranking using the MELs:

Step 1: MELs are calculated for all efficiency intervals, and the most attractive efficiency with the lowest efficiency loss is selected. Let us assume A_{i_1} is selected considering $1 \leq i_1 \leq n$.

Step 2: The selected A_{i_1} is eliminated; the MEL for the remaining (n – 1) an efficiency interval is calculated again and the most attractive efficiency interval is determined. Let us assume A_{i_2} is selected considering $1 \leq i_2 \leq n, i_2 \neq i_1$.

Step 3: Here, A_{i_2} is eliminated, the MEL for the remaining (n – 2) an efficiency interval is calculated again, and the most attractive efficiency interval A_{i_2} is selected.

Step 4: The elimination process is repeated until a single efficiency interval A_{i_n} remains. Here the ">" sign means "superior," and the ranking is $A_{i_1} > A_{i_2} > \cdots > A_{i_n}$.

In this study, while the CP11 (Marport) terminal was found fully efficient, having lower and upper limit efficiency values at all α cut levels equal to 1.00, the efficiencies of the remaining 21 terminals were measured as fuzzy numbers. The elimination steps mentioned above were applied to rank the fuzzy efficiency scores of the terminals found inefficient using the minimax regret approach. When MEL values are ranked from lowest to highest, the ranking of the fuzzy efficiency scores from best to worst is obtained as presented in Table 7.

Results and Discussion

The efficiencies of 22 container terminals operating in Turkey between 2015 and 2017 were evaluated using the FDEA method. Using the lower and upper limit values calculated at five different α cut levels together with Zimmermann's (2011) set of α cuts approach, the lower and upper limit efficiency values were determined with the FDEA model proposed by Wang et al. (2005). Later, the MEL values were calculated with the minimax regret approach and the efficiencies of the container terminals were ranked from best to worst.

The results of the FDEA calculations indicate that the CP11 container terminal is efficient at all α levels and the other terminals are relatively not efficient. In order to rank the inefficient container terminals, the MEL values should be considered. The values in Table 7 rank the MELs of the container terminals from best to worst. Here, a terminal with less efficiency loss is ranked as a better terminal. According to the MEL values, the CP11 container terminal is ranked first and the CP12 terminal is ranked second. The CP17 terminal has the lowest efficiency loss values at three α levels (0.00, 0.25, 0.50) and is ranked third in these cases, while it is ranked fourth and fifth at the 0.75 and 1.00 α levels, respectively. The CP5 terminal ranked third at α=0.75 and the CP4 terminal ranked third at α=1.00, having the lowest efficiency losses at these levels, respectively.

Table 7. Maximum efficiency loss (MEL) values of the container terminals according to α levels

α=0.00			α=0.25			α=0.50			α=0.75			α=1.00		
No.	**DMU**	**MEL**	**No.**	**DMU**	**MEL**	**No.**	**DMU**	**MEL**	**No.**	**DMU**	**MEL**	**No.**	**DMU**	**MEL**
1	CP11	0	1	CP11	0	1	CP11	0	1	CP11	0	1	CP11	0
2	CP12	0.0306	2	CP12	0.0443	2	CP12	0.0574	2	CP12	0.0699	2	CP5	0
3	CP17	0.1288	3	CP17	0.1264	3	CP17	0.1226	3	CP5	0.1166	3	CP4	0
4	CP4	0.3122	4	CP4	0.2636	4	CP4	0.2092	4	CP17	0.1176	4	CP12	0
5	CP20	0.3536	5	CP5	0.3153	5	CP5	0.2174	5	CP4	0.1477	5	CP17	0
6	CP6	0.3689	6	CP20	0.3154	6	CP20	0.2705	6	CP20	0.2170	6	CP20	0
7	CP13	0.3845	7	CP6	0.3383	7	CP6	0.3048	7	CP6	0.2680	7	CP14	0
8	CP5	0.4108	8	CP13	0.3562	8	CP13	0.3237	8	CP13	0.2861	8	CP6	0
9	CP16	0.4817	9	CP7	0.4694	9	CP7	0.4094	9	CP14	0.3391	9	CP13	0
10	CP7	0.5315	10	CP16	0.4743	10	CP14	0.4398	10	CP7	0.3514	10	CP1	0
11	CP14	0.5607	11	CP14	0.5096	11	CP16	0.4662	11	CP1	0.3962	11	CP7	0
12	CP19	0.5687	12	CP19	0.5497	12	CP1	0.4973	12	CP3	0.4410	12	CP3	0
13	CP22	0.5793	13	CP22	0.5637	13	CP19	0.5299	13	CP16	0.4571	13	CP16	0
14	CP1	0.6458	14	CP1	0.5785	14	CP22	0.5456	14	CP19	0.5089	14	CP19	0
15	CP15	0.7958	15	CP3	0.7250	15	CP3	0.5834	15	CP22	0.5243	15	CP22	0
16	CP9	0.8076	16	CP9	0.7707	16	CP9	0.7335	16	CP9	0.6957	16	CP9	0
17	CP18	0.8304	17	CP15	0.7788	17	CP15	0.7575	17	CP15	0.7300	17	CP15	0
18	CP3	0.8665	18	CP18	0.8157	18	CP18	0.8001	18	CP18	0.7832	18	CP18	0
19	CP10	0.9478	19	CP10	0.9439	19	CP10	0.9389	19	CP10	0.9324	19	CP10	0
20	CP2	0.9638	20	CP2	0.9562	20	CP2	0.9475	20	CP2	0.9374	20	CP2	0
21	CP8	0.9639	21	CP8	0.9600	21	CP8	0.9555	21	CP8	0.9502	21	CP8	0
22	CP21	0.9977	22	CP21	0.9839	22	CP21	0.9703	22	CP21	0.9569	22	CP21	0

The CP10, CP2, CP8, and CP21 terminals had the highest efficiency loss values and they ranked as the last four terminals at all α levels. Considering that the CP10, CP2, and CP8 container terminals are the terminals that handle the fewest containers, it is normal for these terminals to be less efficient compared to others. The CP21 container terminal, on the other hand, had just been operationalized. Although the number of containers currently handled by this terminal is relatively high, the number of handled containers in 2015 and 2016 was low compared to other terminals in the same period; thus, CP21 appears to be the terminal with the lowest efficiency at all α levels. It is thought that this container terminal will be ranked more highly in the following years due to the increasing number of handled containers.

It is seen that the number of handled containers (Y1), used as an output variable, is the most important variable in efficiency ranking. Therefore, it can be argued that as the number of containers handled by a container terminal increase, that terminal's efficiency ranking will also increase.

In the future, other studies on efficiency measurements at ports handling different types of cargo (bulk, liquid, chemical, etc.) could be conducted using different FDEA models. More detailed results and new inferences could be obtained regarding efficiency since the discrimination ability of FDEA is better than that of classical DEA, and such results obtained via FDEA could offer information and guidance for the administrators of container terminals.

Conclusions

Container transportation is one of the most used types in maritime transportation. As container transportation has increased gradually, it becomes important in terminals handling such cargoes. The increasing number of large container ships causes the ports to operate in a competitive environment. Therefore, measuring the efficiency of container terminals is very important for international trade. In this study, fuzzy data envelopment analysis (FDEA) was conducted to determine the relative efficiency of the container terminals operating in Turkey.

The efficiencies of the container terminals were measured by the model prepared with 4 input variables and 1 output variable. The input and output variables of the container terminals have been converted to fuzzy numbers with set of α cuts approach. Then the comparison and ranking of the container terminals was performed with the help minimax regret method. According to the results obtained from the study, only one container terminal is efficient at all α levels. This paper has also found that FDEA provides an effective method of evaluating relative port efficiency.

Compliance with Ethical Standards

Authors' Contributions

EY and ALT contributed to design and implementation of the research, to the analysis of the results and to the writing of the 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.

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RESEARCH ARTICLE

Determination of growth conditions for *Chlorella vulgaris*

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ABSTRACT

Microalgae known as third generation technology for biofuel in nature are used as a renewable bioenergy source. Microalgae and crop plants have common for the production of organic compounds by using sunlight and carbon dioxide. In addition, microalgae are capable of being reproducing in the whole year allowing the more product yield than those of plants. Therefore, microalgae are more favorable feedstock since they have some advantages such as photosynthetic efficiency, biomass productivity and oil content. In this study, the most proper conditions for the growth of microalgae *Chlorella vulgaris* were studied. After medium optimization, at 25°C and pH of 9 using 24-hour-illuminating/day, the best growth conditions for *C. vulgaris* giving the maximum biomass productivity was found to be 205 mg/L for 10 days. Determination of optimal microalgal growth conditions may lead to an increase in the industrial applications of *C. vulgaris*.

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Introduction

Microalgae are micrometer-sized aquatic organisms, generally in the range of 1–400 μm, and they are one of the oldest forms of life on the planet (Tijani et al., 2015). They have chlorophyll to be responsible for photosynthesis, their cells are covered with pigment-reproducing cells as well (Tan et al., 2018). Moreover, they account for approximately 50% of the whole photosynthetic activity on Earth. Because, some microalgae have rapid growth such as double in number within hours, thus, having a short harvest time as less than 10 days (Bach and Chen, 2017).

Microalgae particularly require optimum light, carbon dioxide, temperature, pH and nutrients to grow. While the light gives energy, carbon dioxide provides the carbon source for the production of biomass. Microalgae are able to produce different types of renewable biofuels such as biomethane with the anaerobic digestion process, biohydrogen, biodiesel with transesterification process and biochar production with pyrolysis process (Tan et al., 2018). Thus, microalgae can be accepted as an alternative and promising feedstock for energy production along with the overuse of fossil fuels. A progressive decline of resources of fossil fuels and an increase in climate change caused an obligatory situation to search for a renewable and sustainable energy source. Therefore, biofuels are

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considered as the best choice for that purpose (Jankowska et al., 2017). The fossil fuel energy is approximately 78.3% of the whole global energy consumption. Moreover, fossil fuel is a great contributor to CO_2 emissions which are directly linked to global warming. Biodiesel is a clean-burning fuel and is obtained from plants such as soybeans, oil palms, jatropha and microalgae (Elrayies, 2018). The development stages of biofuels are classified into three generations in terms of their feedstock. The first generation of biofuels is obtained from food crops such as sugar cane, sugar beets, rapeseed, soybeans, oil palms and corn. However, the undesirable competition had occurred between food and fuel in time. This is due to the place intended for the production of food that was shifted to the production of food crops used for biofuels. The second generation biofuels are obtained from lignocellulosic agriculture, forest residues and non-food crop feedstocks which also termed as energy crops such as jatropha and pongamia. However, some deficiencies had been occurred such as low productivity of biomass, need for excess water and arable land, and also low energy required pretreatments. In recent years, a new biofuel generation had been appeared and known as the third generation biofuels which are obtained from microalgae. Microalgae culture as feedstock has more advantages compared that the second generation needs the more land, such as having high growth rate, the need for less amount of water for the growth as compared to terrestrial crops, having a high photosynthetic efficiency; having more oil productivity (Faried et al., 2017; Jankowska et al., 2017; Elrayies, 2018). In this study, the proper growth conditions of *Chlorella vulgaris* was investigated.

Materials and Methods

Microalgae

Chlorella vulgaris was provided by courtesy of Prof. Dr. Meltem Conk Dalay from Ege University Bioengineering Department, Turkey. The strain was preserved in the BG11 medium which has composition as follow: $NaNO_3$ (1.5 g/L), $K_2HPO_4{\cdot}3H_2O$ (0.04 g/L), $MgSO_4{\cdot}7H_2O$ (0.075 g/L), $CaCl_2{\cdot}2H_2O$ (0.036 g/L), Na_2CO_3 (0.02 g/L), citric acid (0.006 g/L), Ferric ammonium citrate (0.006 g/L), EDTA (0.001 g/L), and A5 + Consolation (1 mL/L) that consists of H_3BO_3 (2.86 g/L), $MnCl_2{\cdot}H_2O$ (1.81 g/L), $ZnSO_4{\cdot}7H_2O$ (0.222 g/L), $CuSO_4{\cdot}5H_2O$ (0.079 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.390 g/L) and $Co(NO_3)_2{\cdot}6H_2O$ (0.049 g/L) (Suthar and Verma, 2018). The BG11 medium was used as a growth medium in each experimental set-ups.

Medium Optimization in Erlenmeyer Flasks

Obtaining the most proper growth conditions was performed with 100 mL of BG11 medium within 250 mL of Erlenmeyer flasks in shaker-incubator (Microtest, Turkey). The different range of pH (5, 7, 9), temperature (25, 35, 45 °C) and illumination period (24 h light/day, 12h-dark-and-12-h light/day, 24 h dark/day) were used using one variable at a time method. Microalgal cell biomass was separated from the medium by centrifugation at 5000 rpm for 15 min. Dry cell weight was used as a marker for biomass production.

Analysis

Microalgal cell biomass was separated from medium by centrifugation (Centurion, England). BG11 culture medium containing microalgae was centrifuged at 5000 rpm for 15 min, washed twice with distilled water then, the cell pellet was dried at 105°C until a constant weight was observed. Dry weight was measured by filtering a 10 mL of the culture by preweighted filters. After rinsed with ammonium bicarbonate, the filters were dried at 105°C overnight. Dry cell weight was used as a marker for biomass production.

For cell analysis, the culture broth was sampled daily and absorbance was recorded spectrophotometrically at 650 nm.

Results and Discussion

Optimization of pH

In order to determine the optimum pH for *C. vulgaris,* the microalgae production has occurred in Erlenmeyer flasks in the range of pH of 5-9. In this study, the suitable value of pH with a maximum biomass concentration of 150 mg/L after 10 days for *C. vulgaris* was found as 9. Once the pH goes up and the sufficient amount of CO_2 is injected into the microalgal growth medium, pH would back to optimum and lead to an increase in the microalgal biomass. In another study, it was reported that the best growth pH of *C. vulgaris* was 9. It was interpreted that H^+ ion gradients control transport processes throughout the cellular membranes and perform the metabolic activities in the cytoplasm and organelles (Suthar and Verma, 2018).

Optimization of Temperature

The temperature range (25, 35, 45 °C) was used to determine the optimal temperature for *C. vulgaris*. The optimum temperature for the highest biomass concentration of 182 mg/L after 10 days was decided as 25°C. The less growth has appeared at 35 and 45°C. Microalgal growth rapidly decreased when the temperature exceeding the optimum temperature. Therefore, the growth did not occur above 35°C.

It was previously reported that the growth rates for *C. vulgaris* at 28°C and 25°C are not greatly different (Serra-Maia et al., 2016). It was also discovered that temperature below 30°C enhanced the solubility of CO_2 in growth medium thus, dissolving the CO_2 resulted in higher biomass production because of an increase in the growth rate of culture (Mathimani et al., 2017). Moreover, it was declared that the growth rate of *C. vulgaris* at 35°C exhibited a 17% decrease according to those at 30°C and further temperature caused a sharp interruption of microalgal growth and eventually cell deaths (Converti et al., 2009).

Optimization of Illumination Period

The microalgal growth was observed during different illumination conditions at 25°C and a pH value of 9. Continuously illumination (24 h light/day) of the microalgal culture provided more rapid growth and higher biomass concentration of 205 mg/L for *C. vulgaris* after 10 days in this study. Previously, it was remarked that the lipid percent and biomass productivity were higher in 18:6 h light compared to 12 h and 24 h light when keeping the other conditions constant (Ebrahimian et al., 2014). However, it was also reported that there was no significant change in the lipid content of *C. vulgaris* when the dark cycle increases from 8 h to 12 h (Luangpipat and Chisti, 2017). In another study, it was shown that the best light:dark cycle for *C. vulgaris* was 16:8 h/day (Sasi, 2009). The cell growth at optimized conditions (pH=9, 25°C and continuous illumination) was shown in Figure 1.

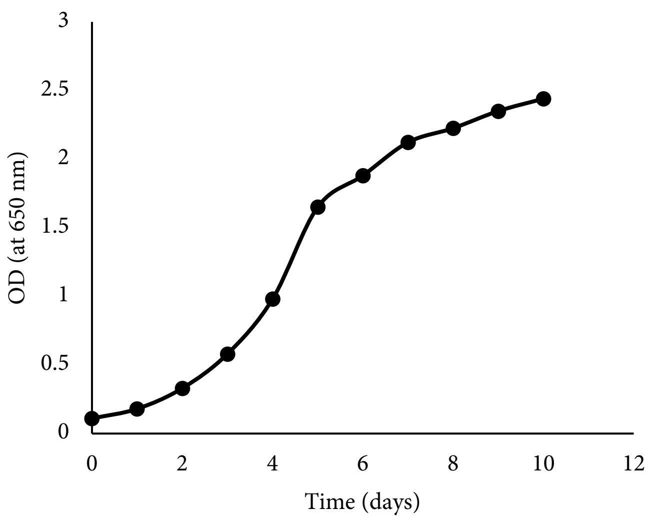

Figure 1. Cell concentration at optimized conditions

Conclusion

In this study, the best conditions with the highest biomass concentrations were determined to be pH 9, 25°C and 24 hour light for the biomass growth of *C. vulgaris* regarding a biomass concentration of 205 mg/L. Microalgae are one of the important sources for the production of biodiesel due to they possess high oil content and there is a limitation about keeping important places of plant-based oil such as safflower, canola and peanut in the food industry. This study focused on the biomass concentration of *C. vulgaris* providing its optimum growth conditions. In the future, the utilization of microalgae as a feedstock of renewable fuels is considered to increase.

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Compliance with Ethical Standards

Authors' Contributions

Author ID designed, performed and managed statistical analyses of the study and wrote the manuscript. The author 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.

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RESEARCH ARTICLE

Determination of fish consumption habits of consumers: Case study of Mary city, Turkmenistan

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ABSTRACT

The aim of this study was to determine the habits of families towards fish consumption in urban areas of Mary city in Turkmenistan. The main data of this study has been compiled by asking 20 questions to 267 consumers who lived in Mary city. Predetermined questions were applied to randomly selected people as question-answer. Sample size of this study was determined with unclustered probability sampling method. According to the research findings, yearly fish consumption of the examined consumers was determined as 3.28 kg per capita. Mostly preferred fish are catfish, herring and grey mullet respectively. Consumers preferred bazaars the most and markets the least as a place to purchase fish. 97% consumers consume fresh fish while 64.79% consume fish by frying. While 56.55% of consumers think that fish prices are high, 34.46% of consumers think it is normal. At the end of the study, it is obtained some results relevant to participant's average monthly income, educational background, their professional status, marital status, types of supplying and consuming fish, cooking methods and attitude towards fish consumption.

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Introduction

With the increase of the world's population, urbanization and the rise of social welfare, the demand for animal products is increasing day by day with the increase of the phenomenon of healthy and balanced nutrition. The researches emphasize that at least 40-50% of the daily protein requirement should be obtained from animal-based nutrients (Kiziloglu et al., 2013). However, it is still known that animal protein consumption is insufficient in many countries today. Fish and other seafood products are an important option in order to close the animal protein deficiency. Fishery products are an important resource for closing this deficit as an animal nutrient with high nutritional value and increasing its production through culture

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in addition to natural stocks (Dogan, 2002). Fish in particular is an indispensable food item in terms of human nutrition and accordingly human health (Ikenweiwe et al., 2011). Fish meat consists mainly of protein, fat and water. However, fish meat contains a significant amount of vitamins, iron and essential amino acids (Gogus and Kolsarici, 1992; Wang et al., 2009; Adeli et al., 2010). Fish oil (omega-3) consumed in high amounts was found to reduce the risk of developing many chronic diseases, especially cardiovascular disease (Trondsen et al., 2004).

Fish constitutes a major source of animal protein in many nations. Global fish consumption is 20.3 kg per capita and it is about 3.3 kg per capita in Turkmenistan (FAO, 2018). It is show that Turkmen people are consumed fishes in low levels. According to FAO (2018) annual fish production is about 15 thousand tons in Turkmenistan. Fish and seafood products contribute to food security at a limited level. Seafood products play an important role exclusively in the diet of the people living in the coastal area.

The aim of this study is to obtain data on the behavior and fish consumption habits of consumers in Mary city center, one of the important cities of Turkmenistan. Therefore, it is aimed to reveal the taste, preference and thoughts of consumers that are effective in their properties and consumption. Thus, this research is intended to contribute to a different approach by setting an example for future fish consumption research.

Materials and Methods

Primary and secondary data sources were used in the preparation of this research. The primary data source of the research is horizontal cross-sectional data obtained via survey from families residing in the urban area of Mary city. The survey form used in the study was developed in accordance with the purpose of the research by using studies that had previously been done for similar purposes. In this study conducted in 2019, a survey of 20 questions was applied to consumers. In the scope of the survey, it was aimed to determine the educational status of the consumers, their monthly income, their yearly fish consumption, the types of fish they consume and love the most, the characteristics they pay attention to when buying fish, and their thoughts on the way they consume and cook fish. In this study, the research, articles, papers, reports prepared before, as well as the statistical data published by the relevant institutions were utilized.

Sampling methods

Sufficient sample size to represent the population in the study was determined by using "unclustered probability sampling method" (Collins, 1986).

$$n = t^2 \times [1 + (0.02)(b - 1)] \times (p \times q)/e^2 \quad (1)$$

Where;

n: Sample size,

t: T table value corresponding to 95% significance level,

b: Sampling stage (taken as 1 since the method is single-stage),

p: Occurrence probability of the relevant case within the main mass taken as 50%,

q: Non-occurrence probability of the relevant case (1-p),

e: Accepted margin of error (The margin of error was taken as 6% in this study).

In the equation, when B=1 is taken, the equality is transformed into the following form:

$$n = t^2 \times (p \times q)/e^2 \quad (2)$$

According to this formula, the sample size is calculated as follows:

$n = 1.96^2 \times (0.50 \times 0.50) / 0.06^2$

$n = 267$

The data was transferred to the computer using SPSS and Microsoft Excel package programs, and descriptive statistical analyses (frequency, mean and percentage) were performed on the findings. The likert type scale was used to evaluate the factors that consumers care about in buying and consuming fish, as well as their level of knowledge about fish. In the study, expressions on the attitude scale were evaluated on a five point scale (Bilgin, 1995).

Results and Discussion

Socio-demographic characteristics of consumers

It is important to know the socio-demographic characteristics of fish consumers in order to investigate the consumption levels and habits of fish consumers. Table 1 includes some socio-demographic characteristics of fish consumers. Accordingly, 59.55% of consumers are women and 40.45% are men. The result also shows that majority (91.39%) of consumers were married while 8.61% were single. Of the surveyed consumers, 14.98% are in the 18-30 age range, 35.96% are in the 31-40 age range, 40.82% are in the 41-50 age range, and 8.24% are 51 years of age or older. When the number of individuals in the family is examined, 23.22% are 1-3 people, 56.55% are 4-6 people, and 20.23% are 7 or more.

When looking at the educational status of the interviewed consumers, 53.18% of them graduated from high school, 33.71% from vocational high school and 13.11% from university. While looking at the distribution of consumers by occupations, 28.84% are public servants, 20.23% are artisans/merchants, 18.35% are workers, 13.11% are retired, 10.86% are housewives, 5.62% are farmers and 2.99% are from other occupational groups. The monthly income of the surveyed consumers ranges from $140 to $750. 37.07% of consumers have monthly income of less than $200, 43.45% have between $201-500, and 19.48% have $501 and above.

Table 1. Some socio-demographic characteristics of consumers

Characteristics		Number	Rate (%)
Gender	Man	108	40.45
	Woman	159	59.55
Marital status	Married	244	91.39
	Single	23	8.61
Age distribution	18-30	40	14.98
	31-40	96	35.96
	41-50	109	40.82
	Over 51	22	8.24
Educational status	High school	142	53.18
	VHS	90	33.71
	University	35	13.11
Occupation	Public servant	77	28.84
	Artisan /merchant	54	20.23
	Worker	49	18.35
	Retired	35	13.11
	Housewife	29	10.86
	Farmer	15	5.62
	Other	8	2.99
Number of people in the household	1-3	62	23.22
	4-6	151	56.55
	7-+	54	20.23
Monthly household income	0 - 200 $	99	37.07
	201 - 500 $	116	43.45
	501 $ <	52	19.48

Consumers' fish consumption status and habits

Information on the fish consumption characteristics of the interviewed consumers is given in Table 2. When the frequency of fish consumption is examined, 33.33% of consumers consume fish once a month, 31.84% several times a year, 21.72% every fifteen days, 8.61% once a week and 4.50% 2-3 times a week. The average yearly fish consumption of the surveyed consumers is 3.28 kg per capita. This value in the world is about 20.3 kg/year and it is about 3.3 kg/year in Turkmenistan (FAO, 2018). The amount of fish consumption was the same the country average. Yearly per capita fish consumption was calculated 12.2 kg/year in the study conducted by Karakaya and Kirici (2016) in Bingöl, Turkey, in the study conducted in India by Bhuyan et al. (2017) was 14.27 kg/year, and in another study conducted in Australia by Farmery et al. (2018) was 9.6 kg/year. Compared to these consumption figures, it can be said that the yearly fish consumption per capita in the research area is quite low.

Table 2. Consumers' fish consumption characteristics

Characteristics		Number	Rate (%)
Frequency of fish consumption	2-3 times a week	12	4.50
	Once a week	23	8.61
	Every 15 days	58	21.72
	Once a month	89	33.33
	Several times a year	85	31.84
Amount of fish consumption (kg/year)	0-2 kg	122	45.69
	2-4 kg	84	31.46
	4-6 kg	25	9.36
	6-8 kg	14	5.24
	8-10 kg	10	3.75
	More than 10 kg	12	4.49
Preferred fish species	Catfish	100	37.45
	Herring	54	20.23
	Carp	47	17.60
	Grey mullet	38	14.23
	Other	28	10.49
Way of fish consumption	Fresh	259	97.00
	Processed	8	3.00
Fish cooking methods	Frying	173	64.79
	Baking	64	23.97
	Steaming	26	9.74
	Other	4	1.50
Fish consumption season	Summer	50	18.73
	Winter	156	58.43
	Both summer and winter	61	22.84
Reasons for consuming fish	Being delicious	119	44.57
	Being healthy	93	34.83
	Being cheap	34	12.73
	Having a habit	21	7.87

Within the scope of the research, 37.45% of consumers stated that they consumed catfish, 20.23% herring, 17.60% carp, 14.23% grey mullet and 10.49% other fish species as a primarily.

When the way consumers consume fish is examined, 97.00% of consumers consume fish as fresh and 3% as processed. In various studies on the subject, the rate of fresh consumption of fish by consumers is 85.12% in Mexico (Pérez-Ramírez et al., 2015), 97% in Erzurum and Van cities, Turkey (Gungor and Ceyhun, 2017), and 76% in Indonesia (Firmansyah et al., 2019).

When the preferred fish cooking methods are sorted from top to bottom, the preferred method is frying with 64.79%, followed by baking with 23.97%, steaming with 9.74% and other cooking methods with 1.50%.

In terms of healthy eating, fish should be consumed in every season. It is seen that the majority of consumers consume fish in winter season (58.43%). However, 18.73% of the surveyed consumers stated that they consumed fish only in summer season, while 22.84% stated that they consumed fish both in summer and in winter season. In previous studies reported that consumers consume fish meat maximum in winter seasons (Erdal and Esengun, 2008; Terin et al., 2016; Kizilaslan, 2019; Saka and Bulut, 2020).

Of the individuals surveyed, 44.57% prefer fish for being delicious, 34.83% for health, and 12.73% for cheap and 7.87% for habit.

Consumers stated that the freshness of the fish is very effective when buying fish with an average score of 4.87. The taste of the fish (4.41 points), price (3.90 points) and the fishbone condition (3.46 points) were mentioned by consumers as effective factors in their preference for fish (Table 3).

Table 3. Distribution of factors affecting consumers' purchase of fish according to their importance

Factors	Attendance Ratings * (%)					Average Score
	1	2	3	4	5	
Freshness	0.37	0.37	1.12	5.62	92.51	4.87
Taste	3.37	5.24	6.74	16.10	68.54	4.41
Price	5.24	9.36	14.61	31.46	39.33	3.90
Fishbone condition	13.48	14.61	15.36	25.47	31.08	3.46

Note: *(1: Not important at all; 5: Very important)

Figure 1 provides information about consumers' preferred fish markets. According to the survey results, 89.88% of consumers buy fish from the bazaar, 7.5% from the market and 2.62% from peddlers. 56.5% of participants find the variety of fish in the market adequate, while 43.5% do not find the variety adequate.

Consumers' confidence levels in the places where they buy fish were also examined. Accordingly, 63.67% of consumers generally expressed confidence in bazaars, 43.06% in markets and 40.82% in peddlers (Table 4).

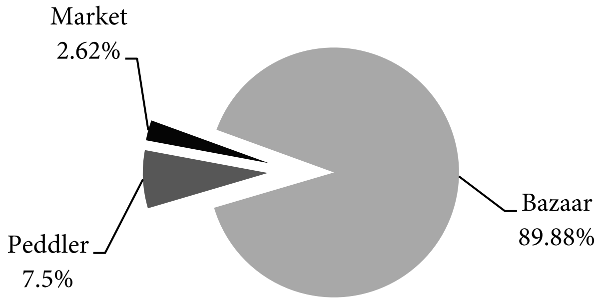

Figure 1. Consumers' preferred fish markets

Table 4. Consumers' confidence levels in places where they buy fish

Places	Always (%)	Usually (%)	Sometimes (%)	Rarely (%)	Never (%)
Bazaar	29.96	33.71	11.24	13.85	11.24
Market	13.85	29.21	13.85	13.50	29.59
Peddler	26.59	14.23	31.84	15.36	11.98

56.55% of the surveyed consumers found that fish prices were generally expensive, while 34.46% stated that they were normal and 8.99% were cheap (Table 5). 81% of consumers in the study conducted in Kenya by Esilaba et al. (2017), 73% of consumers in the study conducted in Kahramanmaraş, Turkey by Ercan and Sahin (2016), stated that fish prices were expensive. It can be said that the result obtained from the research is similar to other study results.

Table 5. Consumers' thoughts on fish prices

Thought	Number	Rate (%)
Very Expensive	64	23.97
Expensive	87	32.58
Normal	92	34.46
Cheap	24	8.99
Total	267	100.00

The status of consumers' participation in certain statements regarding fish consumption was given in Table 6. Consumers were asked to respond to the judgments on fish consumption in Table 6 as "strongly disagree" "disagree" "undecided" "agree" and "strongly agree". When consumers ' responses to these judgments were examined, it was determined that 71% of consumers participated in the statement that fish is an important food item in human nutrition.

It was found that 94% of consumers participated in the statement that fish is healthy. 56% of consumers participated in the statement that I find fish prices high. Approximately 20% of participants that thought they have consumed enough fish, while 54% stated that they have not consumed enough fish. It was determined that 32.96% of consumers strongly disagreed and 34.08% disagreed with the statement that ads affect my fish consumption.

Table 6. Status of consumers to participate in statements on fish consumption

Statements	Attendance Ratings * (%)					Average Score
	1	2	3	4	5	
Fish is an important food item in human nutrition.	4.49	3.75	20.22	35.21	36.33	3.95
Fish is healthy	0.37	0.37	4.49	54.68	40.07	4.34
I find fish prices high	12.36	14.98	15.73	32.96	23.97	3.41
I consume enough fish	29.59	25.09	25.47	10.11	9.74	2.45
Ads affect my fish consumption	32.96	34.08	20.97	7.49	4.49	2.17

Note: *1: Strongly Disagree, 2: disagree, 3: Undecided, 4:Agree 5: Strongly Agree

Conclusion

Fish is one of the oldest food sources of humans. Fish with a high nutritional value in terms of our health are consumed today by being loved and the demand is increasing every day. The increase in fish demand is directly related to the knowledge of consumer characteristics and preferences. Therefore, studies in this area are becoming increasingly important.

In this study, fish consumption status of consumers in Mary city of Turkmenistan was examined. According to the findings, the average amount of yearly fish consumption of the individuals surveyed was found to be 3.28 kg per person. The average fish consumption is about 3.3 kg per capita in Turkmenistan. Previous studies demonstrate that fish consumption is influenced by many factors such as sociodemographic background, personal health status, society, household income and education level (Trondsen et al., 2004; Olsen et al., 2007). High fish prices and weak seafood consumption culture are important factors affecting fish consumption in research area. However, the important reason for the low consumption of fish is that the research area is far from the sea. This also reduces the variety of seafood's in the market.

As a result of the research the consumption rate of fresh fish was determined as 97%. This trend is similar with other studies on consumption of seafood. A significant number of consumers stated that they prefer fish because it is healthy and delicious. Fish consumption is known to have a positive effect on certain diseases. The health researches revealed that consumption of fish oil (omega-3) reduced the risk of many diseases, particularly cardiovascular diseases (Trondsen et al., 2004). The most preferred fish consumed in research area is catfish because it is cheap, nutritious and has taste well.

The purchasing of fish is influenced by many factors such as freshness, taste, smell, price, health, nutrition and quality. A research conducted in France has shown that quality and freshness are the most important factors in the sale of fish (Botrel, 2007). Results show that the most important criteria influencing fish choice by consumers as taste, nutritious and price.

The level of education is an important element in consumers' choice of fish. Also, the socioeconomic situation, differences in the household income and their occupations can cause differences in consumption areas (Salehi, 2006). According to the findings 46.82% of consumers are graduates from higher education. 33.33% of consumers stated that they consumed fish once a month, 31.84% several times a year, 21.72% every fifteen days, 8.61% once a week, and 4.50% 2-3 times a week.

When the research results are evaluated in general, the majority of consumers think that the fish is nutritious and healthy. However, a significant number of respondents surveyed stated that fish prices were high. In order to increase the consumption of fish, it is important to have different varieties regularly at fish outlets and to offer them to consumers at reasonable prices. Fish have many benefits in terms of human health. Therefore, the importance of fish consumption in terms of human health should be emphasized. For this reason, with effective training and extension services, consumers should be directed to healthy nutrition. In addition, more extensive surveys into consumer tendency to fisheries will be important sources of initiatives to increase consumption.

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Compliance with Ethical Standards

Authors' Contributions

AA designed the study. GB wrote the first draft of the article and performed 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.

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Marine Science and Technology Bulletin

SHORT COMMUNICATION

First report of *Symphodus melops* (Linnaeus, 1758) with maximum length in the Black Sea

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ABSTRACT

An individual of corkwing wrasse *Symphodus melops* (female) was captured by trammel nets on May 15, 2020, at 20 m depths from Ordu, the southern Black Sea coast. The total length and weight were measured as 320 mm and 520 g. The specimen was female with a gonad weight of 42 g and it was determined to be eight years old. This is the first record for the *S. melops* from the Black Sea. Its total length and weight were the maximum observed values for the species for the World. The paper is considered to contribute to fisheries biology and international scientific literature.

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Introduction

Labridae has 504 species in the world (Parenti and Randall, 2011), there are 20 species in the Turkey coast (Bilecenoğlu et al., 2014) and eight of them live in the Black Sea (Keskin, 2010). Corkwing wrasse (*Symphodus melops* Linnaeus, 1758) is a native species to the eastern Atlantic Ocean, the Adriatic Sea and in the Mediterranean Sea (Fischer et al., 1987) but not reported in the Black Sea coast. Corkwing wrasse lives in coastal waters at depths of 1 to 30 m, near the rocky substrate and seagrass beds (Muus and Nielsen, 1999). Corkwing wrasse feed with various benthic invertebrates and they reach the first reproductive length of 7-10 cm (1 year-old) for female and 13-15 cm (2-3 years old) for males (Fischer et al., 1987). Coloration very variable, the ground color of the male is greenish or blue while females are brownish to yellowish (Muus and Nielsen, 1999). The maximum length has been reported as 28 cm (Quignard and Pras, 1986; Fischer et al., 1987).

In this study, the first record of *Symphodus melops* for the Black Sea region and the maximum length and weight has been reported.

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Material and Methods

One female specimen of corkwing wrasse (Figure 1) was caught by using a trammel net at a depth of 20 m on May 15, 2020 from Fatsa, Ordu (41°03'44.17"N, 37°30'40.00"E) the northern Black Sea (Figure 2). Total length (TL) and weight (W) were measured to the nearest 0.1 cm and 0.1 g, respectively. Gonad weights, egg counts, and diameters were also determined.

Meristic characters were measured with digital caliper the nearest 0.01 cm sensitivity. Sixteen metric measurements from *S. melops* were performed. Fifteen morphometric characters were evaluated as TL%. Regression analysis of differences body parts against TL of the fish was drawn by the least square method. Dependent and independent variables, TL and morphometric measurements were transformed using log 10.

Results

The total length and weight of the sampled individual were 320 mm and 520 g, respectively. The morphometric properties of the *S. melops* were proportional to the total length. The smallest ratio was eye size (3.8 %), and the highest ratio was the standard length (89.1%). The body depth of the species is 33.8 % of the total length (Table 1).

The pectoral fin position is ahead of the location of the first dorsal and pelvic fin. Scales cycloid cover the entire body. The head length is about 27.8% of the total length (Table 1).

Seven meristic characters were examined. The lists of meristic characters used for the analysis of *S. melops* are presented in Table 2.

Figure 1. *Symphodus melops* 320 mm total length and 520 g weight from the Black Sea

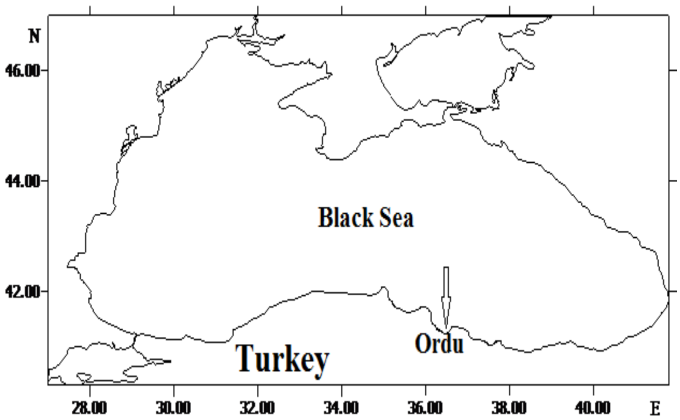

Figure 2. Sampling location from Ordu, Turkey

The swim bladder was measured 65 mm length and 36 mm diameter of *Symphodus melops* for a total length of 32 cm fish (Figure 3).

On the first gill arch has 14 on the upper part, 16 on lower gill raker (Figure 4).

Age determination was carried out by using the individual's vertebrae, and age of the specimen was determined to be 8 years (Figure 5).

The gonads of the sampled female individual were determined to have matured, and the total gonad weight was measured as 42 g. The number of eggs was 7526 /g (fecundity = 316092 eggs), and the average egg diameter was measured as 576.1 µm ± 81.2 (minimum: 314.3 – maximum: 679.44).

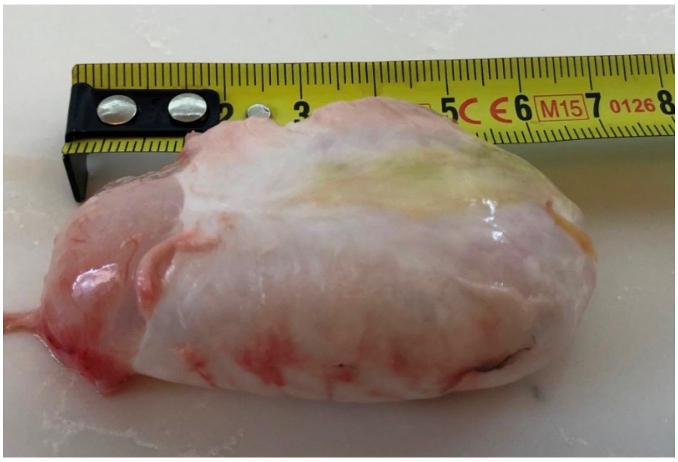

Figure 3. The swim bladder of *Symphodus melops*

Table 1. Some metric properties of *Symphodus melops*

Characters (mm)	Values	TL%
Total length	320	-
Standard length	285	**89.1**
Head length	89	27.8
Post-orbital distance	38	11.9
Eye diameter	12	**3.8**
Pre-dorsal distance	95	29.7
Length of dorsal fin basis	142	44.4
Pre-anal distance	177	55.3
Length of anal fin basis	67	20.9
Depth of anal fin	25	7.8
Max. body depth	108	33.8
Caudal peduncle minimal depth	42	13.1
Pectoral length	51	15.9
Pre pectoral length	82	25.6
Pelvic length	45	14.1
Pre pelvic length	106	33.1
Total weight (g)	520	-
Sex	Female	-
Gonad weight (g)	42	-

Table 2. Meristic features of *Symphodus melops*

Meristic features	Values
Dorsal fin	XV, 11
Pelvic fin	I, 5
Anal fin	III,10
Pectoral fin	14
Caudal fin	14
Gill rakers	14
Linea lateral	34

Figure 4. The first gill arch of *Symphodus melops*

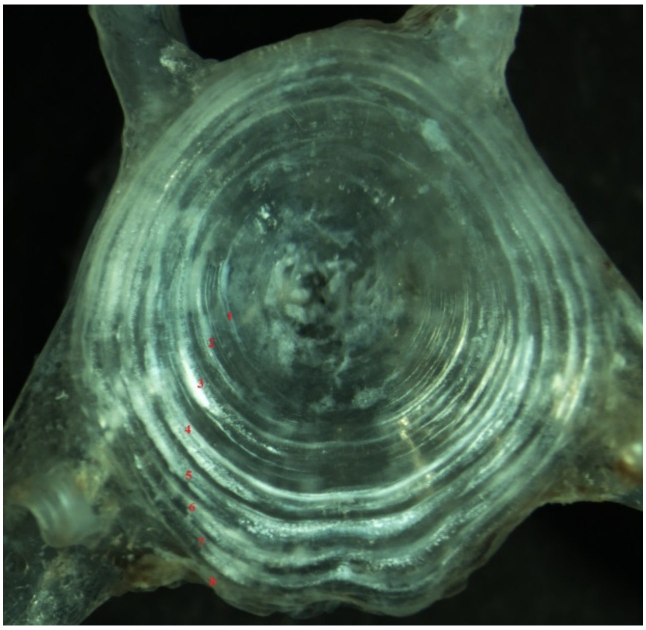

Figure 5. Cross-section of a vertebra of an eight years old *Symphodus melops* (32 cm TL) captured along the Fatsa region (southern Black Sea) on May 15, 2020

Discussion

The captured specimen of *S. melops* was 320 mm in total length and 520 g in total weight. Fischer et al. (1987) determined that this species is mostly in the range of 10-20 cm and the maximum length is 28 cm. Froese and Pauly (2020) are stated that the species can reach 28 cm standard length (Quignard and Pras, 1986; Froese and Pauly, 2020). The maximum total length for *S. melops*, is similar to other studies. This paper reports the largest individual so far registered in the world for *S. melops*. Fischer et al. (1987) reported 16 cm maximum length for *Symphodus (Crenilabrus) cinereus* which belongs to the same family and is very similar to *S. melops*.

Fischer et al. (1987) reported that there are 14-17 spine rays and 8-10 soft rays in the dorsal fin, three spine rays and 8-11 soft rays in the anal fin of the species. They also stated that there are 13-16 gill rakers on the first-gill arch and 31-37 cycloid structure scales on the lateral line. The meristic characters shared similarities with Fischer et al. (1987) findings. In addition, all descriptive characters are similar to Froese and Pauly (2020). The species presents sexual dimorphism with brownish females and larger, more colorful males (Quignard and Pras, 1986). The sampled female in the reproductive period in this study also has a more brownish color.

Labridae is one of the largest and most morphologically and ecologically diversified fish families in the world (Parenti and Randall, 2000; Hanel et al., 2002; Nelson, 2006; Tiralongo and Tirnetta, 2018). *S. melops* was reported by Bilecenoğlu et al. (2014) in the Aegean Sea. It is known that some species living in the Mediterranean in recent years have migrated through the Turkish Straits system and adapted to the Black Sea (Aydın and Sözer, 2016). It is known that 8 species are belonging to the same family in the Black Sea (Keskin, 2010). Therefore, *S. melops* which prefers similar ecological conditions, are likely to adapt to the Black Sea. The gonads of the sampled female individual were determined to have matured, and the total gonad weight was measured as 40 g. Considering these data, it can be said that the species spawned in the Black Sea. Torstensnes (2016) stated that the species was able to spawn at 12-18 ^{0}C between April and September, thus supporting the possibility of this species spawning in the Black Sea ecosystems. The females produce about 50000 eggs/per year (Darwall et al., 1992). In this study, the number of eggs was estimated as 316092 for this individual. Torstensnes (2016) reported the egg diameter as 0.75-0.8 mm for *S. melops*. In this study, the egg diameter was determined as 0.57 mm. This value is smaller than Torstensnes (2016).

Quignard and Pras (1986) reported a maximum age of 9 years while Sayer et al. (1996) reported 7 years. This article reports the maximum age of the species as 8, similar to the previous two studies.

Conclusion

This is the first document for the presence of *S. melops* in the Black Sea. In addition, the largest (320 mm) and the heaviest (520 g) individuals were recorded in the world. The information presented here is considered to contribute to fisheries biology and international scientific literature.

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.

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RESEARCH ARTICLE

Catch comparison of traditional and experimental deep water cast nets with different mesh sizes for whiting (*Merlangius merlangus euxinus*)

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ABSTRACT

In the present study, the commercial (16 mm nominal mesh size) and experimental deep water cast nets with different mesh sizes (20 mm, 24 mm, 28 mm and 32 mm nominal mesh size) were tested to compare the captured fish lengths and catch amounts for whiting (*Merlangius merlangus euxinus*). Experiments were carried out between July, 11 and August, 29, 2018 in Rize province of the south-eastern Black Sea Region. A commercial fishing boat was chartered for 20-day sea trials and in total, 66 set operations were done for all nets. Generalized Linear Mixed Models (GLMM) was used to compare fish sizes caught with experimental and commercial deep water cast nets. Results showed that more than 70 percent of the individuals caught in cast nets with 16 mm, 20 mm and 24 mm size were below the minimum landing size of whiting (13 cm). Among tested nets, only cast net with 32 mm size caught significantly less individuals under the minimum landing size. Finally, the results obtained from this study are discussed in terms of sustainable fisheries of whiting in the Black Sea.

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Dalgıç, G., Kutay, M. Ş., Eryaşar, A. R. (2020). Catch comparison of traditional and experimental deep water cast nets with different mesh sizes for whiting (*Merlangius merlangus euxinus*). *Marine Science and Technology Bulletin*, 9(2): 130-135.

Introduction

Whiting (*Merlangius merlangus euxinus* N., 1840) is one of the most important commercial fish species for the Black Sea and a significant part of the whiting landings are done in the south-eastern Black Sea Region (TurkStat, 2018). Although the gill nets are mostly used for whiting fishery in the region, deep water cast nets (DWCN) are used extensively by the small-scale fishing fleet especially in the summer and autumn when the catch efficiency of gill nets is decreased or in areas where the gill nets are not possible to use. But, there are no prohibitions and regulations regarding the features (e.g. mesh size, twine diameter) and usage of this fishing gear in the Turkish Fisheries Regulations (TFR) for commercial purpose (Anonymous,

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2016a). However, according to TFR for recreational purpose (Anonymous, 2016b), using of cast nets are free in Turkish coastal waters if it complies with the captured fish length, amount and area prohibitions. In addition, the cast net to be used for recreational purpose cannot exceed 3 meters from the ground when closed and the mesh size cannot be smaller than 28 mm (Anonymous, 2016b).

Observations made with commercial boats using DWCN for whiting fishery showed that large number of individuals were caught below the minimum landing size (MLS; 13 cm). For a sustainable fishery, these juvenile individuals should be released from fishing gear. Studies on DWCN used for whiting fisheries are very limited in the literature and, in these studies, technical aspect and catch efficiencies of the commercial DWCN were examined (Emanet and Ayaz, 2018; Karadurmuş, 2019). However, no other study investigated the effect of DWCN with different mesh sizes on catch amount and size selectivity in the literature. In this study, it was aimed to determine appropriate mesh size which select whiting individuals successfully under the minimum landing size (MLS) from the DWCN. For this purpose, the traditional (16 mm nominal mesh size) and experimental DWCN with different mesh sizes (20 mm, 24 mm, 28 mm and 32 mm nominal mesh size) were tested to compare fish lengths in determining appropriate mesh size.

Material and Methods

In the present study, the commercial DWCN (16 mm nominal mesh size) were compared with DWCN with different mesh sizes (20 mm, 24 mm, 28 mm and 32 mm nominal mesh size). Experiments were carried out between July, 11 and August, 29, 2018 in Rize province of the south-eastern Black Sea Region (Figure 1). The commercial boat "Orkan 53" (9 m overall length with 190 HP engine power) was used in 20-day sea trials and tested DWCN were operated by commercial fishermen who work in the region. The DWCN were left depths between 48-105 m. Time of the fishing operation was between 14:00 and 19:00. A total of 66 successful set operations were carried out during the 20-day sea trails. Each set consists of operation of the DWCN which were having 5 different mesh sizes to the same place, respectively. In total, 330 operations (66 x 5) were done. Each operation was started after finding the places with eco-sounders where the DWCN were left. The DWCN which were fixed on the polling rope left in the water with the method of dropping them from the sea surface to the point determined by eco-sounders and waited to reach the bottom. As soon as the DWCN reached to the bottom, they were immediately pulled up with the help of a crane or by hand. Before the operation, the number of operations to be performed was determined by taking the wind and currents into consideration.

Figure 1. Study area

All tested DWCN were rigged at fisherman store. The DWCN were conical in shape and they were usually constructed with 7 panels. The net material was PE and all of them were black in color. Twine diameter of nets was 210d/3. There were 22 bridle ropes which were made of PP material and were 5 mm thick in the DWCN. The bridles were collected in a stainless chrome swivel by passed through the ring at the top end of the net (Figure 2). During the operations, the pulling rope which was 6 mm in diameter and made of PP material attached to the chrome swivel of the DWCN. Heights of tested nets were 5.5 m and their weights were ranged from 4 to 6 kg. When the DWCN are fully opened, they cover an area of approximately 50 m^2. The technical plan of the commercial cast net is given in Figure 3.

Figure 2. Parts of the cast net

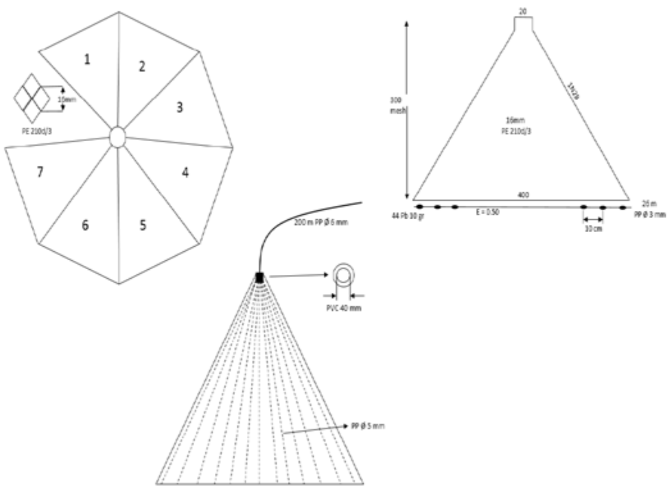

Figure 3. Technical plan of the commercial cast net

In order to prevent weight loss and deformity in the whiting individuals, ice was added to the container and the first body form and weight of the fish was tried to be preserved. At the end of the operation, the number of fishes were counted and wet weight was determined to the nearest 0.01 g. The total length of fish was measured using a fish scaling board prepared for the nearest half cm.

Kruskal-Wallis H test was used to determine whether there is a statistical difference between the DWCNs in the amount of captured whiting. If there is a significant difference between the nets, Dunn multiple comparison test was performed to determine which of the binary groups the cause of this difference was. Generalized Linear Mixed Models (GLMM) was used to compare fish sizes caught with experimental and commercial cast nets (Holst and Revill, 2009). The working of the models and the interpretation of the results were made according to the method reported by Holst and Revill (2009) and other studies (Eryaşar and Özbilgin, 2015; Eryaşar, 2018). In addition, in the statistical comparison of the lengths of the captured species in commercial and experimental nets, student's t-test was used (Godoy et al., 2003). Statistical analysis was performed using the R program (R Development Core Team, 2019).

Results

During the study, no other species was caught with DWCN except for the target species (whiting). The total amount of captured whiting was found to be 168 kg. In the study period, the minimum amount of fish caught with nets during a day period was 1.2 kg and the maximum was 21.3 kg. The maximum catch of whiting was observed in the DWCN with 20 mm mesh size, followed by 16 (commercial), 24, 28 and 32 mm nets, respectively (Table 1). In addition, there was a statistical difference between the DWCNs in the amount of captured whiting ($P<0.05$).

The minimum and maximum lengths of fish captured with the DWCNs were 6 and 18 cm, respectively. In addition, the length class between 10 and 12 cm were the most abundant in the catch (Figure 4). The mean length of whiting individuals which were caught by the DWCN with 16 mm, 20 mm, 24 mm, 28 mm and 32 mm mesh sizes were found as 11 cm, 11 cm, 12 cm, 13 cm and 14 cm, respectively (Table 1). Furthermore, a statistically significant difference was found in terms of length of the captured individuals among the DWCNs (for all DWCN groups) (t-test, $P<0.05$). When we compare the length of the captured fish in the DWCNs according to the minimum landing size for TFR, more than 70% of the individuals caught in 16, 20 and 24 mm mesh sizes was below 13 cm (Table 1). In addition, the GLMM showed to the best fit the data with the logit-quadratic model for all DWCN groups (Table 2). However, it was observed that only DWCN with 32 mm mesh size caught significantly less individuals under the MLS and this reduction was length-related for whiting according to logit-quadratic model ($P<0.05$) (Table 2; Figure 5). DWCN with 28 mm mesh size catch significantly fewer whiting individuals under 12.5 cm (Logit-quadratic model; $P<0.05$) (Figure 5).

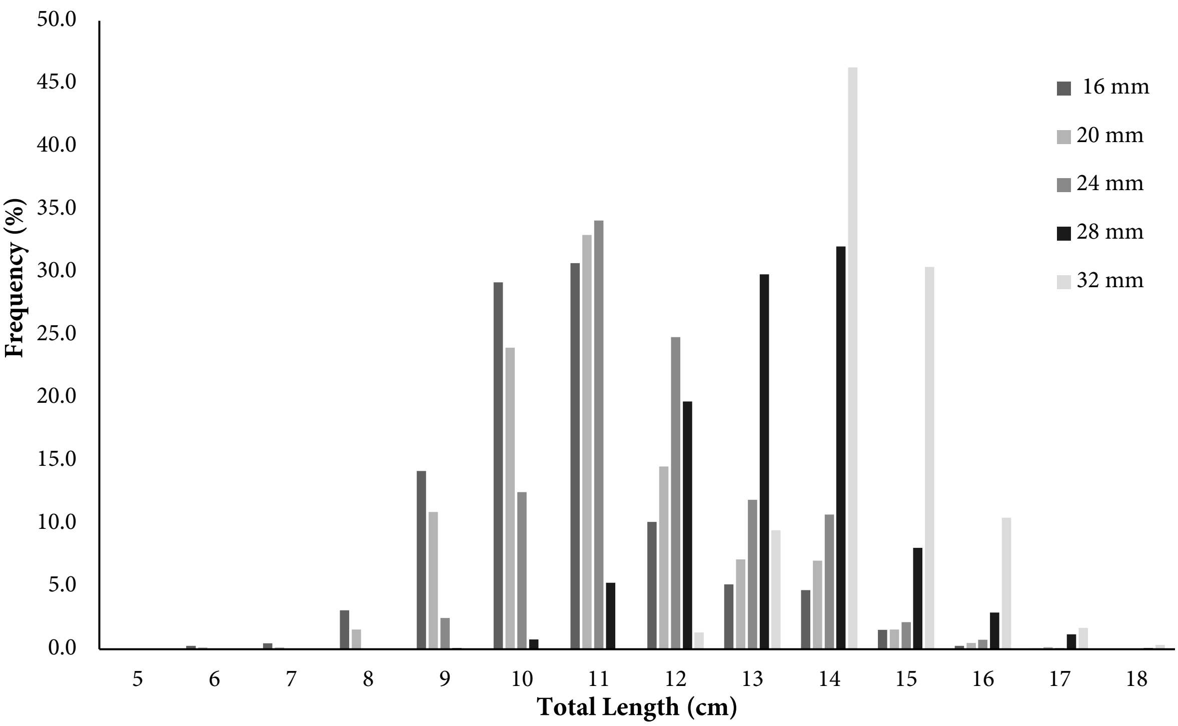

Figure 4. Length frequency distributions of tested DWCNs

Table 1. Catch data for traditional and experimental DWCNs

Mesh Size	Total Number	Total Weight (kg)	Average Weight (S.E.)	Mean Length (S.E.)	Individual Rate Under MLS
16	4264	44.74	2.48 (0.10)	11 (0.02)	0.88
20	4845	54.18	2.72 (0.10)	11 (0.02)	0.84
24	3473	40.72	2.05 (0.09)	12 (0.02)	0.74
28	1266	21.97	1.05 (0.04)	13 (0.03)	0.26
32	308	6.74	0.34 (0.02)	14 (0.05)	0.14

Table 2. Coefficient values and significance (P) based on Generalized Linear Mixed Modelling (GLMM) for experimental DWCNs

Coefficient			Value	SE	DF	t-Value	p-Value
20 mm	Quadratic	$\beta 0$	-4.288	1.095	10	-3.917	0.003
		$\beta 1$	0.647	0.193	10	3.359	0.007
		$\beta 2$	-0.022	0.008	10	-2.585	0.027
24 mm	Quadratic	$\beta 0$	-26.704	2.569	10	-10.395	0.000
		$\beta 1$	4.108	0.433	10	9.499	0.000
		$\beta 2$	-0.153	0.018	10	-8.514	0.000
28 mm	Quadratic	$\beta 0$	-57.362	7.997	10	-7.173	0.000
		$\beta 1$	7.983	1.233	10	6.473	0.000
		$\beta 2$	-0.272	0.047	10	-5.771	0.000
32 mm	Quadratic	$\beta 0$	-88.308	6.021	10	-14.667	0.000
		$\beta 1$	11.231	0.841	10	13.348	0.000
		$\beta 2$	-0.354	0.029	10	-12.052	0.000

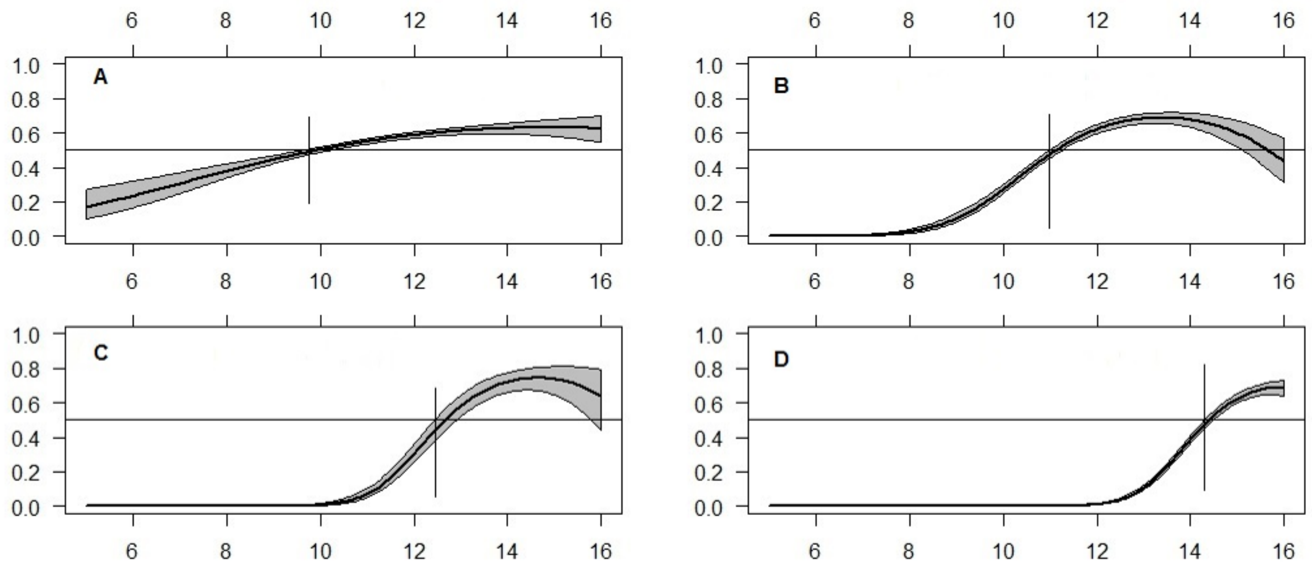

Figure 5. GLMM modeling of the size of whiting, *Merlangius merlangus euxinus*, for experimental DWCNs (20 mm (A), 24 mm (B), 28 mm (C), and 32 mm (D) mesh size) showing differences in the catch at length. Catch ratio of 0.5 (horizontal line) indicates commercial and experimental DWCN catch equal numbers of individuals. The solid line gives the mean; the grey band gives the 95% confidence level. The vertical line shows the length below which the reduction of the catch is significant.

Discussion

The present study is firstly evaluated the effect of different mesh sizes on catch amount and captured length of whiting in the DWCN. In addition, studies on DWCNs are very limited and, in these studies, technical aspect and catch efficiencies of the traditional DWCNs used for whiting fisheries were examined (Emanet and Ayaz, 2018; Karadurmuş, 2019). Emanet and Ayaz (2018) reported that the average catch amount of whiting was 1.9 kg/operation in their samplings for the months between June and October. Karadurmuş (2019) reported this value as 0.14 and 0.42 kg/operation, respectively, for the months of July and August when the current study was also conducted. When the data in this study was examined for the commercial DWCN, the average catch amount was found as 0.68 kg on the operation basis. This value is close to the value reported by Karadurmuş (2019) but it is quite different from the outcome reported by Emanet and Ayaz (2018). Furthermore, there are also differences among studies in terms of the number of bycatch species. Karadurmuş (2019) reported 12 bycatch species in commercial DWCN, while Emanet and Ayaz (2018) reported only one. In our study, there were no other species in the catch except for whiting at all tested DWCNs. These differences may be caused due to the area (others were conducted in Trabzon and Ordu provinces) and seasonal differences. Besides, features of fishing gear, bio-diversity, and environmental conditions (e.g. strong deep currents) in different fishing areas may also contribute to this result.

Except 28 and 32 mm mesh sizes, majority of individuals caught with commercial and other experimental DWCNs (20 and 24 mm nets) were seen to be under the MLS. This outcome indicates that mentioned DWCNs in the present study have a very low selectivity. In contrary of this result, other similar studies (Emanet and Ayaz, 2018; Karadurmuş, 2019) showed that majority of whiting individuals caught with commercial DWCN was found to be above the MLS. This situation is thought to arise from difference of stock structure of target species in different areas where studies were carried out. Furthermore, differences in features of cast nets may also contribute this outcome. For example, Emanet and Ayaz (2018) used commercial DWCN with 14 mm bar length in their experiment and reported that 15% of captured individuals in total catch was under the MLS. In the present study, this value was found as 26% with same bar length (28 mm mesh size). In addition, Kalaycı and Yeşilçiçek (2014) reported that 13% of the captured individuals was under the MLS in their study conducted with the gill nets with 16, 18, 20, and 22 bar length used for whiting fisheries in the south-eastern Black Sea. The results obtained from this study with 16 mm bar length (32 mm mesh size) are similar to the rate reported in the mentioned study.

When we examine the catch amount of the all tested DWCNs, the commercial one which has the smallest mesh opening had caught less whiting compared with 20 mm mesh size. This can be explained by small mesh openings in the commercial gear would cause more water resistance. As such a situation would cause deceleration in the fishing gear while it sinks and it is likely to change the gear's catch performance.

Conclusion

In conclusion, it had been seen that the size selectivity of the commercial DWCN used in the region is quite low. In addition, in line with the information obtained from the findings of this study, it is recommended to use the DWCN with 32 mm mesh size for sustainable whiting fishery in the region. However, there is no regulation regarding the features and usage of this fishing gear in the Turkish Fisheries Regulations (Anonymous, 2016a). Because of important source of income for small-scale fishery in the region for long years, the sustainability of this fishery is extremely important. For this purpose, related provisions should be found in the fisheries regulations. In this context, there are some points in order to take into consideration if the Fishery Management Authority recommend to use the DWCN with 32 mm mesh size for the sustainability of whiting fisheries. First, according to our results, fisherman is likely to experience economic losses when switching to the recommended fishing gear, as the fish under MLS (13 cm) has a commercial value due to demand of the restaurants and insufficient controls in the area. In such a situation, there may be problems in the voluntarily usage of this fishing gear with large mesh size by fishermen. Therefore, in case of increasing the controls, undersized fish sales would be banned both in market and restaurants. Also closing some fishing areas where the juvenile whiting individuals are seen extensively in the catch composition is essential. The fishermen in the area may not earn good salary from undersized fish sales with these actions, so more selective fishing gears can be provided to use compulsory by the fleet. Finally, by-catch species was not caught with the DWCN. High species selectivity of the DWCN is an important feature of an ideal fishing gear in the ecosystem approach to fisheries. In addition, products can be caught as live or with minimal damage by this fishing gear. When compared to passive gear such as bottom gill net used extensively for whiting fishing in the study area (Kalaycı and Yeşilçiçek, 2014) and active fishing gear such as trawl used in the Black Sea (Zengin et al., 2019), it is considered that the negative effects of the DWCN on the by-catch species and benthic ecosystem are too less.

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Compliance with Ethical Standards

Authors' Contributions

GD and ARE designed the study, MŞK performed and managed statistical analyses, ARE wrote the first draft of 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.

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Marine Science and Technology Bulletin

RESEARCH ARTICLE

Length-weight relationships of 12 fish species from the Köyceğiz Lagoon, Turkey

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ABSTRACT

This study provides the length-weight relationships of 12 fish species that belong to ten families from the Köyceğiz Lagoon, Turkey; *Dicentrarchus labrax* (Linnaeus, 1758); *Diplodus annularis* (Linnaeus, 1758); *Diplodus sargus* (Linnaeus, 1758); *Dussumieria elopsoides* Bleeker, 1849; *Engraulis encrasicolus* (Linnaeus, 1758); *Oreochromis niloticus* (Linnaeus, 1758); *Gobius niger* Linnaeus, 1758; *Mullus barbatus barbatus* Linnaeus, 1758; *Sparus aurata* Linnaeus, 1758; *Siganus rivulatus* Forsskål & Niebuhr, 1775; *Solea solea* (Linnaeus, 1758); *Trachinotus ovatus* (Linnaeus, 1758). A total of 720 fish samples were collected with fish barrier, trammel net, beach seine and cast-net. The growth type of *D. annularis* (b=3.148), *D. elopsoides* (b=3.089), *G. niger* (b=3.154), *S. solea* (b=3.124) was determined as positive allometry and *E. encrasicolus* (b=2.814), *D. labrax* (b=2.764), *T. ovatus* (b=2.901) was determined as negative allometry whereas *D. sargus* (b=2.995), *M. barbatus barbatus* (b=3.003), *O. niloticus* (b=3.088), *S. aurata* (b=3.009) and *S. rivulatus* (b=3.079) showed isometry. The results of this investigation will contribute to further fishery studies in the Köyceğiz Lagoon.

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Introduction

Lagoons and wetlands, which are of great ecological importance, are special ecosystems and undertake many functional tasks. The coastal lagoons located between the land and the sea are under the influence of both terrestrial and marine factors and are the transition zones between seawater and freshwater environments (Bianchi, 1988). Lagoons are also important for the economy due to providing a wide array of ecosystem services in addition to being the home of so many different species. Some of these services include fisheries, nutrient cycling and flood protection (Miththapala, 2013).

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Lagoons play a positive role in increasing fish stocks as they are the places where the larvae of many fish species provide their nutrition, shelter and sustainability (Whitfield, 1999).

Length-weight relationships (LWRs) have an important role in fish stock management (Froese, 2006) and are also useful for comparing life history and morphological aspects of different populations from other regions (Goncalves et al., 1997). The LWRs provide predicting the weight corresponding to a given length and to allow for the comparison of fish growth in different habitats (Bagenal and Tesch, 1978; Morato et al., 2001; Tsoumani et al., 2006). LWRs may be useful to determine whether somatic growth is isometric or allometric (Ricker, 1975). Also the LRWs data provide the estimation of population size of a fish stock (Dulčić and Kraljević, 1996). Though there are some studies on length weight relationships in the Black Sea (Samsun et al., 2006; Kasapoğlu and Düzgüneş, 2013), the Sea of Marmara (Keskin and Gaygusuz, 2010; Bok et al., 2011) the Aegean Sea (İlkyaz et al., 2008; Bilge et al., 2014; Ates et al., 2017), the eastern Mediterranean Sea (Cicek et al., 2006; Sangun et al., 2007; Gökçe et al., 2010) and in the Turkish Lagoons few studies on LWRs of fish species in the Homa Lagoon (Acarli et al., 2014); in the Beymelek Lagoon (Sümer, 2012) have been conducted.

The aim of this study is to determine the LWRs of 12 fish species sampled from the Köyceğiz Lagoon, Southeastern Aegean Sea.

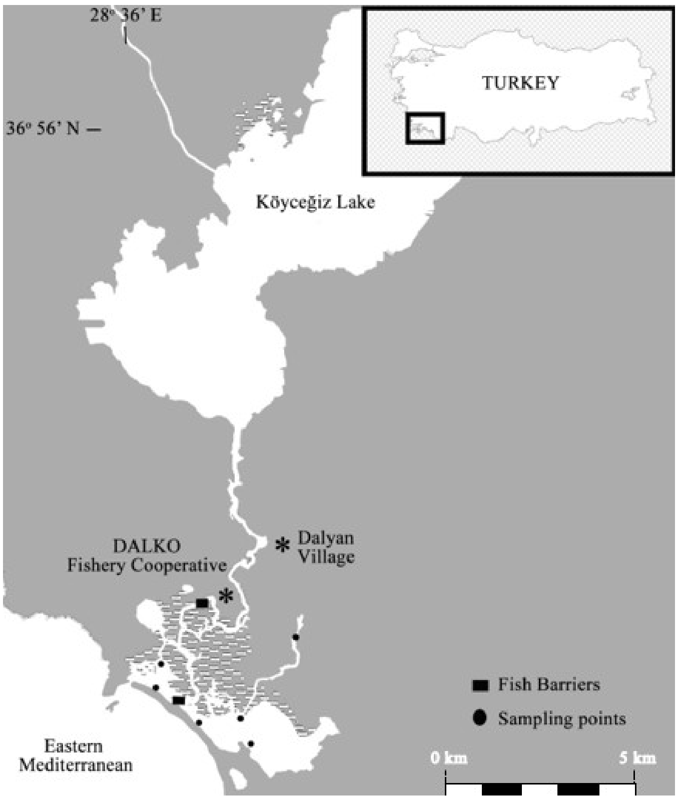

Figure 1. Sampling points of Köyceğiz Lagoon, Muğla, Turkey

Material and Methods

The Köyceğiz Lagoon system covers 5400 hectares lake area, 1150 hectares marsh-looking delta area and is connected to sea via a 14 km long canal. The width of the canal varies between 5-70 meters and the depth between 1-6 meters (Buhan, 1998). A total of 720 fish samples were collected with fish barrier (40 mm mesh size), trammel net (32 mm mesh size), beach seine (10 mm mesh size) and cast-net (32 mm mesh size) between January 2017 and December 2017 in the Köyceğiz Lagoon, Turkey (Figure 1). Fish species were identified at the species level and validated by referencing FishBase (Froese and Pauly, 2017). Total length (TL) of fish species were recorded to the nearest centimetre (0.1 cm), and body weight (W) was measured with precision balance of (0.01 g accuracy).

The parameters a and b of relationships of the equation $W = aL^b$ (Ricker, 1975) were estimated through logarithmic transformation;

$$\log W = \log a + b \log TL$$

where W is weight (g), TL is total length (cm), a is the intercept and b is the slope of the linear regression. Parameters a and b were calculated by least-squares regression, as was the coefficient of determination (r^2). The significance of the b-values for each species was tested by Pauly's t-test to confirm that it was significantly different from the predictions for isometric growth (b=3) (Pauly, 1984). Pauly's t-test was calculated as:

$$t = \left(\frac{SD_{\log TL}}{SD_{\log W}}\right) \times \left[\frac{|b-3|}{\sqrt{1-r^2}}\right] \times \sqrt{(n-2)}$$

where SD_{logTL} is the standard deviation of the $logTL$ values, SD_{logW} is the standard deviation of the $logW$ values, n is the number of fish species used in the computation. The value of b is different from 3 if t value is greater than the tabled t values for n-2 degrees of freedom (Pauly, 1984).

Results

A total of 12 fish species from ten families namely Carangidae, Cichlidae, Dussumieriidae, Engraulidae, Gobiidae, Moronidae, Mullidae, Siganidae, Soleidae and Sparidae were sampled in this study. The sample size ranged from 29 individuals for *D. elopsoides*, to 196 individuals for *E. encrasicolus*. The b value of the LWRs ranged from 2.274 for *D. labrax* to 3.154 for *G. niger*. The curves of length-weight relationships for studied species are given in Figure 2. The growth type of *D. annularis, D. elopsoides, G. niger, S. solea* was determined as positive allometry (b>3) and *E. encrasicolus, D.*

labrax, T. ovatus was determined as negative allometry ($b<3$) whereas *D. sargus, M. barbatus barbatus, O. niloticus, S. aurata* and *S. rivulatus* showed isometry ($b=3$). The determination coefficient (r^2) ranged from 0.94 for *S. aurata* to 0.99 for *D. elopsoides*. The coefficient of determination (r^2) was very high for all studied species. Length-weight relationship parameters *a* and *b*, standard deviation of *b* (SD_b), 95% confidence interval (CI) for *b*, correlation coefficient (r^2), number of sample (n), length range and weight range for each of the twelve species were presented in Table 1.

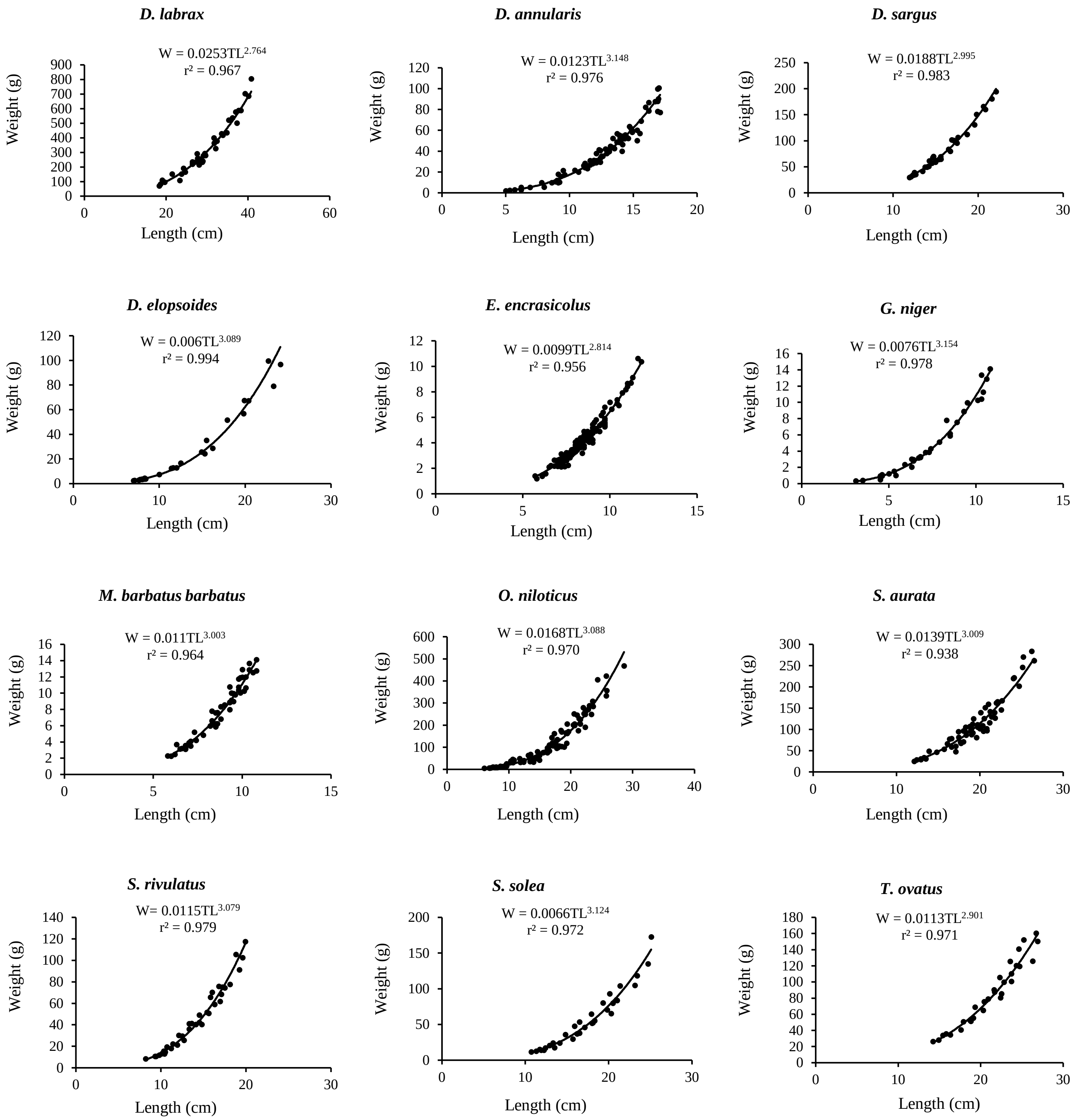

Figure 2. The curves of length-weight relationships for studied species from Köyceğiz Lagoon

Table 1. Parameters of length-weight relationship for twelve fish species from the Köyceğiz Lagoon

Species	n	TL range (cm)	W range (g)	*a*	*b*	r²	SD_b	%95CI of *b*	Pauly's *t*-test
D. labrax	39	18.3-40.8	69.76-805.32	0.0253	2.764	0.967	0.049	2.59-2.94	*t*=2.8002, P<0.05
D. annularis	81	5.0-17.1	1.85-100.57	0.0123	3.148	0.976	0.061	3.04-3.26	*t*=2.6505, P<0.05
D. sargus	35	11.9-22.1	29.40-194.25	0.0188	2.995	0.983	0.031	2.86-3.13	*t*=0.0685, P>0.05
D. elopsoides	29	7.0-24.1	2.41-99.50	0.006	3.089	0.994	0.045	2.99-3.19	*t*=1.9158, P<0.05
E. encrasicolus	196	6.5-11.8	2.09-10.61	0.0099	2.814	0.956	0.033	2.72-2.90	*t*=4.1570, P<0.05
G. niger	34	3.1-10.8	0.33-14.10	0.0076	3.154	0.978	0.070	2.98-3.33	*t*=1.8252, P<0.05
M. barbatus barbatus	53	5.8-10.8	2.25-14.10	0.0110	3.003	0.964	0.043	2.84-3.17	*t*=0.0387, P>0.05
O. niloticus	93	6.0-28.6	5.31-467.85	0.0168	3.088	0.970	0.093	2.98-3.20	*t*=1.5513, P>0.05
S. aurata	61	12.1-26.5	25.15-283.82	0.0139	3.009	0.938	0.062	2.81-3.21	*t*=0.0916, P>0.05
S. rivulatus	37	8.2-19.9	8.42-117.51	0.0115	3.079	0.979	0.047	2.93-3.23	*t*=1.0623, P>0.05
S. solea	32	10.7-25.1	11.42-172.51	0.0066	3.124	0.972	0.057	2.92-3.32	*t*=1.7488, P<0.05
T. ovatus	30	14.9-26.9	28.14-160.36	0.0113	2.901	0.971	0.040	2.70-3.09	*t*=1.7198, P<0.05

Note: *n*: sample size; *TL*: total length (cm); *W*: total weight (g); *a*: intercept; *b*: slope of the regression line; r^2: coefficient of determination; SD_b: standard deviation of *b*; *CI*: confidence intervals.

Discussion

The length-weight relationships in fish could be affected by many factors such as season, habitat, temperature, salinity, gonad maturity, sex, diet, food availability and length range (Tesch, 1971; Moutopoulos and Stergiou, 2002; Froese, 2006). The growth coefficient (*b*) values in LWR identify the growth type of the fish species. The value of $b \neq 3$ shows allometric growth where the fish becomes thinner with increasing length, $b = 3$ shows isometric growth and fish transform into more robust with increase in length (Bagenal and Tesch, 1978). The *b* value is expected to range from 2.5 to 3.5 (Froese, 2006). The *b* values of studied species are in reported range by Froose (2006).

Growth types were found similar to present study for *D. labrax* (Crec'hriou et al., 2013; Mahé et al., 2018), *D. annularis* (İlkyaz et al., 2008), *D. sargus* (Ceyhan et al., 2009), *D. elopsoides* (Erguden et al., 2009), *E. encrasicolus* (Aka et al., 2004; Samsun et al., 2006), *O. niloticus* (Nobile et al., 2015), *G. niger* (Cicek et al., 2006; Bilge et al., 2014), *M. barbatus barbatus* (Djabali et al., 1993; Giacalone et al., 2010), *S. aurata* (Stergiou and Moutopoulos, 2001), *S. rivulatus* (Gabr et al., 2018), *S. solea* (Cerim and Ateş, 2020) and *T. ovatus* (Abdallah, 2002; Moutopoulos et al., 2013). In contrast, there are different results of growth types for *D. labrax* (Dulčić, and Glamuzina, 2006), *D. annularis* (Sangun et al., 2007), *D. sargus* (Maci et al., 2009), *E. encrasicolus* (Veiga et al., 2009), *O. niloticus* (Mehak et al., 2017), *G. niger* (Bok et al., 2011; Kasapoğlu and Düzgüneş, 2013), *M. barbatus barbatus* (Maci et al., 2009; Gökçe et al., 2010), *S. aurata* (Ceyhan et al., 2009; Crec'hriou et al., 2013), *S. rivulatus* (Ates et al., 2017), *S. solea* (Türkmen, 2003) and *T. ovatus* (Santos et al., 2002). Length-weight relationships obtained in the present study are compared by different researches in other locations and some differences determined (Table 2). It can be think that the differences are about sampling method, fish condition, seasonality, length range, sex, gonadal maturity, sample size and stomach fullness.

Table 2. Comparison of length-weight relationships of studied species from different locations

Species	n	TL range (cm)	*a*	*b*	r^2	Location	References
D. labrax	422	24.5-88.0	0.0079	3.065	0.976	Eastern Adriatic estuarine systems	Dulčić and Glamuzina, 2006
	67	27.0-53.0	0.0150	2.880	0.924	French Catalan coast	Crec'hriou et al., 2013
	111	8.5-33.9	0.0150	2.947	0.999	Homa Lagoon	Acarli et al., 2014
	417	16.0-83.0	0.0340	2.701		North-eastern Atlantic Ocean	Mahé et al., 2018
	39	18.3-40.8	0.0253	2.764	0.966	Köyceğiz Lagoon	Present study
D. annularis	154	10.3-15.0	0.0370	2.677	0.90	North-Eastern Mediterranean Coast	Sangun et al., 2007
	161	9.8-16.0	0.0253	3.012	0.95	Gulf of Gabes, Central Mediterranean	Ghailen et al., 2010
	15	7.0-16.7	0.0220	2.957	0.994	Sea of Marmara	Bok et al., 2011
	121	3.9-15.5	0.0100	3.190	0.994	Homa Lagoon	Acarli et al., 2014
	81	5.0-17.1	0.0123	3.148	0.976	Köyceğiz Lagoon	Present study
D. sargus	33	14.9-26.7	0.0342	2.808	0.850	South coast of Iskenderun Bay	Can et al., 2002
	33	16.0-32.3	0.0144	3.061	0.980	Gökova Bay, Aegean Sea	Ceyhan et al., 2009
	368	10.0-40.0	0.0170	3.000	0.934	French Catalan coast	Crec'hriou et al., 2013
	124	11.0-38.5	0.0110	3.145	0.975	Southern Ionian sea, Greece	Dimitriadis and Konstantinidou, 2018
	35	11.9-22.1	0.0188	2.995	0.983	Köyceğiz Lagoon	Present study
D. elopsoides	59	9.9-16.4	0.0055	3.123	0.987	İskenderun Bay, Mediterranean Sea	Erguden et al., 2009
	29	7.0-24.1	0.006	3.089	0.994	Köyceğiz Lagoon	Present study
E. encrasicolus	212		0.0050	2.970	0.872	Saros Bay	İşmen et al., 2007
	759	5.8-14.0	0.0008	3.822	0.950	North Aegean Sea, Greece	Karachle and Stergiou, 2008
	1588	5.9-14.6	0.0124	2.711	0.944	Black Sea	Kasapoğlu and Düzgüneş, 2013
	68	7.0-11.3	0.0070	2.917	0.999	Homa Lagoon	Acarli et al., 2014
	196	6.5-11.8	0.0099	2.814	0.956	Köyceğiz Lagoon	Present study
G. niger	272	2.1-12.2	0.0047	3.394	0.946	Northeastern Mediterranean	Cicek et al., 2003
	112	6.8-15.8	0.0180	2.856	0.953	Black Sea	Kasapoğlu and Düzgüneş, 2013
	34	3.1-10.8	0.0076	3.154	0.978	Köyceğiz Lagoon	Present study
M. barbatus barbatus	2693	5.3-19.0	0.0074	3.123	0.962	Black Sea	Kasapoğlu and Düzgüneş, 2013
	1565	8.7–21.5	0.0071	3.165	0.894	Northeastern Mediterranean Sea	Özvarol, 2014
	53	5.8-10.8	0.0110	3.003	0.964	Köyceğiz Lagoon	Present study
O. niloticus	125	9.1-18.5	0.0393	2.720	0.910	Indus River, Pakistan	Naeem et al., 2010
	261	11.5-47.0	0.310	3.250	0.967	Eight floodplain lakes of Agusan Marsh	Jumawan and Seronay, 2017
	93	6.0-28.6	0.0168	3.088	0.970	Köyceğiz Lagoon	Present study
S. aurata	59	14.6-45.0	0.0266	2.736	0.966	Gökova Bay, Aegean Sea	Ceyhan et al., 2009
	105	13.5-18.3	0.0090	3.150	0.999	Homa Lagoon	Acarli et al., 2014
	61	12.1-26.5	0.0139	3.009	0.938	Köyceğiz Lagoon	Present study
S. rivulatus	84	15.5-25.0	0.016	2.964	0.97	Beymelek Lagoon	Sümer, 2012
	2004	11.2-30.2	0.0114	3.061	0.98	Red Sea, Saudi Arabia	Gabr et al., 2018
	37	8.2-19.9	0.0115	3.079	0.979	Köyceğiz Lagoon	Present study
S. solea	21	11.0-22.1	0.0098	3.002	0.988	Northern Aegean estuarine systems	Koutrakis and Tsikliras, 2003
	73	8.7–20.5	0.0070	3.053	0.999	Homa Lagoon	Acarli et al., 2014
	1136	3.9-31.1	0.0079	3.064	0.991	Southern Aegean Sea	Cerim and Ateş, 2020
	32	10.7-25.1	0.0066	3.124	0.972	Köyceğiz Lagoon	Present study
T. ovatus	26	14.1-26.8	0.0120	2.897	0.90	Beymelek Lagoon	Sümer, 2012
	33	15.7-44.0	0.0089	2.937	0.994	Tropical north-eastern Atlantic	Oliveira et al., 2015
	30	14.9-26.9	0.0113	2.901	0.971	Köyceğiz Lagoon	Present study

Note: *n*: sample size; *TL*: total length (cm); *a*: intercept; *b*: slope of the regression line; r^2: coefficient of determination.

Conclusion

Consequently, growth parameters such as length-weight relationship provide some indication of resource utilization and the effectiveness of management strategies. Thus, fisheries management should be designed on biological data to understand the status and to manage of fish stocks. Köyceğiz Lagoon is important fishing area for local fisherman. This study provides the first basic information of the length-weight (LWRs) relationships of the studied species for the Köyceğiz Lagoon. These results will be useful for fisheries research, management and conservation in the Köyceğiz Lagoon.

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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.

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RESEARCH ARTICLE

Comparison of electronic length frequency analysis (ELEFAN) for estimation of growth parameters for lollyfish, *Holothuria* (*Holothuria*) *atra* and sand sea cucumber, *Holothuria* (*Thymiosycia*) *arenicola* (Holothuroidea: Echinodermata) in the north Arabian Sea, Pakistan

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Seasonal Growth
Sea Cucumber

ABSTRACT

To estimate the growth parameters, the non-seasonal von Bertalanffy and Hoenig seasonal von Bertalanffy models were fitted to the length frequency data of *Holothuria atra* and *H. arenicola* obtained from coastal areas of Karachi in the northern Arabian Sea, Pakistan between January and December 2014. The Hoenig seasonal von Bertalanffy growth parameters were estimated as L_∞=36.1 cm total length (TL), K=0.75 year^{-1} for *H. atra* and as L_∞=34.9 cm TL, K=0.70 year^{-1} for *H. arenicola*. *H. atra* individuals reached 73.9% of their maximum total length at the one year old. For *H. arenicola* it was calculated as 72.7%. Monthly mean growth rate of *H. atra* at the same ages was calculated higher than *H. arenicola*. The seasonal oscillation in growth rate for *H. atra* (C=0.50) was larger than it was for *H. arenicola* (C=0.37). The time of the year when the growth is slowest corresponded to the middle of April (WP=0.30) in *H. atra* and at the beginning of August (WP=0.60) in *H. arenicola* may be the result of the extended both reproduction and poor nutrition periods due to monsoonal effects on the marine environment. The relatively high growth rates (K>0.7 year^{-1}) of these two species may have important implications for high survival rate, particularly in environmental condition where cause biological stress and marine confusion but may also increase their potential as a candidate species for aquaculture.

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Introduction

A total of 25 sea cucumber species have been recorded from the coastal waters of Pakistan, so far and seven of them are documented as commercially important species (Tahera and Tirmizi, 1995; Purcell, 2010; Purcell et al., 2012; Ahmed and Ali, 2014, 2020; Ahmed et al., 2016a, 2020b; Moazzam and Moazzam, 2020). Currently, sea cucumber fishing occurs all over the world, but, there is no traditional sea cucumber fisheries in Pakistan (Siddique and Ayub, 2015; Moazzam and Moazzam, 2020). Two of the commercially important sea cucumber species are lollyfish, *Holothuria* (*Holothuria*) *atra* Jaeger, 1833 and sand sea cucumber, *Holothuria* (*Thymiosycia*) *arenicola* Semper, 1868 found in the coastal waters of Pakistan. *H. atra* known also as the black sea cucumber is found in the tropical Indo-Pacific region, its range extends from the Red Sea and East Africa to Australia. It inhabits the inner and outer flats, back reefs, shallow lagoons, sand-mud and rubble, and sea grass beds between 0 and 20 m. In few Pacific Island nations, the body wall, intestines and/or gonads are consumed in traditional diets or in times of hardship (Purcell et al., 2012).

H. arenicola is also found from the Indo-Pacific to the tropical Western Atlantic. The species is reported from tropical Australia, Mozambique, the Red Sea, Ascension Island, Virgin Islands, Antigua, Barbados, Tobago, Belize, Bermuda, Tortugas, Jamaica, Puerto Rico, U.S. (Mosher, 1980; WoRMS, 2020). These two holothurians are found on the seabed, in shallow waters on reefs and under intertidal rocks, sand flats and in seagrass meadows at depths of up to 20-30 m (Mosher, 1980; Pourvali et al., 2014; WoRMS, 2020). *H. atra* stocks in many regions are now being exploited and are often marketed under the name bêche-de-mer or trepang which means “cooked and dried sea cucumbers” (Purcell et al., 2012).

Relatively little information is available on the biology and ecology of these sea cucumbers belong to the class Holothuroidea in the northern Arabian Sea coasts of Pakistan. There are some works about the growth of sea cucumbers; including the weight–length relationships (WLRs) and condition factor (CF) based growth features in holothurian species such as *Ohshimella ehrenbergii* Selenka, 1867, *H. arenicola*, *H. atra*, *H. pardalis* Selenka, 1867 and *H. verrucosa* Selenka, 1867 from this region (Siddique et al., 2015; Ahmed et al., 2018a, 2018b). In addition to these, a detailed study on the population dynamics of sea cucumbers has been carried out on the *H. arenicola* stocks in Manora and Buleji rocky shores in the northern Arabian Sea, Pakistan so far (Siddique and Ayub, 2015). It was reported that an increase of the gonad index (GI) of *H. arenicola* was observed during spring and early summer, followed by a decrease in autumn and winter, which showed the spawning followed by resting phase and also the GI values were reported as a significant negative correlation with salinity and non-significant correlation with temperature (Siddique and Ayub, 2015). Moreover, unimodal distribution, bimodal distribution and three modes were determined for *H. arenicola* by seasons and areas (Siddique and Ayub, 2015). In other geographical regions, previous studies have been conducted for *H. atra* to determine reproduction biology features including sexual and asexual reproduction from Heron Reef (Harriott, 1982, 1985), spawning season, size at sexual maturity and fecundity from Red Sea (Chao et al., 1994; Abdel-Razek, 2005), ecology including distribution, habitat preference and feeding activity from Rongelap Atoll (Bonham and Held, 1963), Bahamas (Mosher, 1980), and Gulf of Mannar (Asha et al., 2015) and also environmental variability, population biology and fishing pressure from South Tarawa Lagoon, Republic of Kiribati (Tamaroa, 2010).

Age determination of holothuroid echinoderms is difficult because of their soft-bodied, polymorphic shape, and the small size of hard structures that could exhibit growth rings (Watanabe et al., 2014; Sun et al., 2019). Analyses of length frequency data (LFDA) can be used for estimating age classes and growth parameters (Pauly and David, 1981), and can also be applied to calculate growth of holothuroid echinoderms (Olaya-Restrepo et al., 2018). An additional challenge for estimating growth of holothuroid echinoderms arises due to the seasonal growth pattern, and a modified von Bertalanffy growth model has been developed to incorporate this (Hoenig and Hanumara, 1982). Seasonal growth patterns have been reported for different *Holothuria* species, such as *H. arguinensis* Koehler and Vaney, 1906 (Olaya-Restrepo et al., 2018), *Isostichopus badionotus* (Selenka, 1867) (Poot-Salazar et al., 2015) and *Australostichopus mollis* (Hutton, 1872) (Morgan, 2012). However, no seasonal growth information exists for the mentioned unexploited *Holothuria* species inhabiting the Arabian Sea. The aim of this study was to investigate seasonal growth rate of *H. atra* and *H. arenicola* in the north Arabian Sea.

Material and Methods

Study Area and Sampling

Holothuria atra (n=221) and *Holothuria arenicola* (n=258) specimens were collected on monthly basis from Buleji (24°50’20.41” N, 66°49’24.15” E) and Sunehri (24°52’33.49” N, 66°40’40.20” E) (Figure 1) coasts from January to December 2014 except June and July due to rough sea in monsoon season. The sample was collected from intertidal zone by hand-picking through forceps at low tide.

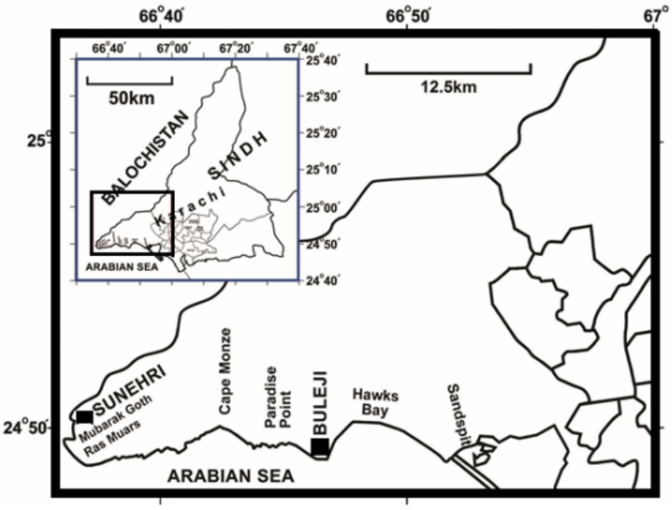

Figure 1. Sampling stations, Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan

Collected specimens were kept alive in water filled containers and then were transported to the laboratory and shifted in well aerated aquaria. For taxonomic studies and identification, morphological features were examined and microscopic studies were conducted. Identification of species of sea cucumbers was based on characteristics body morphology of the sea cucumbers, using the guidebooks from Selenka (1867) and Samyn et al. (2006).

Ossicles were taken from three positions (dorsal and ventral body walls, and tentacles); wet mounts were prepared by placing a small piece of skin tissue on slide and adding few drops of 3.5% bleach, the slides were then rinsed with drops of distilled water. The slides were examined under the Nikon LABOPHOT-2 microscope at 10×10 magnifications. Microphotography was also performed through Fujifilm 16 MP digital camera (see Ahmed et al., 2018a, 2018b for more details). Length (cm) data were collected for each sea cucumber after allowing the sea cucumber to relax in water for 5 min. Total length from mouth to anus was measured with the flexible ruler.

Von Bertalanffy Growth Function Parameter Estimation

Growth in length has been described using the von Bertalanffy (1938) growth function, based on either observed or back calculated length at ages. The length frequency distribution analysis (LFDA) package is also a PC based computer package for estimating growth parameters from length frequency distributions. Version 5.0 of LFDA includes methods for estimating the parameters of both non-seasonal and Hoenig seasonal versions of the von Bertalanffy growth curve (Kirkwood et al., 2003).

The standard or non-seasonal von Bertalanffy (1938) growth function (VBGF) is:

$$L_t = L_\infty\left(1-e^{-k(t-t_0)}\right) \quad (1)$$

Seasonal growth or five parameters von Bertalanffy growth model (5 Parameters VBGF) was described using the Somers's (1988) version of the VBG equation:

$$L_t = L_\infty\left[1-e^{\left[-K(t-t_0)-\left(C\frac{K}{2\pi}\right)\sin 2\pi(t-t_S)+\left(C\frac{K}{2\pi}\right)\sin 2\pi(t-t_S)\right]}\right] \quad (2)$$

where, L_t is length at age t, L_∞ is the asymptotic length to which the sea cucumber growth, K is the growth-rate parameter, t_0 is the nominal age at which the length is zero, C is the relative amplitude ($0 \leq C \leq 1$) of the seasonal oscillations, t_S is the phase of the seasonal oscillations ($-0.5 \leq t_S \leq 0.5$) denoting the time of year corresponding to the start of the convex segment of sinusoidal oscillation.

The time of the year when the growth rate is slowest, known as the winter point (*WP*), was calculated as:

$$WP = t_S + 0.5 \quad (3)$$

Seasonal VBG curves were fitted to the length distributions after first indicating a range of values of L_∞ and K and reducing iteratively the range to maximize the goodness of fit (*Rn*) of the curves to the data. *Rn* was calculated as:

$$Rn = \frac{10^{\frac{ESP}{ASP}}}{10} \quad (4)$$

where *ASP* is the available sum of peaks, computed by adding the best values of the available peaks, and *ESP* is the explained sum of peaks, computed by summing all the peaks and troughs hit by the VBGF curve.

Analysis of the length data were fitted to length frequency distributions grouped in 2 cm total length size classes using the Electronic LEngth Frequency ANalysis (ELEFAN) procedure in the PC-based computer package Version 5.0 of Length-Frequency Distribution Analysis (Kirkwood et al., 2003). The ELEFAN procedure first restructures length frequencies and then fits a VBGF curve to the restructured data. Both seasonal and non-seasonal VBGF curves were fitted to the seasonal length distribution after providing a range of values for the parameters to be estimated and then iteratively reducing the range until the goodness of fit of the curve to the data is maximized.

Reliability of Growth Parameter Estimates

Having estimated a set of growth parameters, one would like to evaluate their reliability. A possible test is the so-called phi-prime test (*Φ'*) known as growth performance index. This test is based on the discovery by Pauly and Munro (1984) that *Φ'* values are very similar within related taxa. So, the growth performance comparisons were made using the growth performance index (*Φ'*) which is preferred rather than using L_∞ and *K* individually (Pauly and Munro, 1984) and is computed as:

$$Q' = \log(K) + 2\log(L_\infty) \quad (5)$$

Results

Population Structure

A total of 221 *Holothuria atra* and 258 *Holothuria arenicola* were sampled between January and December 2014. The total length ranged between 12 and 34.5 cm (mean 19.7±0.38 cm, 95% confidence interval: 19.0-20.5 cm) for the *H. atra* (Figure 2) and between 13 and 33 cm (mean 23.3±0.30 cm, 95% confidence interval 22.7-23.9 cm) for the *H. arenicola* (Figure 3). The length composition data revealed that most individuals in both *H. atra* (76.9%) and (72.5%) *H. arenicola* species ranged from 14 to 24 cm. Larger *H. atra* individuals (>22-24 cm) were not obtained in January, February, March and October. Smaller *H. arenicola* individuals (<16-20 cm) were not determined in August, September, October and December. Size frequency distributions were significantly different (Kolmogorov-Smirnov two-sample test; d=0.395, $P<0.0001$) between two species. The mean total length of *H. arenicola* was significantly (t-test: 4.814E13, $P<0.0001$) greater than the mean total length of *H. atra*.

Seasonal and Non-Seasonal Von Bertalanffy Growth Parameters

The Hoenig seasonal and non-seasonal VBGF curves parameters obtained from the LFDA for each species are summarized in Table 1. The *Rn* value of the non-seasonal growth curve for both *H. atra* and *H. arenicola* improved when the seasonal growth curves were fitted (Table 1), suggesting that, at least for our data, these two sea cucumbers exhibit seasonal growth patterns. This seasonality in growth was also apparent in the results of the relative amplitude values (C=0.50 for *H. atra* and C=0.37 for *H. arenicola*) of the seasonal oscillations and in Figure 4B and Figure 5B where sinusoidal pattern could be observed in the Hoenig seasonal von Bertalanffy growth curve.

The slowest growth period started at the middle of April for *H. atra* (*WP*=0.30; Figure 6). For *H. arenicola*, however, the start of slow growth period was at the beginning of August (*WP*=0.60; Figure 6).

The calculated growth performance indices (*Φ'*; Table 1) of seasonal growth for *H. atra* (*Φ'*=2.989) was greater than the *H. atra* (*Φ'*=2.930) (Table 1).

Table 1. Seasonal and non-seasonal von Bertalanffy growth parameters estimated from the length frequency distribution analysis and maximum life span for *Holothuria atra* and *Holothuria arenicola*. L_∞, asymptotic total length (cm); *K*, growth coefficient (year^{-1}); t_0, age at zero length; WP, winter point; *C*, amplitude of growth oscillation; *Rn*, goodness of fit index; *Φ'*, growth performance index; A_{95}, the life span to attain 95% of L_∞, calculated from the VBG equation.

Parameters	*Holothuria atra*		*Holothuria arenicola*	
	Hoenig Seasonal	Non-Seasonal	Hoenig Seasonal	Non-Seasonal
L_∞ (cm)	36.09	35.37	34.86	34.86
K (year^{-1})	0.75	0.76	0.70	0.74
t_0 (year)	-0.23	-0.27	-0.19	-0.15
WP	0.30	-	0.60	-
C	0.50	-	0.37	-
Rn	0.296	0.263	0.502	0.423
Φ'	2.99	2.99	2.93	2.95

Age Structure and Growth Rate

Age–length key calculated from the Hoenig seasonal VBGF curves parameters both *H. atra* and *H. arenicola* individuals are showed in Figure 7. Length for the first age class (0 years old) was estimated as 14.2±1.38 cm (between 7.7 and 21.3 cm) for *H. atra* and 13.1±1.41 cm (between 4.8 and 18.6 cm) for *H. arenicola* by LFDA method with ELEFAN model.

The calculated mean total length in the age classes showed that the *H. atra* individuals reached 73.9% of their maximum total length (L_{max}=34.5 cm) at the second age class (one year old; 10.3±0.39 cm). For *H. arenicola* it was also calculated as 72.7%. This fast growth characteristic of small individuals was also apparent in the growth curves in Figure 4 and Figure 5, where it could be observed that the slight slope in the larger

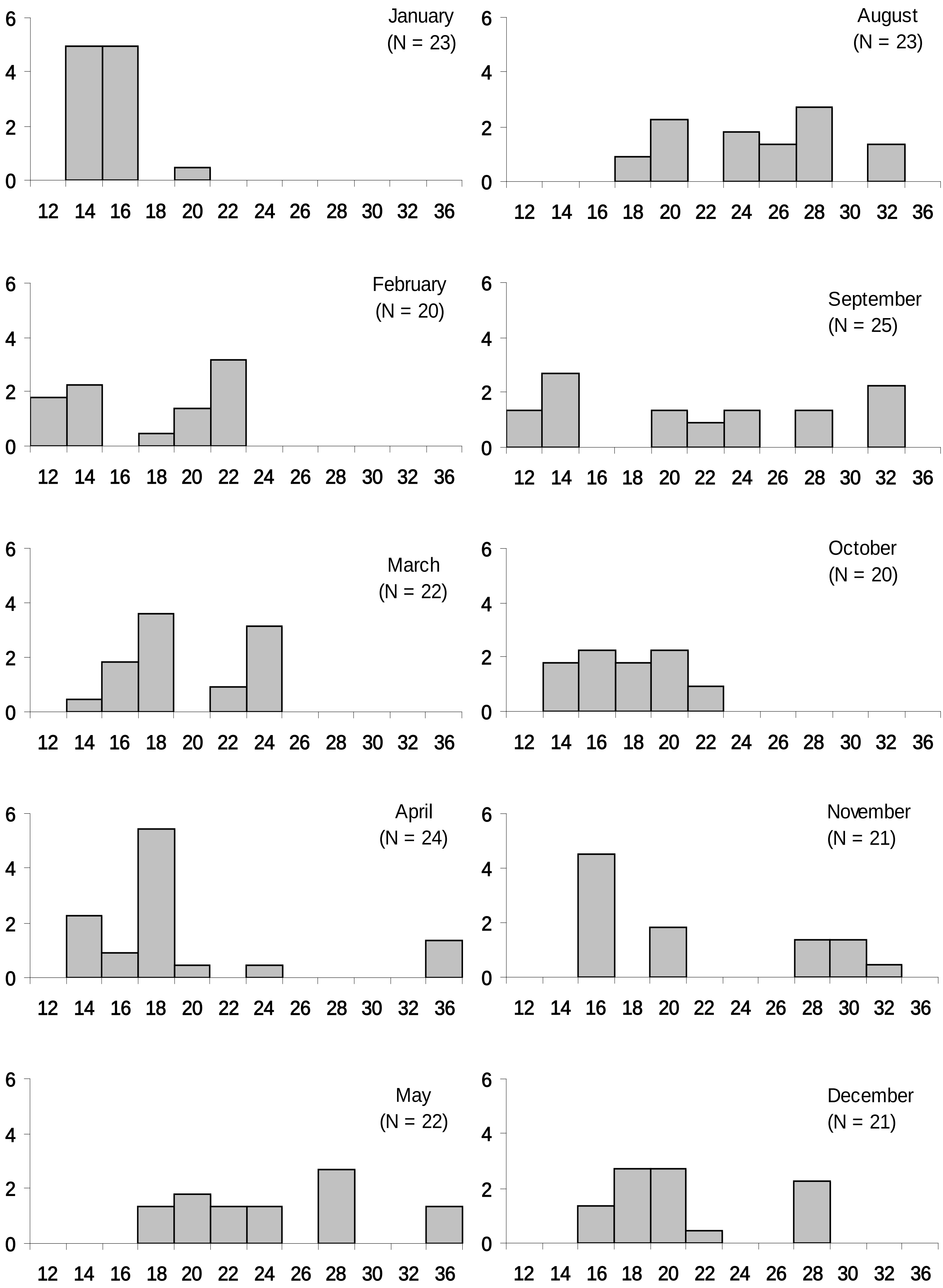

Figure 2. Monthly total length (2 cm size classes) frequency distributions (in percentages) of *Holothuria atra* between January and December 2014

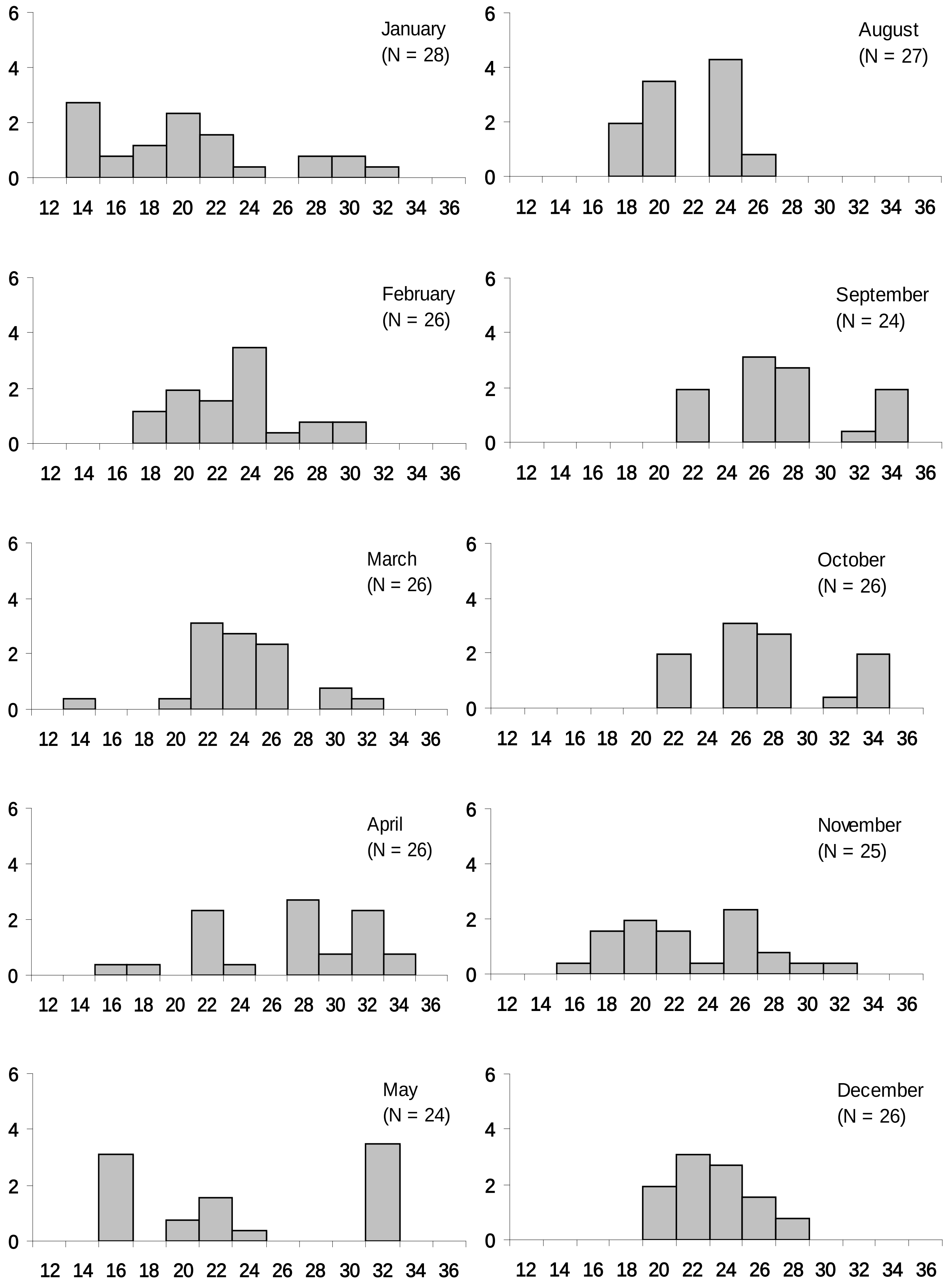

Figure 3. Monthly total length (2 cm size classes) frequency distributions (in percentages) of *Holothuria arenicola* between January and December 2014

individuals compared to smaller sea cucumber leading to small individuals grew faster than large ones.

Monthly mean growth rate of *H. atra* was calculated as 1.18 cm/month for 0-365 days (0 age class), 0.97 cm/month 365-730 days (1 age class), 0.45 cm/month for 730-1095 days (2 age class) by using estimated average total length values in the ages. For *H. arenicola*, however, it was calculated as 1.09 cm/month for 0-365 days (0 age class), 0.91 cm/month 365-730 days (1 age class), 0.46 cm/month for 730-1095 days (2 age class). In older individuals (>1095 days old), it almost tended to steady state for two species.

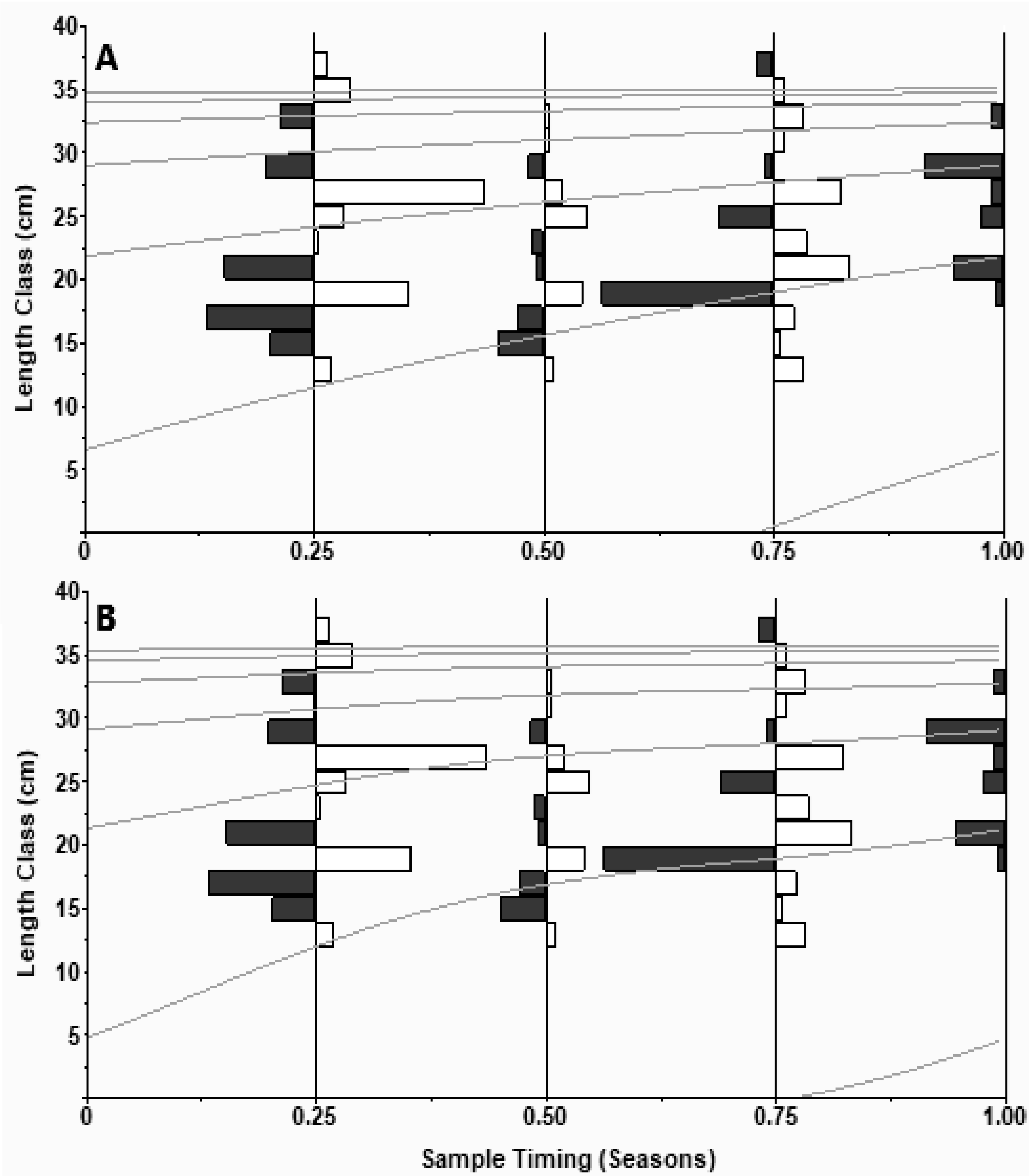

Figure 4. Growth curve (lines) from the von Bertalanffy model with non-seasonal (A) and the Hoeing seasonal (B) by using electronic length-frequency analysis (ELEFAN), to length-frequency data of *Holothuria atra* sampled from Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan between January 2014 and December 2014

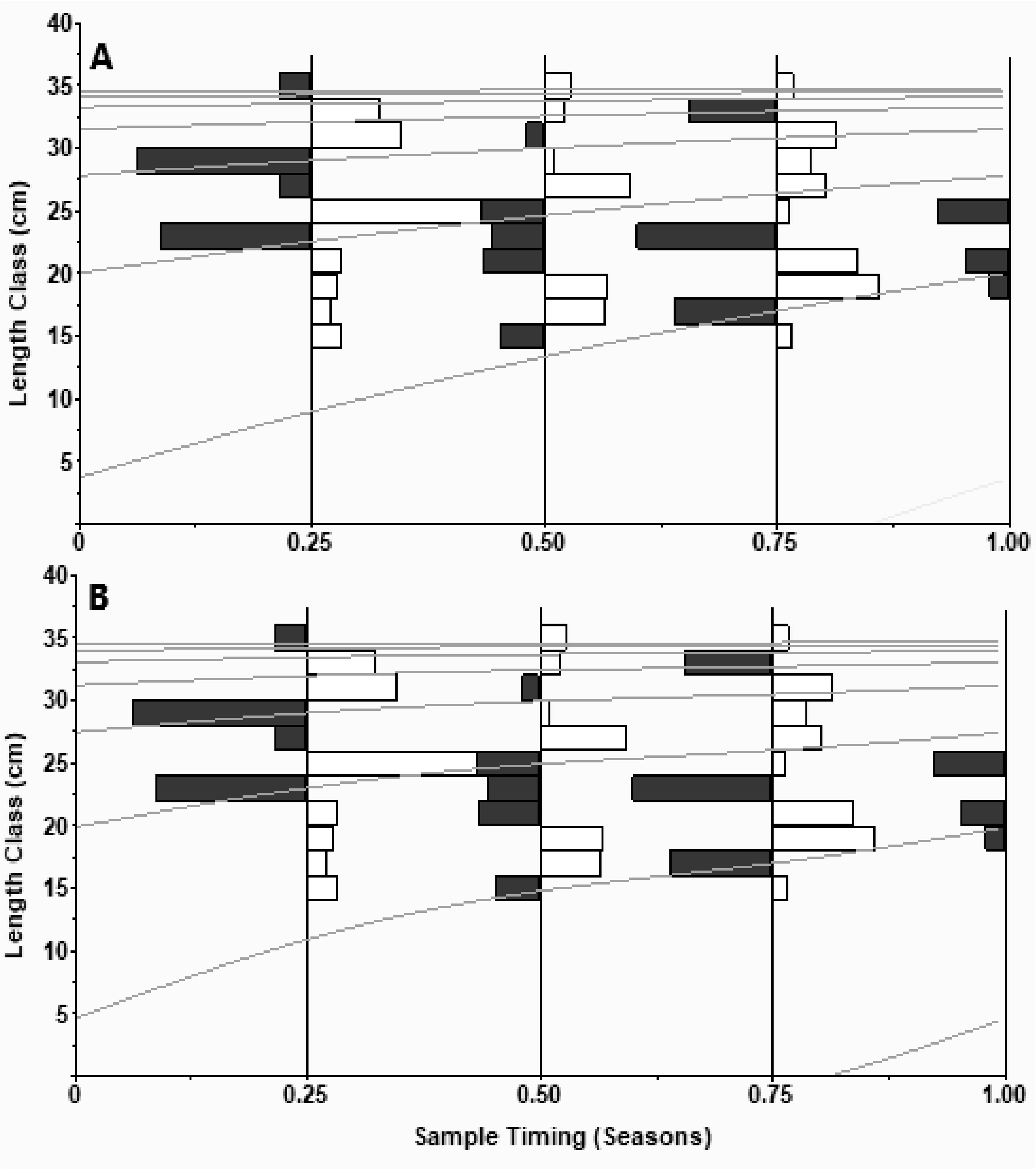

Figure 5. Growth curve (lines) from the von Bertalanffy model with non-seasonal (A) and the Hoeing seasonal (B) by using electronic length-frequency analysis (ELEFAN), to length-frequency data of *Holothuria arenicola* sampled from Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan between January and December 2014

Discussion

To the best of our knowledge this is the first study to calculate the Hoenig seasonal growth curve parameters by using ELEFAN, to length-frequency data and age–length key of two holothurians, *H. arenicola* and *H. atra*. Our LFDA results showed that both *H. arenicola* and *H. atra* have similar length-frequency distribution and modes. Different spatial and temporal variation of LFDA and/or modes was reported for *H. atra* from Manora and Buleji in the previous study (Siddique and Ayub, 2005). According to Siddique and Ayub (2005), the small-sized individuals (<12 cm) were equally abundant at both sites, the medium-sized individuals (12-20 cm) were more (37.7% of the total population) at Buleji than at Manora (20.6%) and the larger-sized individuals (>22 cm) were higher in numbers at Manora (21.2%) than at Buleji (2.4%). Moreover, bimodal distribution (consisting of small and medium-sized individuals in summer and autumn), three modes (winter and spring) from Manora and unimodal distribution from Buleji were reported. The mean total length of examined individuals in Buleji (mean length: 11.6±3.5 cm, between 3.8 and 37.6 cm) was also reported as higher than Monara (mean length: 14.1±7.6 cm, between 2.7 and 41.6 cm) during the period from April 2011 to November 2012. In our study, the mean total length (mean: 23.3±0.30, between 13.0 and 33.0 cm) of examined individuals were about 10-11 cm larger than the study of Siddique and Ayub (2005) and also, we have not observed small-sized individuals (<12 cm). In the present study, the sea cucumbers smaller than 10-12 mm could not be obtained in the sampling area both two species, most probably due to the differences in behavioral characteristics (e.g. burrowing cycle) by size. Mercier et al. (2000) reported that juveniles *H. scabra* ≤10 cm burrowed at sunrise and surfaced at sunset, whereas individuals >10 cm burrowed and surfaced a few hours earlier (in the middle of the afternoon). According to these results described above, it can be concluded that the growth and length composition for examined species in the research region differ significantly between years and between regions due to the marine environmental variability such as site health, e.g., water quality (dissolved oxygen and nutrients), hydrodynamics (monsoonal effect, current, water movement). Secondly, because of the different natural mortality and migration or recruitment levels. Migration of *H. atra* from one area to another was reported by different researchers due to rolling with the water movement (Tamaroa, 2010) and also due to lack of dissolved oxygen and nutrients in any environment (Uthicke, 2004).

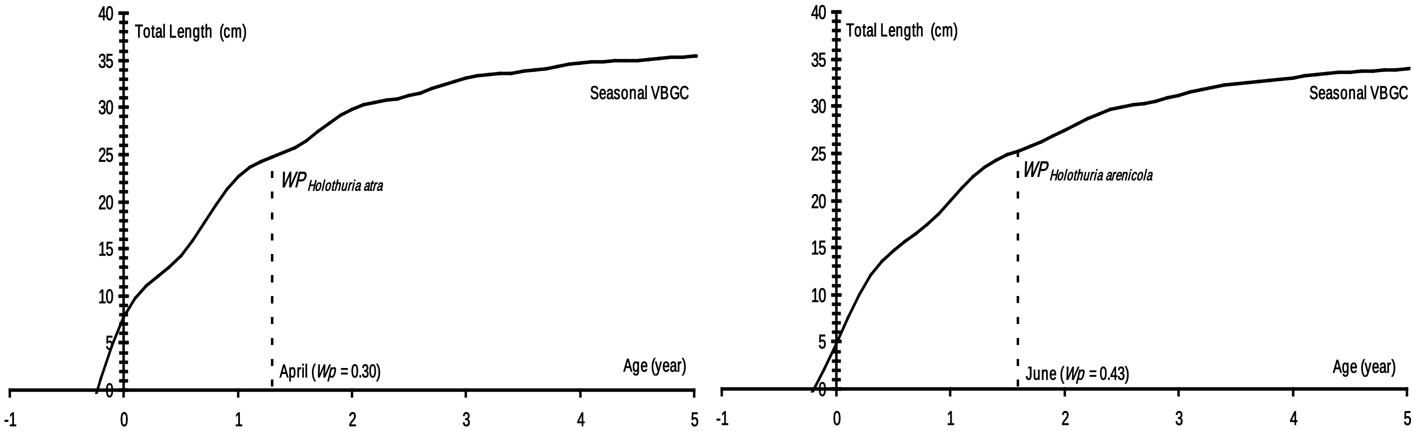

Figure 6. Winter point (*Wp*) of the seasonal oscillations in the seasonal von Bertalanffy growth function curves for *Holothuria atra* and *Holothuria arenicola* in the north Arabian Sea, Pakistan

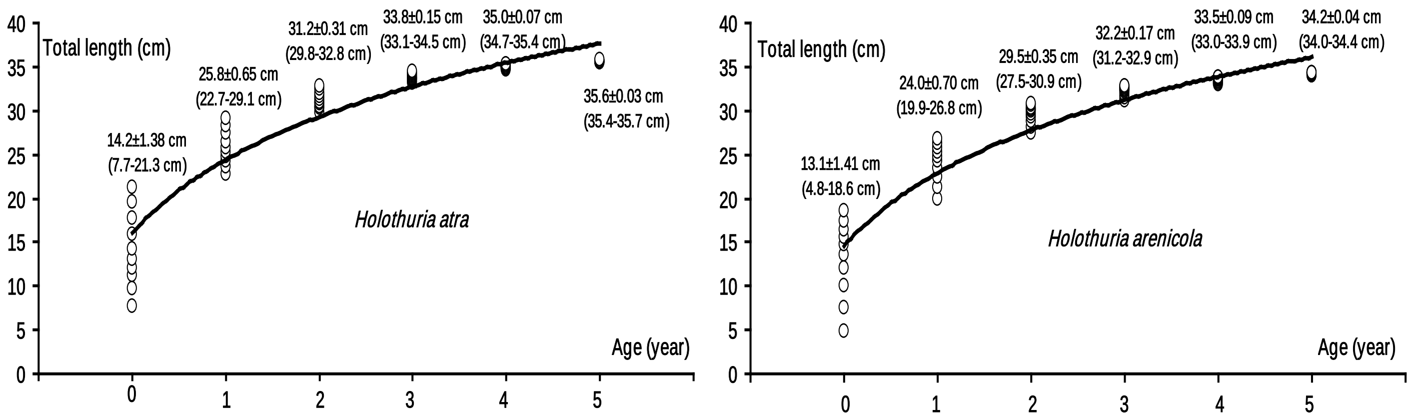

Figure 7. Length–age key (estimated length for each year class) from the seasonal von Bertalanffy growth function curves parameters both *Holothuria atra* and *Holothuria arenicola* in the north Arabian Sea, Pakistan. Values as mean ± std. err. (minimum–maximum)

The observed growth rates (K>0.70) indicate that examined two species achieve asymptotic size quickly, even faster some of the other holothurians, such as *I. badionotus* (K=0.2), *I. fuscus* (K=0.18), *H. pulla* (K=0.24). On the other hand, similar growth parameters were also reported for many other holothurian species such as *H. arguinensis* (K=0.88), *I. badionotus* (K=0.7) (Table 2). The general assumptions for different length-based growth models for the growth determination of holothurians reported as (Olaya-Restrepo et al., 2018): *i*) species reproduce and recruit once per year; *ii*) species reach a specific size; and *iii*) species grow isometrically (b=3). Most commercial sea cucumber species fulfill the first two assumptions, but usually they do not exhibit isometric growth (Olaya-Restrepo et al., 2018). For *H. atra* and *H. arenicola*, negative allometric growth has been reported (Siddique et al., 2015; Ahmed et al., 2018b). However, much larger species such as *I. badionotus* are long lived and usually have relatively low values for K. In such cases it could be expected that life span is extended considerably. For *I. badionotus* in Sisal, at asymptotic population size was reported ten years while K was as low as 0.20 year^{-1} (Poot-Salazar et al., 2015). Faster growth rates may have important and positive effects on the mortality of holothurians such as *Australostichopus mollis* (Hutton, 1872), primarily under high stress conditions and in disturbed environments (Morgan, 2012) and so, the fast growth could be considered as an adaptive advantage. Namely, larger sea cucumbers would be capable of moving and stabilizing themselves in more mobile sediments, such as sandy substrates, and they would also be more likely to escape predation (Mercier et al., 2000; Olaya-Restrepo et al., 2018). In our study, calculated K (0.75 year^{-1}) and L_∞ (36.1 cm) values for *H. atra* were found to be larger than the K (0.11 year^{-1}) and L_∞ (32.4 cm) values reported in the previous study, conducted between 1975 and 1976 on the seaward reef bench of Ananij Island, Enewetak Atoll, Marshall Islands (Elbert, 1978) (see: Table 2). This difference between our study and Elbert's study, which was carried out about 42 years ago, may have resulted from the difference between biotic and abiotic factors such as prey availability, predators, genetic variation, salinity, habitat structure, etc. and also may be due to the difference of the method used to calculate of the growth. When there is a seasonal growth pattern for holothurian species belonging to the same family in a geographical region, the estimations of L_∞ and K may differ significantly between the seasonal and non-seasonal models. In the present study, the non-seasonal VBG model provided realistic results. However, when seasonality was included (with the Hoenig model), more reliable values were obtained, which confirmed the seasonality in the growth of *H. arenicola* and *H. atra*.

Table 2. Growth parameters of different sea cucumber species belong to different family. L_∞, asymptotic total length (cm); K, growth coefficient (year^{-1}); Φ', growth performance index

Family	Species	L_∞ (cm)	K (yr^{-1})	Φ'	Area	Ref.
Stichopodidae	*Isostichopus badionotus*	31.9	0.60	2.786	Celestun, Mexico	1
	Isostichopus badionotus	38.0	0.20	2.461	Sisal, Mexico	1
	Isostichopus badionotus	23.5	0.70	2.587	Progreso, Mexico	1
	Isostichopus fuscus	36.1	0.18	2.370	Gulf of California, Mexico	2
Stichopodidae	*Stichopus vastus*	31.6	0.55	2.739	Karimunjawa, Indonesia	3
	Stichopus quadrifasciatus	38.7	0.34	2.707	Karimunjawa, Indonesia	4
Holothuriidae	*Holothuria arguinensis*	66.9	0.88	3.595	South Portugal	5
	Holothuria atra	32.4	0.11	2.062	Ananij, Marshall Islands	6
	Holothuria scabra	29.0	0.52	2.641	Bolinao, Philippines	7
	Holothuria pulla	30.7	0.24	2.354	Bolinao, Philippines	7
	Holothuria pardalis	18.0	1.00	2.511	Karachi, Pakistan	8
	Holothuria verrucosa	18.0	0.86	2.445	Karachi, Pakistan	8
	Holothuria atra	36.1	0.75	2.990	Karachi, Pakistan	9
	Holothuria arenicola	34.9	0.70	2.931	Karachi, Pakistan	9

Note: 1) Poot-Salazar et al., 2015; 2) Herrero- Pérezrul et al., 1999; 3) Sulardiono et al., 2012; 4) Sulardiono and Muskananfola, 2019; 5) Olaya-Restrepo et al., 2018; 6) Elbert, 1978; 7) Pauly et al., 1993; 8) Ahmed et al., 2020a; 9) Present study.

The *Rn* and *C* values with visual growth curves both *H. arenicola* (Figure 4B) and *H. atra* (Figure 5B) evidenced that these two species exhibited marked seasonality in growth. Seasonal growth pattern was also reported from different regions for different holothurians such as *H. arguinensis* from South Portugal (Olaya-Restrepo et al., 2018) and *I. badionotus* off the northwest coast of Yucatan state, Mexico (Poot-Salazar et al., 2015), *I. fuscus* at Espiritu Santo Island, Gulf of California, Mexico (Herrero-Perezrul et al., 1999) and also for *H. pardalis* and *H. verrucosa* population along Karachi coast, northern Arabian, Sea Pakistan (Ahmed et al., 2020a). Since there is no information in the literature reporting the parameters showing seasonality in growth for *H. arenicola* and *H. atra*, we were unable to compare our findings with others. However, the major factors that cause seasonality in the growth of marine organisms belong to different taxa such as marine invertebrate including shrimps and sea cucumbers were reported to be *i*) photoperiod, *ii*) variation in water temperature and salinity fluctuating over the year, *iii*) seasonal change in nutrient quality/availability, *iv*) energy input into reproduction during the breeding season (Bilgin et al., 2009a, 2009b; Poot-Salazar et al., 2014; Olaya-Restrepo et al., 2018). Unfortunately, detailed studies neither of productivity along the northern Arabian Sea coasts nor of the sea cucumber species reproduction biology in these regions yet exists (except for the spawning time of *H. arenicola*). For *H. arenicola*, it has been reported that the gonad activity began to increase regularly in March, reached the highest value at the beginning of the summer (in June), and decreased the lowest value at the end of the summer (in August) (Siddique and Ayub, 2015). However, *H. atra*, reproduction features based on gonad development reported in the Red Sea coast of Egypt (Abdel-Razek et al., 2005), in southern Taiwan (Chao et al., 1994), New Caledonian lagoon (Conand, 1993), and at Heron Reff, Greak Barrier Reff (Harriott, 1985). Temperature, salinity, food abundance and photoperiod have all been known as factors regulating holothurian reproductive cycle. The coast of Pakistan is for most of the year influenced by high-salinity surface water (36-38‰) and the sea surface temperature during summer is 28-30°C while during winter, it is 21-24°C (Siddique and Ayub, 2015). Furthermore, there is monsoonal variations in nutrient concentration due to monsoon winds and fluctuations of rainfall (Hussaina et al., 2010). Such variations may also be related to productivity and avai1ability of food and to the reproductive cycle and growth of *H. arenicola* and *H. atra* and also other sea cucumber species in the north Arabian Sea, Pakistan (Ahmed et al., 2020a). All these could be considered as the factors forcing seasonality in growth both *H. arenicola* (Figure 4B) and *H. atra* (Figure 5B) in the study area.

The variations in the period of the slowest growth time (*WP*) generally related to environmental condition which cause biological stress, physiological conditions of the marine animal, fullness of stomach and gonads stages (Bilgin et al., 2009a, 2009b; Ahmed et. al., 2016b, 2020a). Temperature also plays an important role in the reproductive events and the abundance of food, and therefore especially the water temperature indirectly effective on the *WP* of the marine organisms. The seasonal and/or monsoonal variations in the mean condition factors of *H. arenicola*, *H. atra*, *H. pardalis* and *H. verrucosa* was reported from the coasts of Karachi, Pakistan (Siddique et al., 2014; Ahmed et al., 2018a, 2018b) and also the gonad index (*GI*) based reproduction time for *H. arenicola* was reported during spring and early summer from these regions (Siddique and Ayub, 2015). The period of slowest growth for *H. arenicola* corresponded to June when the highest *GI* value occurs (see Siddique and Ayub, 2015). The slowest growth period for *H. atra*, however, was estimated to be April, which is the period with a relatively low water temperature and with the most probably when reproductive cells (gonad development) begin to mature or develop (Harriott, 1985; Conand, 1993; Chao et al., 1994; Abdel-Razek et al., 2005). Since the growth rate of holotorians depends on the food availability and quality of the habitat and/or organic matter content of sediment, dissolved oxygen concentration in the water column and in sediment (Tamaroa, 2010) and also reproduction events, the slow growth of *H. atra* in April may be the result of the extended both poor nutrition and reproduction periods growth but this was not tested in this study. Therefore, the effects of these factors on growth should be examined in the future studies.

Conclusion

Together with this study seasonal growth was first pronounced for both *H. arenicola* and *H. atra*. Although the causes of this seasonality are not tested in this study, the effect of fluctuations in the amount of nutrient and maturity levels are probably important for this situation. The effects of the climatic events as monsoon rain and reproduction activities on growth should be studied in detail in order to provide data for holotorian fisheries management in the north Arabian Sea, Pakistan.

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Compliance with Ethical Standards

Authors' Contributions

The data of this study was provided by the first author. The statistical analysis and other stages with writing of the manuscript were all carried out by corresponding author.

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.

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RESEARCH ARTICLE

Immune responses and growth performance of the aqueous methanolic extract of *Malva sylvestris* in *Oncorhynchus mykiss*

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ABSTRACT

Growth promoting and immune stimulating effects of common mallow (*Malva sylvestris*) aqueous methanolic extract (AME) in *O. mykiss* were examined. Two different concentrations of common mallow AME [0.1 (CM1) and 0.5 (CM5) g kg^{-1} of feed] commixed a basic diet and a control diet without the common mallow extract were fed to rainbow trout for 30 days to evaluate growth rate and immune responses. At the end of the study, fish growth performance was determined as significantly higher in the group fed with 0.1 g kg^{-1} common mallow AME compared with control diet fed group ($P<0.05$). In fish fed with CM5 diet, oxidative radical production (ORP) was the highest ($P<0.05$). Similarly, myeloperoxidase (MPO) activity was increased significantly in CM5 group. Lysozyme (LYS) and phagocytic activities (PA) were not altered in treated fish groups compared to the control group ($P<0.05$). The pro-inflammatory (IL-1β, TNF-α1, IL-8,), anti-infammatory (IL-10), lymphocyte agonist (TGF-β) and cell-mediated immune regulatory, IL-12 cytokines were generally down-regulated insignificantly compared to control. Although, common mallow AME could not elicit cytokine-mediated immunity and resistance to bacterial pathogen, *A. hydrophila* in rainbow trout, elevation in ORP and MPO activities in treated groups and growth promoting effect were noticed at a low dose. Therefore, application AME of common mallow at 0.1 g kg^{-1} of feed as growth promoter and non-specific immunostimulant is advocated for rainbow trout.

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Introduction

According to FAO (2018), among food producing sectors, aquaculture industry is the fastest rising sector in the world. The occurrence of fish disease and use of chemicals such as antibiotics are the most determining factors in aquaculture. However, it is a hard reality that use of chemicals and antibiotics in aquaculture may result in antibiotic resistance in bacteria and finally leads to environmental hazards (Boran, Terzi, Altinok, Capkin, & Bascinar, 2013; Capkin, Terzi, & Altinok, 2015; Terzi et al., 2020). Furthermore, other unwanted characteristics of antibiotics include residual effects that persist in the environment for a long time (Jones, Voulvoulis, & Lester, 2004; Terzi, 2018) and cultured fish species as well (Cabello, 2006; Corum, Durna Corum, Er, Terzi, & Uney, 2018; Corum et al., 2020). Another important issue is, using new techniques such as oxygen supplementation or floating fish farm unit to obtain maximum production efficiency from the unit area by increasing stocking density, could result some immune deficiency problems and diseases (Bilen, Bilen, & Önal, 2015; Bilen, Kızak, & Bilen, 2013). Therefore, discovering environment friendly products and developing their appropriate application plans are of utmost importance currently. On this direction, studies are being undertaken to find out suitable organic products that are environment friendly, sustainable and cost-effective.

Of late, plant-derived immunostimulants have been tested and several works have been performed related to application of medicinal herbs (Almabrok, Amhamed, Mohamed, Bilen, & Altief, 2018; Arslan, Sönmez, & Yanık, 2018; Dawood, Eweedah, Moustafa, & Farahat, 2019; Mohamed et al., 2018; Moustafa et al., 2020). Several medicinal plants have the potential positive effects on growth performance, survival rate and immune system activation in fishes (Amhamed, Mohamed, Almabrok, Altıef, & Bilen, 2018; Bilen, Altief, et al., 2019; Bilen, Özkan, Alagöz, & Yürüten Özdemir, 2018). Traditional medicines are becoming popular in Turkey because of its rich diversity of medicinal plants. Common mallow (*Malva sylvestris*) is an important medicinal plant cultured in Turkey and it has several beneficial effects (Alan & Padem, 1989; Kaya, İncekara, & Nemli, 2004; Özdestan & Üren, 2008; Tugay, Bağcı, Ulukuş, Özer, & Canbulat, 2012). Recently, positive effects of common mallow were demonstrated in European sea bass, gilthead sea bream and common carp (Bilen, Filogh, Ali, Kenanoğlu, & Zoral, 2019; Bilen, Kenanoglu, Terzi, Ozdemir, & Sonmez, 2019). Therefore, the present experiment was performed to evaluate the effects of dietary supplementation of AME of common mallow on growth, and different cytokine and adaptive immune responses, such as oxidative radical production, phagocytic activity, myeloperoxidase activity and lysozyme activity in rainbow trout which is an important commercially cultured finfish in Europe.

Material and Methods

Design of Experiment

Rainbow trout was acquired from Inland and Marine Fish Research and Application Center, Kastamonu University, and maintained for 14 days at laboratory condition for acclimatization. To start the experiment, nine aquaria (300 L) were stocked with rainbow trout (mean weight: 54.97±0.03 g) at 20 number/aquarium. There were three triplicate aquaria assigned randomly for each treatment group. Experimental diet contained common mallow extract at 0.1 (CM1) and 0.5 g kg^{-1} (CM5), whereas the control diet had no supplementation (0 g kg^{-1}, CM0). During 30 days of the study, fish were fed ad libitum with experimental diets twice a day. At the end of the study, fish was sedated using 0.02 mg L^{-1} of phenoxyethanol. The blood samples were collected from the caudal vein and kept in heparin containing tubes. Head kidney was aggregated and immersed directly into liquid nitrogen then stored at -80°C until further use. During the experimental period, water quality parameters of the fish holding aquaria were as follows: temperature 15±0.8°C, dissolved oxygen 9.01±0.3 mg L^{-1}, pH 8.1±0.1, total ammonium N 0.001±0.001 mg L^{-1}, nitrite-N 0.002±0.002 mgL^{1}, and nitrate-N 0.01±0.01 mg L^{-1}, conductivity 424±11 µS.

The experimental procedures were permitted by Kastamonu University, Local Ethics Committee for Animal Research Studies (Protocol Number: 03.04.2017-2017.08).

Preparation of Common Mallow Extract

The extraction was performed according to (Bilen, Ünal, & Güvensoy, 2016). Briefly, common mallow (*Malva sylvestris*) was collected from country side in Kastamonu (41°26'33.20"N, 33°47'30.48"E). The herbs were washed with deionized water and shed-dried. One hundred g sample was extracted with 1 L methanol (40%) and filtered after the solvent was dehumidified by a rotary evaporator. Finally, 3.12 g concentrate was dissolved in 25 mL deionised water. This solution was mixed with the feed at a rate of 0, 0.1 and 0.5 g kg^{-1}. The feed were kept at –20°C until further use.

Growth Efficacy

At the end of the 30-day trial, individual fish was weighed. Growth performances (weight gain, WG; specific growth rate, SGR, feed conversion ratio, FCR; protein efficiency ratio, PER) were determined as follows:

$$WG\ (\%) = \frac{Daily\ feed\ intake\ (g)}{Biomass\ (g)} \times 100$$

$$SGR = 100 \times \left[\frac{(\ln final\ weight - \ln initial\ weight)}{Days\ fed\ (g)}\right]$$

$$FCR = \frac{Feed\ intake\ (g)}{Weight\ gain\ \ (g)} \times 100$$

$$PER = \frac{Wet\ body\ mass\ gain}{Crude\ protein\ intake}$$

Designation of Adaptive Immune Parameters

In the present study, oxidative radical production was measured according to Siwicki and Anderson (1993), lysozyme activity was determined according to Ellis (1990) and myeloperoxidase activity was measured according to Quade and Roth (1997) slightly modified by Sahoo, Kumari, and Mishra (2005). The phagocytic activity (PA) was assayed following Siwicki, Anderson, and Rumsey (1994).

Determination of Cytokine Expression

Total RNA extraction from head kidney sample

Total RNA from 20 µg head kidney sample was isolated using RNeasy Plus Micro RNA isolation kit (Qiagen, Germany) according to manufacturer's protocol. Total RNA was treated with 1 U DNase I (Thermo, Lithuania) and reverse transcribed using Revert Aid RT synthesis kit (Thermo, Lithuania), making 1 µg of template RNA, 15 pmol/µL oligo dT primer, 0 U/µL Revert Aid M-MuLV RT enzyme, 1 mM of dNTP mix, 4 µL 5× reaction buffer and 6 µL of NFW. cDNA was synthesised using thermal cycler (Thermo, Lithuania) for 60 min at 42°C.

qRT-PCR procedure

Rotor-Gene qPCR detection system (Qiagen, Hilden, Germany) and Rotorgene SYBR Green PCR kit (Qiagen, Hilden, Germany) were used for qRT-PCR analysis. Specific primers were used previously determined by Altunoglu, Bilen, Ulu, and Biswas (2017). qRT-PCR mixture contained 0.4 µM of specific forward and reverse primer (β-actin as reference, IL-1β, IL-8, IL-10, IL-12p40, TNF-α1, TGF-β), 0.1 µg template DNA, 12.5 µL of 2× SYBR Green Master Mix and NFW to the final volume of 25 µL. Samples were denatured at 95°C for 10 s, annealed and extended together at 60°C for 40 s. Relative gene expression level was calculated following $2^{-\Delta\Delta CT}$ method as described elsewhere (Altunoglu et al., 2017).

Experimental Challenge Test

All challenge procedure was performed according to Bilen et al. (2016). 14 fish from each tank were injected intraperitoneally with LD_{50} dosage (1×10^8 CFUs mL^{-1}) of *A. hydrophila* (SB-Ah1) (Bilen, Filogh, et al., 2019) and survival was noted during 14 days after injection.

Statistical Analysis

All results were given as mean (±) standard error except survival rates. Differences among groups in terms of growth rate, immune responses and cytokine expressions were tested by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test at $P<0.05$ using SPSS 23.

Results

Results related to effects on growth were summarized in Table 1. FCR and PER did not vary among the experimental groups (P>0.05). All growth parameters such as SGR, weight gain, total feed consumption and final weight were determined significantly advanced in CM1 group (P<0.05) compared to control (CM0). However, there were no differences in these variables between CM5 and CM0 groups (P>0.05).

Table 1. Effects of different doses of methanolic extracts of common mallow on the performances of rainbow trout after a 30-day feeding trial

Measurements	Control (CM0)	CM1	CM5
Initial weight (g)	54.97±0.03	54.97±0.03	54.97±0.03
Final weight (g)	79.13±0.19^b	102.93±0.39^a	84.25±0.67^b
Weight gain (%)	43.96±10.11^b	87.49±11.77^a	53.15±9.78^b
SGR (% day^{-1})	1.21±0.25^b	2.10±0.02^a	1.42±0.1^b
Total feed consumption (g)	739.91±11.51^b	1455.43±21.90^a	912.29±762.32^b
FCR	1.02±0.12	1.01±0.01	1.04±0.03
PER	2.18±0.11	2.20±0.01	2.14±0.09

Note: CM0, CM1 and CM5 indicate common mallow extract doses at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Values are mean±SD of three replicates (n=3); Different superscript letters in a row indicate significant differences among the experimental groups ($P<0.05$). FCR: feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio.

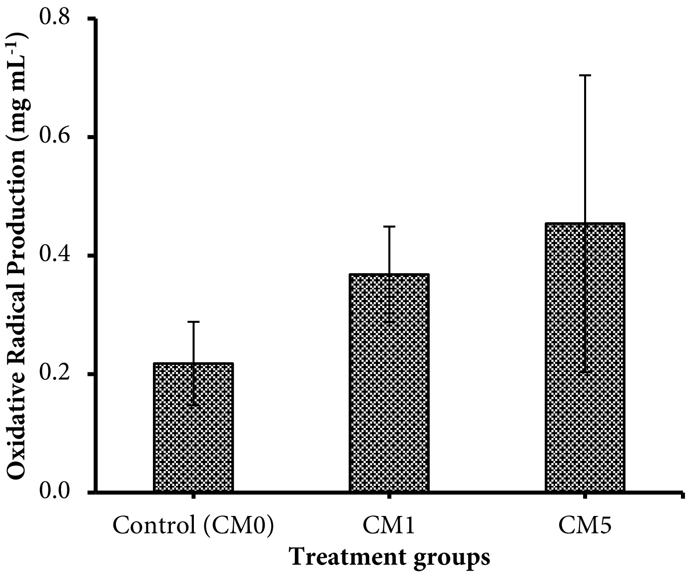

Figure 1. Oxidative radical production in rainbow trout fed with diets supplemented with different doses of common mallow methanolic extract. CM0, CM1 and CM5, common mallow methanolic extract at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Different letters on bars indicate significant differences among groups (P<0.05). Data are presented as mean± SD (n=3)

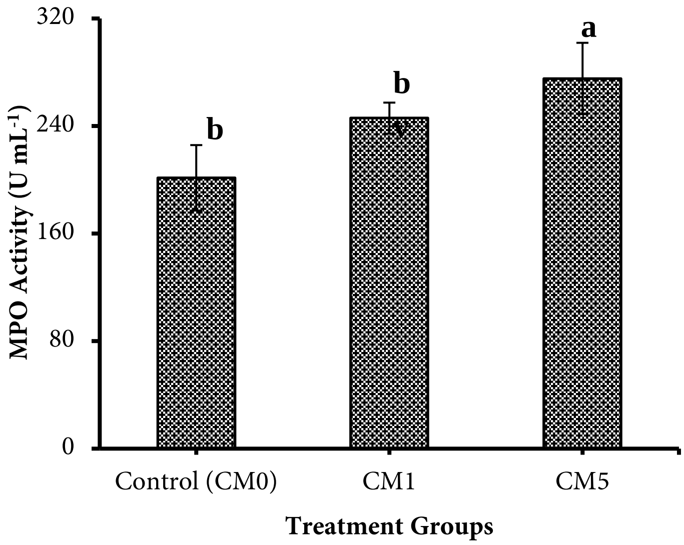

Figure 2. Myeloperoxidase (MPO) activity in rainbow trout fed with diets supplemented with different doses of common mallow methanolic extract. CM0, CM1 and CM5, common mallow methanolic extract at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Different letters on bars indicate significant differences among groups (P<0.05). Data are presented as mean± SD (n=3)

Oxidative radical production was increased in two experimental groups compared to control. The oxidative radical production was highest in CM5 group (0.454±0.25), followed by in CM1 group (0.368±0.08) ($P<0.05$) (Figure 1). MPO activity was determined significantly higher in CM5 group compared to that of CM0 and CM1 groups (Figure 2). Lysozyme activity was not affected by common mallow extract administration in rainbow trout (Figure 3). Similar to LYS activity, phagocytic activity was not influenced by common mallow extract administration in rainbow trout of any treatment group as well as the control (Figure 4).

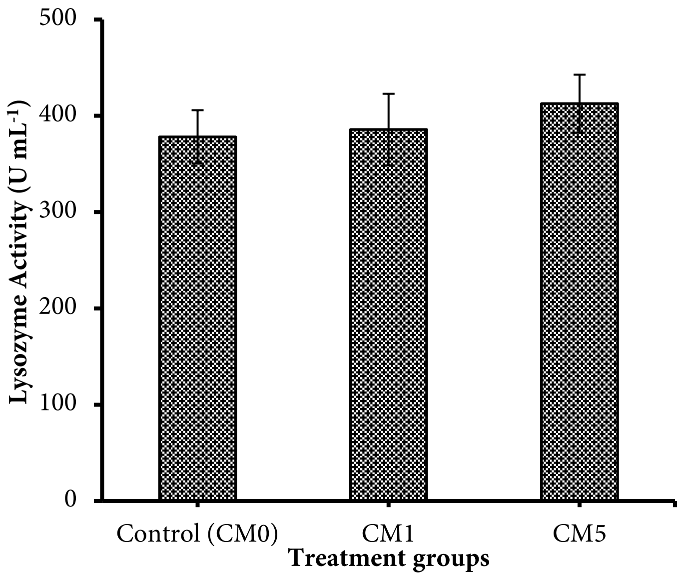

Figure 3. Lysozyme activity in rainbow trout fed with diets supplemented with different doses of common mallow methanolic extract. CM0, CM1 and CM5, common mallow methanolic extract at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Data are presented as mean± SD (n=3)

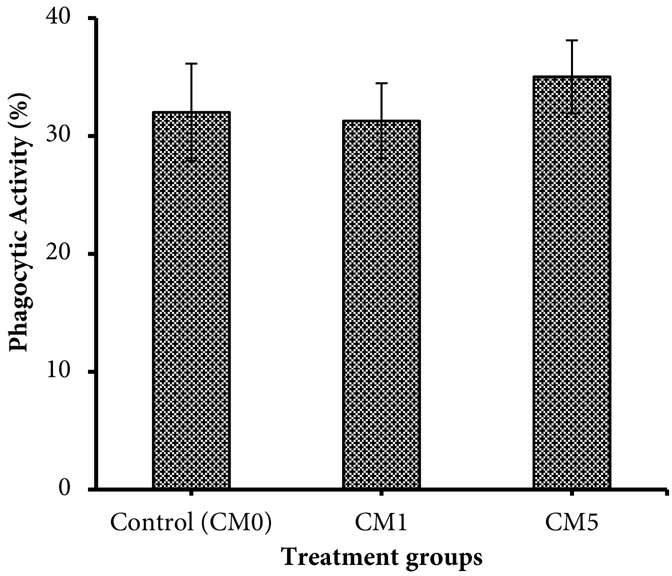

Figure 4. Phagocytic activity in rainbow trout fed with diets supplemented with different doses of common mallow methanolic extract. CM0, CM1 and CM5, common mallow methanolic extract at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Data are presented as mean± SD (n=3)

Cytokine gene expression such as IL-1β, IL-8, IL10, IL-12, TNF-α1 and TGF-β, was analysed using qRT-PCR (Fig. 5). IL-1β, TGF-β, IL-8, IL-10 and TNF-α expression generally decreased in the CM1 and CM5 groups compared with control but this decrease was not significant ($P>0.05$).

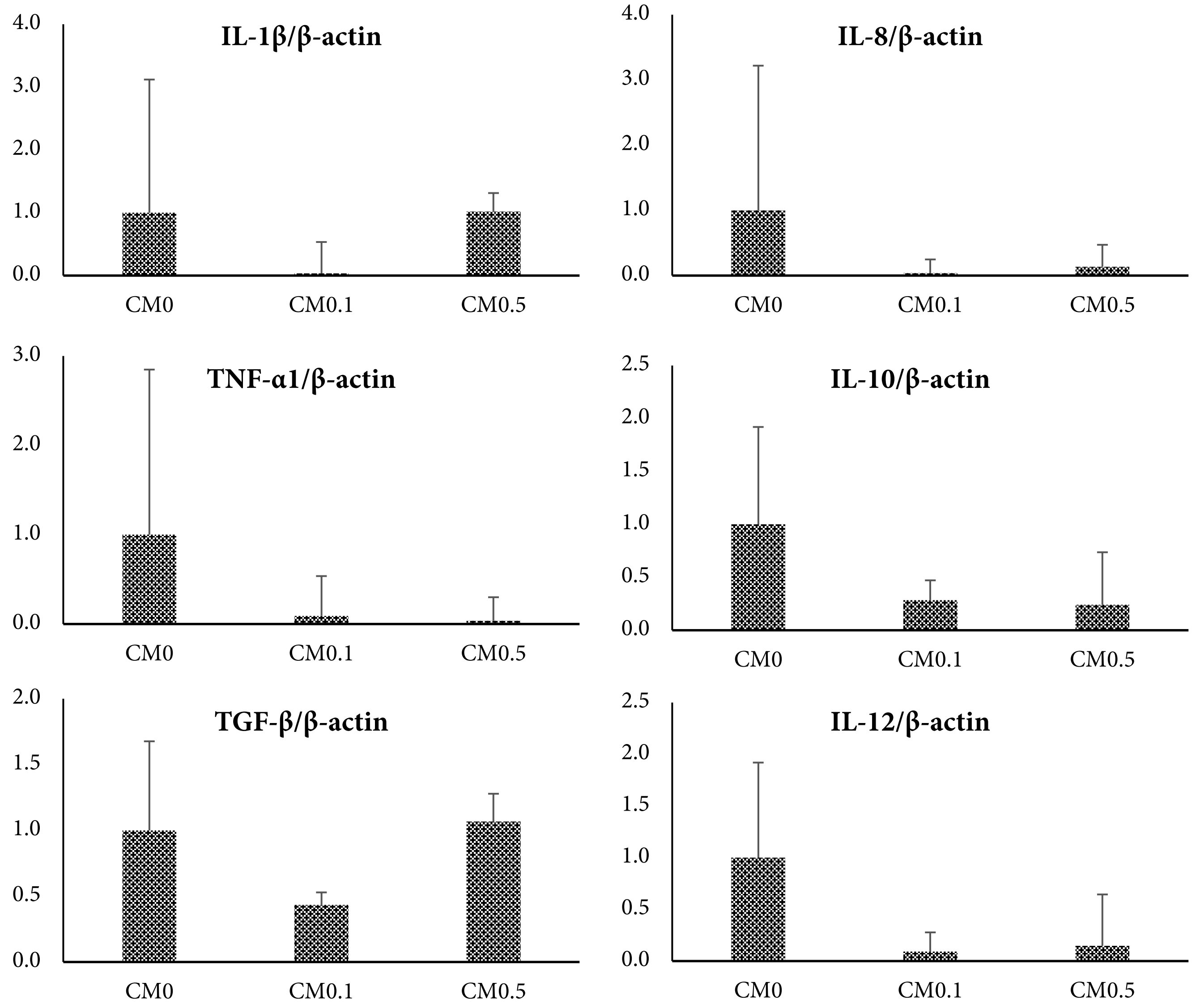

Figure 5. Relative gene expression (mean±SD; n=3) patterns of cytokines in the head kidney cells of rainbow trout fed with diets supplemented with different doses of common mallow methanolic extract. CM0, CM1 and CM5, common mallow methanolic extract at 0, 0.1 and 0.5 g kg^{-1} diet, respectively. Different letters on bars indicate significant differences among groups (P<0.05)

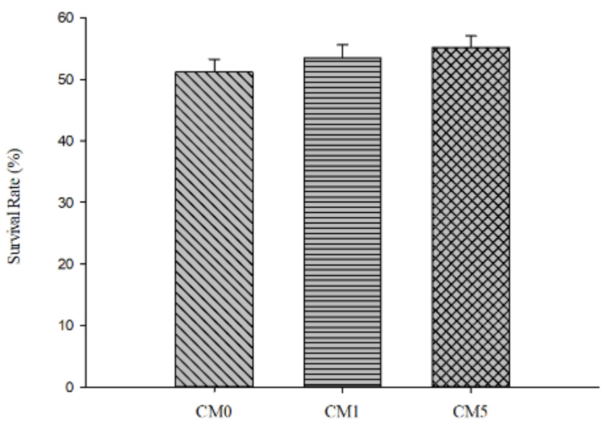

Figure 6. Survival of common mallow extract administered rainbow trout after experimental challenge with *Aeromonas hydrophila*. CM0, CM1 and CM5 indicate black cumin extract doses at 0, 0.1 and 0.5 g kg^{-1} feed, respectively

Survival rate of the fish after challenged with *A. hydrophila* is given in Figure 6. Survival rate was not affected among CM0, CM1 and CM5 groups (*P*>0.05).

Discussion

In this study, we investigated the effect of common mallow extract as a fish feed supplement on growth promotion and immune response in rainbow trout. Previously, beneficial role of common mallow using different application methods was described in fish (Bilen, Filogh, et al., 2019; Bilen, Kenanoglu, et al., 2019; Elbesthi, Yürüten Özdemir, Taştan, Bilen, & Sönmez, 2020). Our results indicated that common mallow extract is effective for adaptive immune responses and growth promotion, but not for innate immune enhancement in rainbow trout and no protection was determined against *A. hydrophila* (P>0.05).

Dietary supplementation of medicinal plant products proved to provide positive effects on fish growth (Sönmez, Bilen, Alak, et al., 2015; Sönmez, Bilen, Albayrak, et al., 2015; Bilen, Altief, et al., 2019; Bilen, Filogh, et al., 2019). In the current study, growth was influenced by administration of common mallow extract. Although, FCR remained unchanged, total feed consumption increased significantly. Similarly, growth promoting effect of common mallow extract was demonstrated in carp (Bilen, Filogh, et al., 2019) and European sea bass (*Dicentrarchus labrax*) (Bilen, Kenanoglu, et al., 2019) and rainbow trout fed with aqueous extract of *Malva sylvestris* (Rashidian, Kajbaf, Prokić, & Faggio, 2019). Moreover, fenugreek seeds at 1% and 2% doses caused an enhancement in feed utilization and SGR in Nile tilapia (*Oreochromis niloticus*) (Abdelhamid & Soliman, 2012). On the other hand, Bilen and Bilen (2012) evidenced that *Cotinus coggygria* and *Laurus nobilis* had no effects on growth in rainbow trout. Mohamed et al. (2018) found growth improvement in carp fed with *Apium graveolens* and Almabrok et al. (2018) determined a dose depended growth promoting effect in carp fed with *Tilia tomentosa*.

ORP, MPO, LYS and PA activities were evaluated in rainbow trout fed diet containing common mallow extract. An elevated ORP was detected in both the experimental groups when compared to control. In the fish of CM5 group, ORP reduction level was the highest. Similarly, ORP was elevated in rainbow trout treated with nettle (Bilen, Soydaş, & Bilen, 2014) and black cumin (Altunoglu et al., 2017) extracts. MPO activity was induced by common mallow extract treatment. Bilen, Altunoglu, Ulu, and Biswas (2016) found that caper extracts caused elevation of MPO in rainbow trout. However, lysozyme, which provides non-specific immune responses to pathogens during infection, was not varied by common mallow extract administration in rainbow trout. Moreover, phagocytic activity was not influenced by dietary common mallow extract administration. A similar result was reported in rainbow trout received changed doses of *Nigella sativa* seed extract (Altunoglu et al., 2017).

In this experiment, we examined transcription of six cytokines belonging to different functional groups. The proinflammatory, IL-1β, TNF-α1, IL-8, anti-inflammatory, IL-10, cell-mediated immune regulatory, IL-12 cytokines and lymphocyte agonist, TGF-β were either down-regulated or remained unchanged in their expression in common mallow treated rainbow trout compared to that of untreated control. Similar to our results, no elevation in IL-1β, IL-8, TNF-α1, IL-10 and IL-12 cytokine gene responses was caused by the administration of *Nigella sativa* in rainbow trout (Altunoglu et al., 2017). Contrary to this, Bilen and Elbeshti (2019) observed elevated expression of related genes with our study in rainbow trout treated with *Cotinus coggygria*, and *Usena barbata* (Bilen, Sirtiyah, & Terzi, 2019). Similar to our study, A. Sönmez, Yürüten Özdemir, & Bilen (2018) determined induced pro-inflammatory cytokine gene expression in rainbow trout leucocytes. Therefore, current results suggest that common mallow extract is not effective in induction of cytokine-mediated immunity in rainbow trout.

No differences in survival rate were observed in common mallow treated and control fish groups tested with *A. hydrophila*. Similarly, there was no effect of oyster mushroom in survival of rainbow trout after exposed to the same pathogen (Bilen, Ünal, et al., 2016). However, Bilen, Filogh, et al. (2019) found a decreasing survival rate in common carp when challenged with *A. hydrophila*. Consequently, our results suggest that administration of dietary common mallow extract to rainbow trout could not enhance fish defiance to this bacterial pathogen.

Conclusion

From this study, it was evident that the methanolic extract of common mallow was unable to persuade innate immunity in rainbow trout. However, growth promoting effect was noticed at a low dose. However, non-specific immune responses (ORP, MPO) were elevated. Thus, use of methanolic extract of common mallow at 0.1 g kg^{-1} of feed as growth promoter is suggested for rainbow trout.

Compliance with Ethical Standards

Authors' Contributions

All authors contributed equally.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experimental procedures were permitted by Kastamonu University, Local Ethics Committee for Animal Research Studies (Protocol Number: 03.04.2017-2017.08).

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RESEARCH ARTICLE

The blue crab (*Callinectes sapidus*, Rathbun, 1896) is spreading in the southern coast of the Black Sea

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ABSTRACT

The natural fauna of the Black Sea changes due to the entrance of different origin species. One of them is the blue crab that the first location information of which reported in 1968 on the Black Sea. Location records of blue crabs have been found in the southern coasts of the Black Sea in recent years. A total of 451 hauls were performed with the hydraulic dredge fishing gear to detect the existence of the blue crab on the coast of Sakarya (Southern Black Sea). Two female individuals were caught (carapace length: 70.09 cm, width: 15.69 cm, weight: 166.9 g; carapace length: 83.42 cm, width: 188.89 cm, weight: 272.1 g). Especially when the location records in recent years are examined, it is seen that this species is spreading to the southern coasts of the Black Sea. The probability of reproduction and adaptation in the Black Sea should be explored by diving and a blue crab-oriented study with traps or diving.

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Ceylan, Y. (2020). The blue crab (*Callinectes sapidus*, Rathbun, 1896) is spreading in the southern coast of the Black Sea. *Marine Science and Technology Bulletin*, 9(2): 168-172.

Introduction

It is well known that the natural fauna of the Black Sea has changed because of the entrance of species with different origins (Pashkov et al., 2012). The deep current from the Sea of Marmara to the Black Sea has carried the salty waters of the Mediterranean to the Black Sea. The increase in water temperature due to both the deep current and climate change make the environmental conditions more suitable for the species of Mediterranean origin the Black Sea. Besides, increased human activities and maritime transport play a role in moving species to other regions. Therefore, it have been reported that many alien species have recently found in the Black Sea (Shefer et al., 2004; Şahin et al., 2009; Sağlam et al., 2011; Turan et al., 2016).

The blue crab (*Callinectes sapidus* Rathbun, 1896), originating from the West Atlantic, was first recorded in the early 20th century in Europe and then in 1949 in the Mediterranean Sea (Enzenroß et al., 1997). It has been reported that the blue crab, which has been reported in different

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locations in the Aegean, Marmara and the Black Sea, is generally spread by ballast waters of the ship (Nehring, 2011). The *Callinectes sapidus* is euryhaline, so the salinity value of the Black Sea is not a criterion that limits its habitat (Guerin and Stickle, 1992). The blue crab, which is emphasized to have an important role in the structure and functioning of the food chain in benthic areas in the west of the Atlantic, is defined as an invasive species for the Mediterranean (Zenetos et al., 2005). Accordingly, it is assumed that this species may have negative effects on the aquatic ecosystems (Sharov et al., 2003). Besides, the small-scale fishing of economically valuable blue crab consumed by humans will naturally be developed in the new area. (Ayas and Özoğul, 2011; Mancinelli et al., 2017). In the literature, this species has been reported in 15 different locations in the Black Sea (Aydın, 2017). Recent studies have shown that it is especially recorded on the southern coast of the Black Sea. In this study, it was aimed to compile the records of the distribution of blue crab in the Black Sea and to give two new location records of the blue crab in the Black Sea.

Material and Methods

It was heard that a few blue crab specimens were caught with hydraulic dredge sand mussels and thrown into the sea on the coast of the western Black Sea, Sakarya, Turkey (Figure 1).

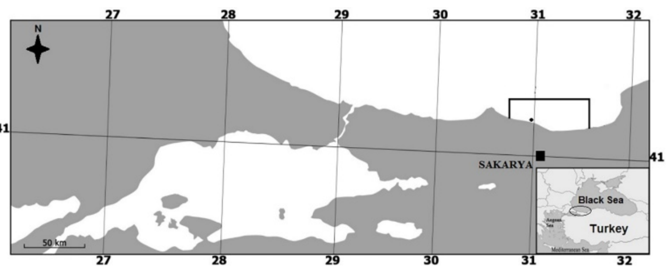

Figure 1. Sampling location (The black dot indicates two new localities)

A meeting was held with the crew of two fishing vessels (1: Sinyor Paşa, 2: Fehmi Reis) to confirm the presence of species in the region and the researcher participated in trips of vessel-1 (December 27-28, 2019- 5-7 January 5-7, 2020) (Figure 2). Observations on vessel-2 were made by the crewmember, and two female individuals (♀♀) were found. The first specimen was caught on December 27, 2019 at 10:35 AM (41° 08' 411" N, 30° 32' 557" E) with the first fishing vessel at a depth of 7 m. The second specimen was caught with the second one at a depth of 11 m at 08:15 AM on December 28, 2019 (41° 09' 484" N, 30° 30' 112" E).

All caught in the collection box were checked in each hauls. The samples obtained in this study were frozen and transferred to Recep Tayyip Erdogan University, Faculty of Fisheries Laboratory and length and weight measurements were carried out in the laboratory.

Figure 2. Fishing vessels used in the research

Results

A total of 451 operations were performed (45-58 hauls in each day) and the total haul time was recorded as 3608 minutes. In all hauls, the prey collected in the collection box was sampled and two individuals (female) were caught. The individuals have 70.09 cm carapace length, 15.69 cm width, 166.9 g weight and 83.42 cm carapace length, 188.89 cm width, 272.1 g weight, respectively (Figure 3). Taxonomic classification was performed according to Williams (1974).

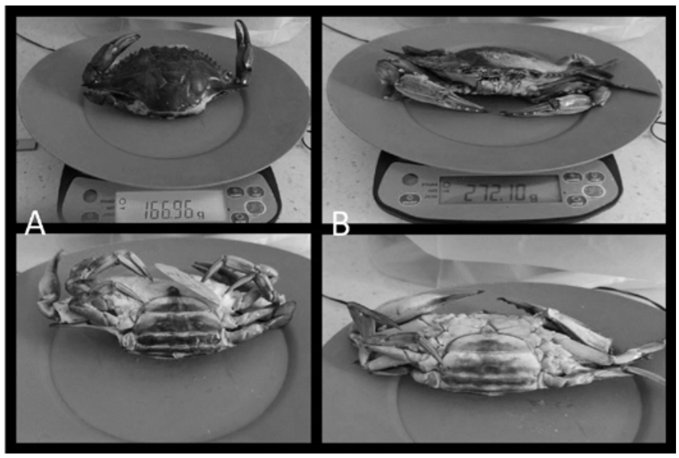

Figure 3. Sampled *Callinectes sapidus* specimens (A: 1, B: 2)

Discussion

The blue crab was first recorded in the Bulgarian coast of the Black Sea in 1968 and then was reported in 11 different locations of the Black Sea until 2014 (Table 1).

According to the records from the southern coasts of the Black Sea, it is understood that the first record was reported in 2014 and then it has been reported from 6 different locations in the last 6 years together with this study (Ak et al., 2014; Yağlıoğlu et al., 2014; Aydın, 2017).

Some researchers reported that the entrance of the blue crab into the Black Sea was provided with ballast water of commercial ships, while others reported that it entered with natural ways. In particular, it is reported that the absence of

Table 1. Location data of blue crab for Sea of Azov and Black Sea

Study	CW	CL	Depth (m)	Fishing method	Location
Bulgurkov (1968)*	16.6	7	5-6	NA	Varna Bay
Shaverdashvili and Ninua (1975)*	19.4	7.5	8-10	Three walled fishing net	Poti
Zaitsev (1998)*	17 20.5	NA	NA	NA	Kerch Strait
Monin (1984)*	20	8	20	NA	Cape Bolshoi Utrish
Zaitsev (1998)*	NA	NA	NA	NA	Varna Bay
IMO (2002)*	NA	NA	NA	NA	Romanian
Diripasko et al. (2009)*	NA	NA	NA	NA	Sea of Azov
Diripasko et al. (2009)*	15	7.5	NA	NA	Sea of Azov
Khvorov (2010)*	18	7	30	NA	Crimean
Diripasko et al. (2009)*	14	7.3	NA	NA	Sea of Azov
Pashkov (2012)	20	8.1	5-6	Gillnet	Lazarevskoye
Yağlıoğlu et al. (2014)	NA NA	NA NA	14 18	Gillnet	Düzce/Turkey Zonguldak/Turkey
Ak et al. (2014)	19.1	9	8-12	Gillnet	Trabzon/Turkey
Aydın (2017)	19.4 19.6	NA	2-10	Trammel net	Ordu/Turkey
Present study	18.9 15.7	83.4 70.1	7 11	Hydraulic dredge	Sakarya/Turkey Sakarya/Turkey

Note: * Information is quotation from Pashkov (2012), CW: Carapace width, CL: Carapace length, NA: No information.

juvenile and adult individuals with eggs seems to support the first theory (Aydın, 2017). On the other hand, Pashkov (2012) reported that the blue crab entered the Black Sea from the Mediterranean basin with both ballast waters and natural ways. Especially the fact that there are too many records on the South coast in a short time strengthens the possibility of spreading with natural ways and reveals the suspicion that it adapted to the Black Sea. Because it is known that adult individuals are very good swimmers so they can migrate new areas hundreds of kilometers away (Castriota et al., 2012).

Almost all records were caught by fishing gears as non-target and a research focus on this species was not conducted. Therefore, questions about the entrance of this species have to remain unanswered. Therefore, it is necessary to investigate the existence of juveniles and the possibility of reproduction of blue crab in the Black Sea by sampling with dives and traps in possible habitats.

Conclusion

The Black sea, which is poor in terms of species diversity, is very vulnerable to alien species. Due to its low salinity, species with less tolerance against environmental factors can settle in this region. Hence, measures must be taken and operated to prevent foreign species that can be transported by the commercial activities of ships from entering this region. Otherwise, much effort will have to be put to understand the effects of an invasion and to struggle with it.

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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.

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Marine Science and Technology Bulletin

SHORT COMMUNICATION

On maximum length record of the chub mackerel (*Scomber japonicus* Houttuyn, 1782) from Northern Aegean Sea (Turkey, eastern Mediterranean)

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ABSTRACT

The accurate estimates of the maximum length of fish within a population are significant issues. Because the parameters related to maximum length, weight and age of fish communities in the ecosystem are constantly used in population dynamics and stock estimation studies, recording of such data is vital for determining the life history of fish. In this connection, a single specimen of *Scomber japonicus* was captured off Kabatepe Bight (Gallipoli Peninsula) with handline by a commercial fisherman on 21 September 2013. This specimen was 370.00 g in total weight and 34.6 cm in total length (32.0 cm in fork length), corresponding to the second maximum size recorded for Turkish waters. This study aims to contribute to the scientific literature.

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Introduction

The chub mackerel (*Scomber japonicus* Houttuyn, 1782) is a cosmopolitan middle-sized species inhabiting the temperate and warm transition waters of the Mediterranean Sea, the Atlantic, Indian and Pacific oceans. It is a schooling and highly migratory species over the continental shelf, distributed from the surface to 300 m depth (Collette and Nauen, 1983).

Concerning Turkish waters, information on the biology of this species come from Marmara (Tuggac, 1957) and Black Seas (Atlı, 1959), Dardanelles (Özekinci et al., 2009), Izmir (Sever et al., 2006; Bayhan, 2007) and Saros Bays (Cengiz, 2012, 2021). In addition, Cengiz et al. (2013) reported the determination of hook selectivity for catching the chub mackerel.

Maximum length and weight are important parameters used in life history studies and fishery science. These measurements are applied directly or indirectly in most stock

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assessment models (Borges, 2001; Cengiz et al., 2019a). Therefore, it is important to regularly update the maximum size of commercially important species (Navarro et al., 2012; Cengiz et al., 2019b) such as the chub mackerel (Yu et al., 2018). In this study, the proved length of *Scomber japonicus* is second maximum size record for Turkish waters.

Material and Methods

A single specimen of *Scomber japonicus* was caught off Kabatepe Bight (Gallipoli Peninsula) (Figure 1) with handline by a commercial fisherman on 21 September 2013. Total length is defined as the measurement taken from the anterior-most part of the fish to the end of the caudal fin rays when compressed dorso-ventrally (Anderson and Gutreuter, 1983). Hereby, the specimen was subsequently measured to the nearest mm and weighted to the nearest g. Unfortunately, the specimen was not preserved as it was sold by a professional fisherman at the fish market.

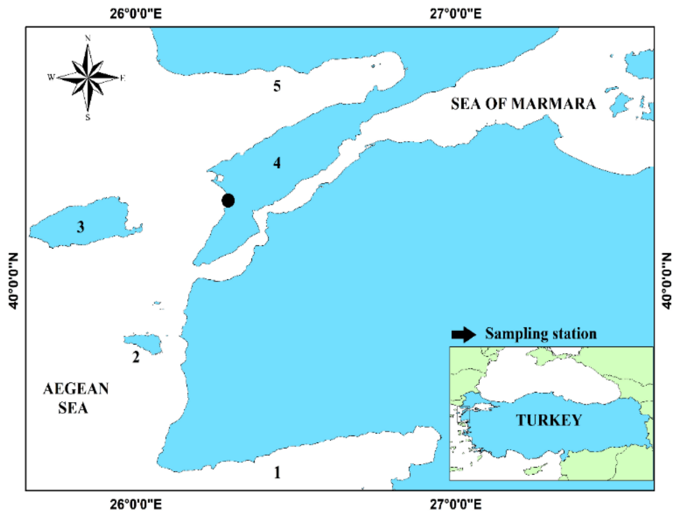

Figure 1. The Northern Aegean coasts of Turkey (1: Edremit Bay; 2: Bozcaada Isl.; 3. Gökçeada Isl.; 4: Gallipoli Peninsula; 5: Saros Bay) and sampling station

Table 1. The comparison of the maximum lengths and weights recorded for *S. japonicus* from different localities

Author(s)	Area	N	L_{max} (cm)	W_{max} (g)
Gonçalves et al. (1997)	South-west coast of Portugal	216	39.5	-
Kiparissis et al. (2000)	Cretan Sea (Greece)	-	31.0	-
Carvalho et al. (2002)*	Azores	349	53.0	2000.00
Santos et al. (2002)	Algarve coast (southern Portugal)	805	47.2	951.50
Sinovčić et al. (2004)	Adriatic Sea (Croatia)	1607	38.8	710.60
Perrotta et al. (2005)	NE Mediterranean	158	39.0	-
	SW Atlantic	392	45.0	-
Karakulak et al. (2006)	Gökçeada Isl. (Northern Aegean Sea)	25	31.2	-
Özaydın and Taşkavak (2006)*	İzmir Bay	129	26.0	157.00
Rosa et al. (2006)	Azores	167	53.0	2192.00
Bayhan (2007)*	İzmir Bay	520	27.2	-
İşmen et al. (2007)	Saros Bay (Northern Aegean Sea)	45	22.0	98.00
Sangun et al. (2007)	Kuzey Doğu Akdeniz	11	22.0	93.06
Gang et al. (2008)*	East Chine-Yellow Sea	352	41.1	-
Ceyhan et al. (2009)*a	Gökova Bay	16	33.0	-
Cengiz (2012)	Saros Bay (Northern Aegean Sea)	402	31.1	314.70
Cengiz (2013)	Gallipoli Peninsula (Northern Aegean Sea)	69	26.4	157.88
Cengiz et al. (2013)	Dardanelles & Gallipoli Peninsula (Northern Aegean Sea)	345	28.6	-
Bilge et al. (2014)	Southern Aegean Sea	31	18.8	-
Cengiz (2021)	Saros Bay (Northern Aegean Sea)	35	29.2	260.22
This study[b]	Gallipoli Peninsula (Northern Aegean Sea)	1	34.6 (FL = 32.0)	370.00

Note: L_{max}: maximum length, W_{max}: maximum weight, *N*: number of individuals,
*Fork length = FL;
[a] Maximum size record for Turkish waters
[b] Second maximum size record for Turkish waters

Results and Discussion

The captured chub mackerel was 34.6 cm in total length (32.0 cm in fork length) and 370.00 g in total weight (Figure 2). The comparison of the maximum lengths and weights recorded for *S. japonicus* from different localities is given in Table 1.

Figure 2. The chub mackerel with 34.6 cm in total length (32.0 cm in fork length) and 370.00 g in total weight

As well known, if a fish population in any ecosystem is exposed to overfishing, fish sizes will gradually be smaller over time (Cengiz, 2020). However, the one individual that subjected to no overfishing pressure could be reached that length (Filiz, 2011). On the other hand, any factor that might possibly influence growth has been shown to have an effect, including nutrient availability, feeding, light regime, oxygen, salinity, temperature, pollutants, current speed, nutrient concentration, predator density, intra-specific social interactions and genetics (Helfman et al., 2009; Acarli et al., 2018).

Conclusion

This work proves that *Scomber japonicus* can grow above the previous maximum data found in the Northern Aegean coasts of Turkey. The researchers involved in fisheries management should consider the paper results.

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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.

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RESEARCH ARTICLE

Factors affecting fish consumption of traditional subsistence fishers in Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Subsistence inland fisheries are underreported in developing countries like Pakistan. This study attempted to find out fish consumption determinants of traditional subsistence fishers in Charsadda district of Khyber Pakhtunkhwa (KP) Province of Pakistan. Data were collected between March and December 2019 through 36 predetermined questions applied to 286 randomly selected households. The data were analyzed through multiple linear regression model. Study found that average fish consumption of the sample households was 3.3 kg per capita per annum, which is higher than Pakistan's national average of 1.9 kg per capita per annum. The most viable reasons of fish consumption among the sample households were that most of them were; subsistence fishers, lived closed to water bodies and had easy access to fishing grounds. Majority of them consumed fish once a month in summer season but consumption increased in winter season. The regression results indicated that fish price, proximity to rivers, and family size have negative, whereas number of fishing equipment's, education and family income have positive effect on fish consumption. Actions are needed to improve fish production in local rivers through hatcheries development and aquaculture encouragement, so that fish meat become affordable to other areas located far away from water bodies.

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Introduction

Inland subsistence fisheries are vital as food and nutritional security in developing countries. It supports the prosperity of millions of poor families worldwide (Kawarazuka, 2010; Moreau and Garaway, 2018; Mohanty et al., 2019). Subsistence inland fisheries provides high quality cheap and accessible animal protein and nutrients (Bennett et al., 2018) for checking hunger and supporting rural development in food insecure communities (Corvalan et al., 2005; Kwasek et al., 2020). However, besides being vital to human nourishment, the per head fish intake in Pakistan is 1.9 kg (Baldwin and Hamstead, 2014) in contrast to the global average of 20.1 kg per capita (Belton et al., 2018). This low per capita fish intake in Pakistan becomes more critical when other means of protein are also inadequate. However, there is remarkable difference in per capita fish intake within Pakistan (Wasim, 2007). In 1975-1976, per capita per annum fish consumption was 0.04, 0.12, 4.25 and 6.80 kg in KP, Punjab, Sindh and Baluchistan provinces of Pakistan, respectively. It increased to 1.00 and 0.71 kg per capita in 2002-2003 in Punjab and KP. However, per head fish intake in the rest of the two provinces i.e. Sindh and Baluchistan further declined to 2.98 and 5.28 kg in 2002-2003, respectively (Wasim, 2007). Besides these variations in data regarding fish consumption, catch from inland fisheries is believed to be greatly underreported especially in the case of Pakistan. Pakistan produced an estimated 185,000 metric tonnes during 2018-2019 and 180,000 metric tonnes during 2017-2018 (Ministry of Finance, 2019). However, production and consumption of the traditional subsistence fishery workers is underreported and is not accounted towards Gross Domestic Product in Pakistan. On the other hand, the surge in global fish production during the last fifty years has improved people's capability to consume healthy and diverse foods (FAO, 2019). Advancement in technology, information and communication has also changed people's perception from traditional yields to essential and nutritious fish meat. But the remarkable differences in fish consumption in different regions making the job of protein provision to low income people in developing countries more challenging.

Numerous research investigations has been undertaken regarding the role of fish in the provision of high quality protein and nutritional security, to low income people particularly in less developed countries. Majority of research studies on fish consumption have based their investigation on the nutritional importance of fish meat (Belton et al., 2018; Bennett et al., 2018; Moreau and Garaway, 2018; Akuffo and Quagrainie, 2019; Mohanty et al., 2019; Kwasek et al., 2020). Some studies have investigated the impact of socioeconomic and demographical factors on people's fish intake (Oliveira et al., 2010; Perez-Cueto et al., 2011; Onurlubas, 2013; Can et al., 2015; Zhou et al., 2015; Kızıloğlu and Kızılaslan, 2016; Wenaty et al., 2018). There is tremendous research gap with respect to fish consumption pattern and preferences of subsistence fishers, especially in the case of Pakistan. Therefore, the basic objective of this investigation is to acquire information on the level of fish intake in this region and study the impact of socioeconomic and demographic features on fish consumption practices of households residing near the vicinity of rivers in district Charsadda, Pakistan. It is presumed that the results of this study will help decision makers regarding planning of nutrition policies for the poor segment of the society.

Material and Methods

Study Area

Charsadda District lies between 34° 3' to 34°28' North and 71° 28' to 71°53' East (Figure 1) with a total area of 996 km^2. It has extreme weather, and summer season continues from May to September. The monsoon period persists from July to September. The district has very old and comprehensive irrigation system and about 80 percent of the area is irrigated through canals. Farmers of Charsadda mostly grow wheat, barley, sugarcane, rice, maize and vegetables. A very distinctive feature of this area is the three major rivers flowing through this land, which has made its terrain very fertile. Agriculture accounts for the major source of employment for the people. Many people combine farming and fishery due to the seasonal character of fishing occupation. People residing near the vicinity of rivers carry out fishing throughout the year in this area. However, there are two key spells with abundant catch. The fish breeding season locally known as "*mainchal*" starts in February and continues till late April, attract large numbers of local people and fishermen (Qasim et al., 2019). The second spell starts in July and continues till October. The common fishing practices include use of hooks, spears, cast and drag nets, spears, and rods.

Data Collection

Primary data were gathered through household survey. In the beginning, a preliminary survey was carried out to investigate fishery related activities. The exploratory survey assisted us in the pre-testing of the questionnaire. After this initial survey, study sites were carefully selected. For the collection of needed information a questionnaire was developed. Considering the exploratory survey and local realities the questionnaire was modified. Prepared interview schedule with the households and female interviewers were recruited. Field observation were also undertaken in order to examine diverse fishery related events, including the use of variety of fishing devices, areas fished, meetings with fishery monitors and kinds and quantity of fish caught. Data so collected were analyzed through regression analysis and descriptive statistics. The data were analyzed using SPSS v20.0 and findings and conclusions were reached.

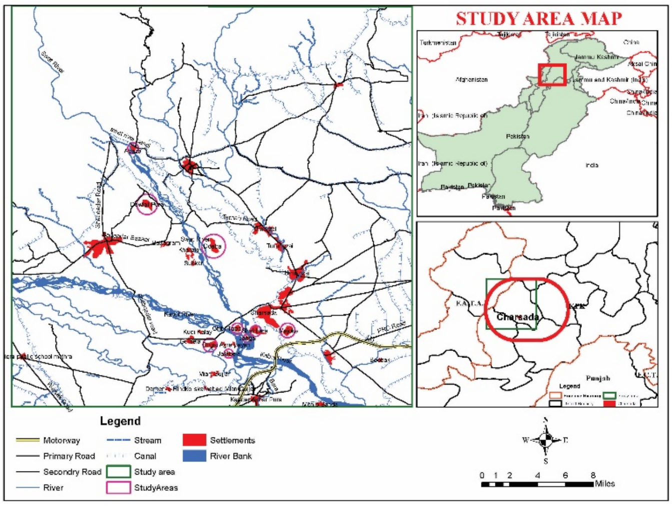

Figure 1. Study area

Sampling Techniques

Primary data were gathered between March and December 2019 through household survey questionnaire and observations. Data were collected from both male and female households. Female interviewers were recruited to collect data from female respondents while respecting cultural considerations. To select a representative sample of respondents two stage cluster sampling was used. Firstly, fishing communities with distinct fishing features, like nearness to water bodies, intensive fishing and presence of large number of fishermen labor were purposively selected. Secondly, sample households were selected from those fishing communities. Targeted villages near the vicinity of River *Swat*, *Kabul* and *Jindi* were selected. The population of Charsadda was 1,616,198 (GoP, 2017) as stated by the Population Census Organization of Pakistan. We used formula in the Eq. (1) suggested by Yamane (1967) for sample size calculation, where n is the sample size, N is population size and level of precision (e) is 6 percent, which presented a sample of 277. However, owing to calculation convenience 286 was taken as a sample size.

$$n = \frac{N}{1+N(e)^2} \tag{1}$$

Data Processing and Analysis

Fish consumption pattern and preferences are affected by socioeconomic, natural, physical and environmental condition of respondents. Many factors including family income, age, household size, access to market, price, health, gender, literacy, marital status, existence of children in a family, employment, consumption season, urbanization etc. have an impact on people's fish intake. However, data were collected from respondents residing proximate to rivers, having higher access to fishery resources than other people. Therefore, we assume that fish intake of current respondents may be higher than the per head fish consumption in Pakistan.

Considering respondents socioeconomic and attitudinal determinants, seven explanatory variables were carefully chosen for the multiple linear regression model;

$$FC = \alpha + \beta_1 Hi_1 + \beta_2 W_{b2} + \beta_3 Fp_3 + \beta_4 S_{h4} + \beta_5 Ag_5 + \beta_6 Ed_6 + \beta_7 Fg_7 + \varepsilon \tag{2}$$

Whereas *FC* is the outcome variable i.e. quantity of fish intake per year per household (kg), α stands for the intercept, and β's are the coefficients of the predictor variables. Hi_1 is the explanatory variable for income (in PKR.), W_{b2} for proximity to water sources (Km), Fp_3 for fish price per kg (PKR.), S_{h4} for family size (number), Ag_5 for respondent's age (years), Ed_6 for education and Fg_7 for number of fishing gears.

Results

Understanding socioeconomic features of the selected subsistence fishing households is necessary as it affect their fish consumption preferences. The overall socioeconomic features of the sample respondents are presented in Table 2.

The quantity of fish consumed show total quantity consumed per family per year. Therefore, to find out the average per capita fish consumption, we divide the total amount of fish consumed by average number of persons per family. This gave the value of 3.3 kg per head per annum, which is greater than 1.9 kg per capita, the national average for Pakistan.

Majority of respondents in the study area practice fishing as a subsistence activity, which plays an important role in their protein intake. About 34 percent (Figure 2) of respondents responded that they consume fish because they reside near the vicinity of rivers and fish for self-consumption. The reason for this high response is that most of them occasionally fish and using small and inefficient gears. Therefore, they catch such a small quantity which they cannot sell in the market. More than 20 percent replied that they consume fish as it is easily available. Lower price was not much important as only about 6 percent responded that they eat fish due to lower price. About 15 percent replied that they eat fish due to its availability in fresh form, whereas 13 consume fish due to being nutritious. Results revealed that 38.8 percent of the respondents eat fish once a month, 32 percent of them eat fish 2 to 3 times a month, 20.6%

Table 1. Justification of variables for fish consumption

Variables	Unit used	Effects on fish consumption	Sources
Household income	Amount in different currencies in different range	Family income affects fish consumption positively (+)	Ahmed et al. (1993) Burger et al. (1999) Barberger-Gateau et al. (2005) Onurlubas (2013) Can et al. (2015) Nguyen and Kinnucan (2018) Akuffo and Quagrainie (2019)
Proximity to water bodies	Scoring method	The lesser the distance the more the fish catch and consumption (-)	Tol (2006) Oliveira et al. (2010) Qasim et al. (2019)
Price	Price of fish per kg	A decrease in price, increase in fish intake (-)	Lebiedzińska et al. (2006) Herath and Radampola (2016)
Age	20-21 22-23 24-25 >25	Age affects fish consumption positively (+)	Watanabe et al. (2004) Kull et al. (2006) Perez-Cueto et al. (2011) Onurlubas (2013) Can et al. (2015)
Education	Different Levels or Uneducated Educated	Education and awareness increase fish consumption (+)	Myrland et al. (2000) Barberger-Gateau et al. (2005) Verbeke and Vackier (2005) Shimshack et al. (2007) Onurlubas (2013) Can et al. (2015)
Family size	Range of family members	Higher the family size higher the level of consumption (+)	Trondsen et al. (2003) Verbeke and Vackier (2005) Onurlubas (2013)
Fishing gears / technology	Number of fishing gears	Number of gears is positively associated with fish consumption (+)	Odada et al. (2004) Lampe et al. (2017)

Note: Source: Literature Survey, 2019

Table 2. Socioeconomic characteristics of respondents

S. No.	Respondents characteristics	Respondents (n=286)		
		Mean St. Deviation	t-test	p-value
1	Average total household income (Rs.)	26,3391±16,934	-5.44	0.000
2	Average family size (number)	9.42±4.43	-5.97	0.000
3	Average number of species caught	3.25±1.88	29.91	0.000
4	Average age of the respondent (years)	40.96±8.60		
5	Fishing experience (years)	19.47±7.06		
6	Average years of formal education	7.74±6.17		
7	No. of fishing gears	3.92±1.76		
8	Amount of fish consumed (Kg)	30.84±27.71		
9	Land holding size	4.6±1.8		

Note: 95% confidence level, Income is measured in PKR, average exchange for the period of January 2020 was approximately $1=PKR.150

four times a month and 8.03 percent more than four times a month. The Indus garua, locally known as Shermai (*Clupisoma naziri, Cluoisoma garua*), a famous indigenous fish of River Kabul is considered as the most delicious fish, however its production is insufficient to fulfill the rising demand. So, majority of people in KP choose to consume common carp (*Cyprinus carpio)*. Secondly, most plentiful and easily captured fish in the rivers of Charsadda is an inexpensive fish locally called "Marmahe", with the common name zig-zag eel (*Mastacembelus armatus*)**.** Due to the taste, and availability in local rivers, 17.80 percent of the selected households want to consume this fish (Figure 2).

Our results showed that six variables were correlated with the outcome variables (Table 3). Thus, the final regression was run with only six variables. Results also indicated that these six variables have high degree correlation with the outcome variable. Of the total six explanatory variables, three were negatively and three positively correlated with the outcome variable. The correlation between fish price, proximity to water sources and the dependent variable was highly negative. Whereas, the correlation between numbers of fishing gears, education and the dependent variable were positive. Family income was moderately correlated with fish consumption. Low correlation was found between family size and quantity of fish intake. A very low correlation was found between age of the respondent and the dependent variable, by reason of which this variable was not entered in the final regression.

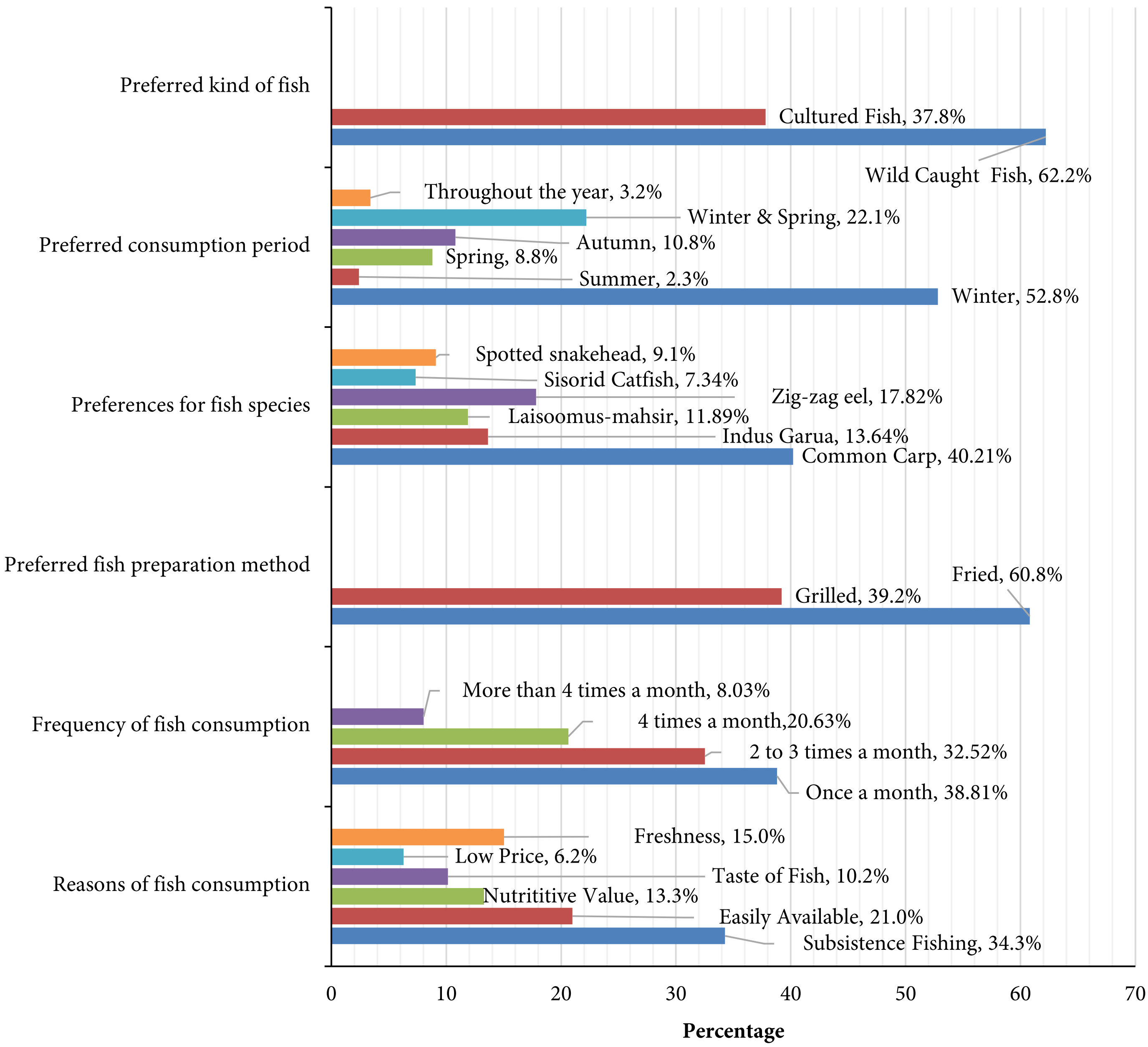

Figure 2. Reasons, frequency and preferences of fish consumption (Source: Field survey, 2019)

The Pearson correlation helped in the selection of variables for the final regression. Before running the regression, we checked the data for the assumptions of multiple linear regression i.e. the existence of linear relationship between dependent and explanatory variables using scatterplots. We checked correlation between independent variables using Variance Inflation Factor. The constant variance of residuals has been checked using scatterplot, the existence of autocorrelation has been checked using Durbin Watson test, and the normality of data has been checked using histograms with superimposed normal curve. Results revealed that all of the predictor variables have significant impact on the dependent variable. Table 4 indicated that about 66 percent of the variation in outcome variable was explained by predictor variables and is evident of R^2 values. Similarly, all the predictor variables have high degree of explanatory power on the dependent variable, evident from the adjusted R square value of more than 60 percent. Precision of the model predictions can similarly be seen from the lower standard errors of the regression. The F-ratio of the predictor variables indicates a better fit to the data model.

The regression output disclosed that of the total six explanatory variables three variables including, fish price, proximity to water sources, and family size have negative impact on the outcome variable. However, number of fishing gears, education and family income have positive impact on fish intake. The magnitude of "t" values also showed greater evidence of a significant difference. Overall, the predictor variables are helpful in explaining the variation in fish consumption, which is evident of the low standard errors.

Table 3. Correlation and other features of independent variables

Variable and description	Variable type	Mean	Pearson correlation value (r)
Fish price (in Kg)	Continuous	218.62±48.30	- 0.712**
Proximity to water bodies (Km)	-do-	3.02±3.20	-0.426**
Number of fishing gears	-do-	3.91±1.77	0.415**
Education (Years)	-do-	7.73±6.16	0.406**
Family size	-do-	9.41±4.42	- 0.277*
Family income (Rs.)	-do-	26,3390±16933	0.353*
Age (Years)	-do-	40.95±8.61	-0.023

Note: *, **, Correlation is significant by 99% & 95% confidence levels, respectively. Fish price was taken at the retail level rather than wholesale.

Table 4. Summary and ANOVA of the regression model

Summary of the Model

R	R^2	Adjusted R^2	Standard error of the estimate	Durbin Watson
0.814[a]	0.663	0.66	33.59	1.255

ANOVA of the Regression

Model	Sum of squares	Degree of freedom	Mean square	F ratio	Significance
Regression	620761	7	88680	78.550	0.000[a]
Residual	313850	278	1129		
Total	934612	285			

Note: a. Predictors: (Constant), Family Income, Proximity to water bodies (km), Number of family members, Education, Price of fish (kg), and Number of fishing gears: b. Dependent Variable, Quantity of fish intake

Table 5. Coefficients of the model explanatory variables

Coefficients	Unstandardized coefficients		Standardized coefficients	t	Significance
	B	Standard error			
(Constant)	183.44	14.39		12.74	0.000
Fish price (Kg)	-0.609	0.047	-0.514	-12.86	0.000
Proximity to water sources (Km)	-3.69	0.66	-0.205	-5.56	0.000
Fishing gears (Number)	2.542	1.39	0.109	2.55	0.011
Education (Years)	1.98	0.34	0.213	5.81	0.000
Family income (PKR)	2.990	0.00	0.095	2.46	0.015
Family size (Number)	-1.72	0.42	-0.133	-3.73	0.000

Discussion

Fisheries and aquaculture production has increased substantially over the last fifty years (FAO, 2016), which has boosted consumer's capability to eat healthy and diversified food worldwide. Though, per head fish intake is not the same worldwide. The results of this study revealed that average annual fish intake in district Charsadda was 3.3 kg per capita, which was higher than 1.9 kg per capita per annum i.e. the national average of Pakistan (Baldwin and Hamstead, 2014). However, this is much lower than the global average of 20.1 kg per capita per annum (Belton et al., 2018). This higher average annual fish consumption shows the importance of the nutritious fish meat in overall protein consumption and food security of traditional subsistence fishers in this region. This could be because of two major reasons; firstly, they live near the vicinity of water bodies and secondly, majority of them can fish which is also because of the first reason.

The study revealed that about 34 percent (Table 4) of respondents consume fish because they reside near the vicinity of rivers and fish for self-consumption. Similar results were recorded in a study conducted by Oliveira et al. (2010) to identify fish consumption of traditional subsistence villagers in Rio Madeira. Moreau and Garaway (2018) also reported that location and season affect fish consumption pattern, especially of poor consumers. This was also evident from the regression results (Table 5), showing a negative relation between proximity to water bodies and fish intake. However, most of them occasionally fish and use small and inefficient gears, due to which their catch was low. This low catch could also be the result of destructive fishing practices (Qasim et al., 2019). Another factor that has contributed to fish consumption was easy access and availability. About twenty one percent (Figure 2) replied that they consume fish as it was easily available. This ease of access to fisheries did not mean that it was easily available in the market but it mean that rivers are open access and they reside near water bodies, which enable them to fish whenever they need. This was also supported by the results indicating lower price as not an important factor in determining fish consumption because majority of them did not purchase fish in the market but consume the wild caught.

A study conducted by Lebiedzińska et al. (2006) reported that the most significant factors of consuming fish were taste and freshness. Herath and Radampola (2016) also find out positive relationship between nutritional value of fish and its consumption. Akuffo and Quagrainie (2019) revealed that fishing have positive effect on households' nutritional quality. However, results of the current study revealed that freshness and nutritional value of fish meat were not much important among subsistence fishers, as only 15 percent like to eat fish due to its freshness and 13 percent due to its nutritional value. This could be due to the low level of education and poverty of these subsistence fishers.

The study also discovered that family income have positive impact on fish intake. In a study carried out by Moreau and Garaway (2018), it was found that rich households consume larger quantities of fish than poor households. In another study, Verbeke and Vackier (2005) stated that poor income group have low fish consumption frequency. However, opposing results were found by Onurlubas (2013), showing a negative relationship between fish consumption and family income.

A study conducted by Herath and Radampola (2016), and Lebiedzińska et al. (2006) revealed that when fish price rise, consumers prefer not to eat fish. Results of the current study also show a negative relation between fish price and consumption, however being subsistence fishers' price was not much important in determining fish consumption.

Can et al. (2015) and Verbeke and Vackier (2005) investigated that higher education indicated higher level of fish consumption, which was confirmed by the current study. Lampe et al. (2017) and Odada et al. (2004) reported positive relationship between number of fishing gears and technology on fish consumption. Results of the current study have also revealed that the number of fishing gears, and fish consumption of subsistence fishers are positively related.

Conclusion

An inland fishery is an essential source of protein, nutrition and well-being for numerous people around the world. This paper is the first attempt to study determinants of fish consumption of traditional subsistence fishers in Charsadda district of Khyber Pakhtunkhwa (KP) Province of Pakistan. However, there is tremendous research gap and much needs to be explored in the future. Fish consumption of traditional subsistence fishers is more than the national average of Pakistan but much lower than the global average and needs to be improved. However, subsistence fishers shall be educated regarding the rational use of fisheries resources.

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Compliance with Ethical Standards

Authors' Contributions

MQ designed and wrote the first draft of the manuscript, SQ performed and managed statistical analysis, NN revised the 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.

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RESEARCH ARTICLE

Seasonal population patterns of *Holothuria Arenicola* Semper, 1868 from Karachi coast, Northern Arabian Sea

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ABSTRACT

The present study was carried out to assess the reproduction weight-length based growth and condition factor of *Holothuria arenicola* Semper, 1868, from January to December 2018, in Karachi coast, Northern Arabian Sea. The sex percentage was determined as 33.3% females and 66.7% males. Length of specimens ranged from 14 and 38 cm and weight of specimens ranged from 21 and 82 g. The relationship was $W = 2.408\ L^{0.9482}$ ($R^2 = 0.999$) for females, $W = 0.989\ L^{0.9482}$ ($R^2 = 0.998$) for males and $W = 1.234\ L^{1.1565}$ ($R^2 = 0.999$) for all individual. The condition factor ranged between 0.84 and 2.62 (mean: 1.52±0.70) in females and between 0.83 and 2.67 (mean: 1.43±0.71) in males. The gonadosomatic index of specimens ranged between 5.50 and 10.66 (mean: 7.95±1.24). The gutted weight of specimens ranged between 2.10 and 11.44 g (mean: 4.91±1.77). Studies about environmental variables and characteristics are necessary in order to complement the understanding of reproduction, the length-weight relationships and condition of sea cucumber in Karachi coast.

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Introduction

Holothuria arenicola Semper, 1868 commonly called the sand cucumber and been on the IUCN Red List (DD - Data Deficient) is commercially important (Conand and Gamboa, 2013). It distributed at some localities in the Western Pacific, including the Red Sea, parts of Asia, the Indian Ocean, the Comoros, along the Pacific coast of Central America, Caribbean and Brazil (Purcell et al., 2012). This species is found in substantially in coastal waters of Pakistan (Tahera and Tirmizi, 1995). Abundant in shallow areas but can also be found in deeper waters, under stones, in coral debris and on sand flats (Mosher, 1980).

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Relatively little information is available on the biology and ecology of these sea cucumbers belong to the class Holothuroidea in the northern Arabian Sea coasts of Pakistan. *H. arenicola* is also found from the Indo-Pacific to the tropical Western Atlantic. The species is reported from tropical Australia, Mozambique, the Red Sea, Ascension Island, Virgin Islands, Antigua, Barbados, Tobago, Belize, Bermuda, Tortugas, Jamaica, Puerto Rico, U.S. (Mosher, 1980).

Sea cucumber hunting is an important source of income for many other communities (Clarke, 2004). Although there are about 66 species of sea cucumbers in the world (Purcell, 2010), important biological parameters for most species are not known. One of these parameters is the allometric coefficient, which is obtained from length–weight relationships (Gerritsen and McGrath, 2007).

The length weight relation (LWR) is an important tool to estimate attributes of the population (Le Cren, 1951; Giacalone et al., 2010). In the literature, there are some studies about some parameters of sea cucumber such as length and weight (Kilada et al., 2000; Purcell and Tekanene, 2006; Herrero Pérezrul and Reyes-Bonilla, 2008; Kazanidis et al., 2010; Hannah et al., 2012; Siddique et al., 2014; Poot-Salazar et al., 2014; Natan et al., 2015; Aydin, 2016; Ram et al., 2016; Ahmed et al., 2016; Ahmed et al., 2018a, 2018b; Siddique and Ayub, 2019).

This study includes the length, weight, condition, gonadosomatic index, gutted weight parameters and length weight relations study on the population of *Holothuria arenicola* from Karachi coast of Pakistan.

Material and Methods

Sample Collection and Identification

A total of 81 *Holothuria arenicola* was collected from Buleji (Rocky Shore) (24°50’20.41” N, 66°49’24.15” E) of Karachi, Pakistan, the area has approximately eight hundred (800) meters long and one hundred fifty (150) meters wide shown in Figure 1 and Figure 2. Ten (10) surveys were conducted from January to December 2018 on seasonal basis, pre-monsoon (March to May), Southwest monsoon (June to September) post-monsoon (October and November) and Northeast monsoon (December to February).

Specimens were collected by hand and transferred to the laboratory in well aerated aquarium for taxonomic studies, morphological features and microscopic examinations were conducted under a microscope at 10×10 magnifications (Nikon LABOPHOT-2). Length (cm) and weight (g) of specimen were measured. Total length from mouth to anus was measured to the flexible ruler and wet weight, gutted weight was measured to the nearest 0.01 g immediately after removing the animal from the water to avoid evisceration.

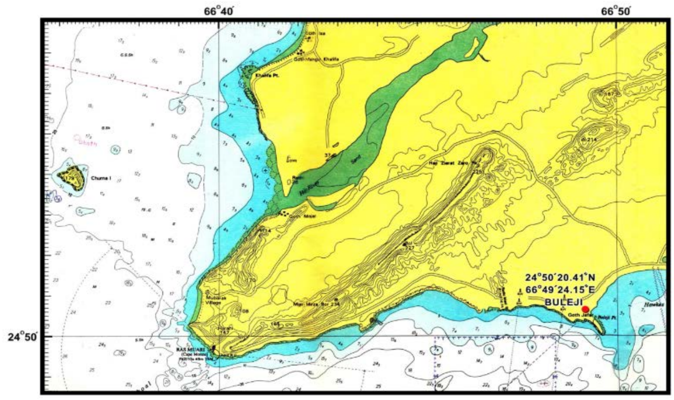

Figure 1. Study area map (Buleji) Karachi coast

Data Analysis

They were grouped according to their weight and length relationships (Chuqunova, 1963). The growth in length and weight were shown in absolute and relative growth parameters. The growth equation of the sea cucumbers equation 1 was derived from equation 2 (Le Cren, 1951).

$$W = a \times L^b \quad (1)$$

$$W = \log a + b \log L \quad (2)$$

Here, W represents the weight “a” and “b” are the coefficients of the logarithmic equation. Fulton’s condition factor is widely used in fisheries and general fish biology studies. This factor is calculated from the relationship between the weight of sea cucumbers and its length, with the intention of describing the “condition” of that individual. The formula is of the form:

$$C = \frac{W}{L^b} \quad (3)$$

where C is Fulton’s condition factor, b=1.1565 for males + females, b=0.9482 for females, and b=1.2214 for males. W is the weight of sea cucumbers, and L is the length. For calculating gonadosomatic index (GSI) the following formula was used:

$$GSI = \frac{Weight\ of\ ovary}{Weight} \times 100 \quad (3)$$

Statistical Analysis

Statistical analysis of data (difference between sexes, seasonal difference) was carried out using SPSS statistical package program for Mac Ver. 23.

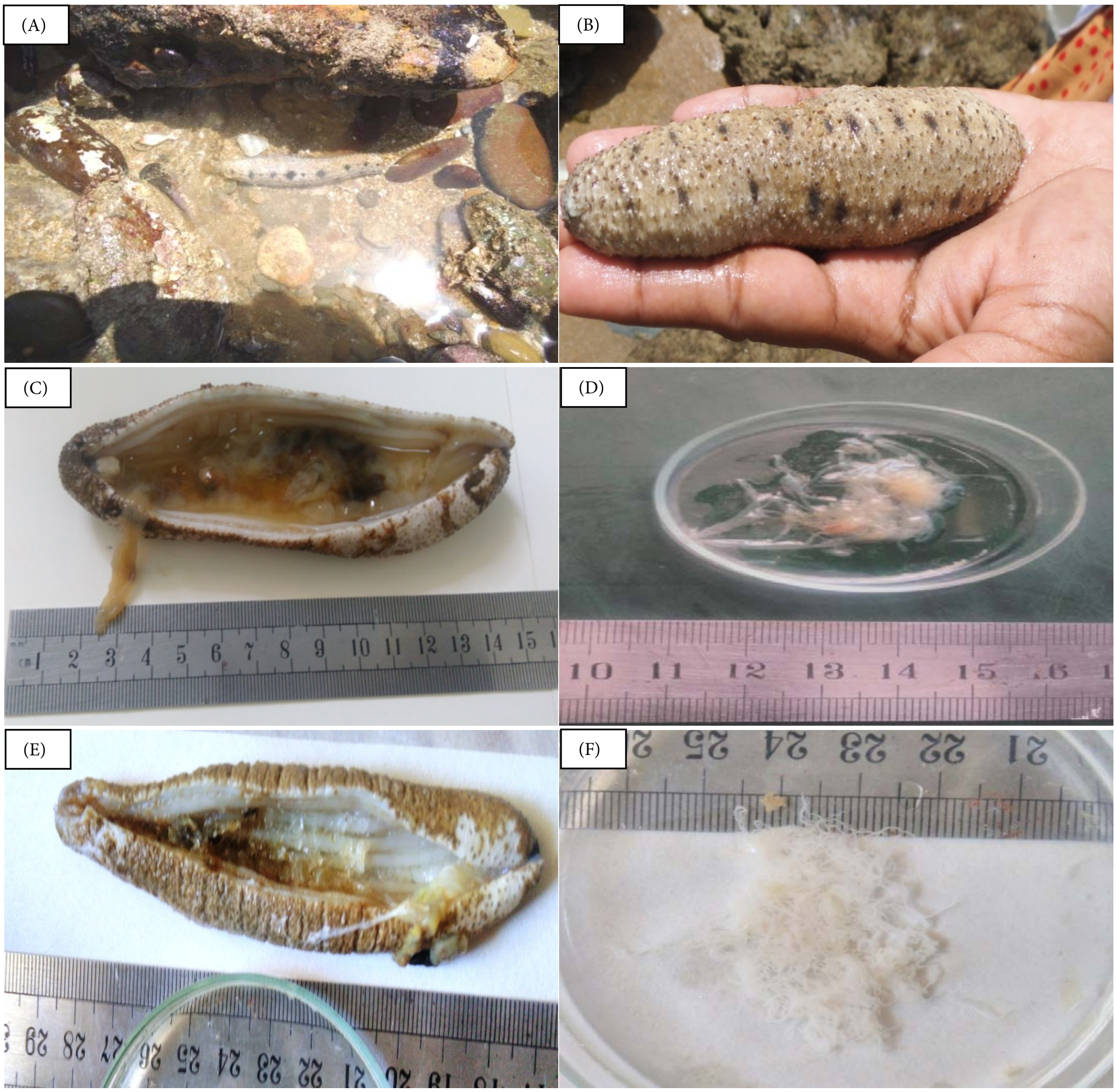

Figure 2. *Holothuria arenicola.* (A) *H. arenicola* with its habitat on Buleji coast, (B) *H. arenicola* dorsal view, (C) *H. arenicola* (female), (D) Gonads (female), (E) *H. arenicola* (Male), (F) Gonads (Male)

Results

There were about 33.33% females and 66.66% males (sex ratio 1:2). The sex distribution of all specimens examines are shown in Table 1. The lengths of specimens ranged between 14 and 38 cm (mean: 22.97±5.65). The weight of specimens varied between 21 and 82 g (mean: 46.40±9.69). The condition factor ranged between 0.84 and 2.62 (mean: 1.52 ±0.70) in females and between 0.83 and 2.67 (with average 1.43 ±0.71) in males. The gonadosomatic index (GSI) of specimens ranged between 5.50 and 10.66 (mean: 7.98±1.24). The gutted weight of specimens ranged between 2.10 and 11.44 g (mean: 4.91±1.77) (Table 1). The differences between the genders were insignificant in terms of length, weight, gonadosomatic index and gut weight in all season ($p>0.05$, ANOVA). The differences between the genders were found significant in terms of condition in all seasons ($p<0.05$, ANOVA).

Length–weight relationships were calculated using the data of all sea cucumber individuals. The relationship was $W = 2.40848216\ L^{0.9482}$ ($R^2 = 0.999$) for females, $W = 0.9893513\ L^{1.2214}$ ($R^2 = 0.998$) for males and $W = 1.23371441\ L^{1.1565}$ ($R^2 = 0.999$) for all individuals. This may be due the cylindrical shape of the animal (Conand, 1989) and it means also that length dose that not grows at the same rate of the weight. Length–weight curves for males and females are drawn in Figure 3.

Table 1. The distribution of sea cucumbers of season, sex, length, weight, condition, gonadosomatic index and gutted weight

Season	Sex	N	L (cm) min-max Average±SD	W (g) (min-max) Average±SD	C (min-max) Average±SD	GSI (min-max) Average±SD	GtW (min-max) Average±SD
Pre Monsoon	♀	5	17.50-27.50/21.20±4.12	36.00-58.00/42.60±9.04	2.40-2.61/2.48±0.08	6.21-8.61/7.66±1.08	3.00-4.40/3.82±0.51
	♂	6	15.50-24.00/20.17±2.99	23.00-52.00/40.50±9.65	0.84-1.07/1.02±0.09	6.83-9.23/7.91±0.94	2.50-4.80/3.78±0.78
	♀♂	11	15.00-27.00/20.58±3.30	23.00-58.00/41.46±8.97	0.84-2.61/1.68±0.07	6.31-9.23/7.79±0.89	2.50-4.80/3.80±0.64
Southwest Monsoon	♀	12	16.00-24.50/20.42±2.48	33.00-54.00/42.25±6.01	2.34-2.67/2.47±0.09	5.50-10.37/7.01±1.36	3.10-6.10/4.03±1.00
	♂	18	14.00-33.00/22.42±6.64	21.00-71.00/45.22±16.57	0.83-1.12/1.00±0.08	5.56-9.76/7.79±1.27	2.10-9.80/4.96±2.10
	♀♂	30	14.00-33.00/21.62±5.40	21.00-71.00/44.03±13.30	0.83-2.67/1.59±0.07	5.50-10.37/7.48±1.34	2.10-9.80/4.59±1.81
Post Monsoon	♀	4	17.50-29.00/22.95±5.44	36.00-54.00/45.70±8.65	2.27-2.53/2.42±0.11	7.07-9.26/7.98±0.95	3.80-5.70/4.55±0.81
	♂	15	19.30-32.00/25.88±5.14	41.00-67.00/52.53±9.55	0.89-1.10/1.00±0.08	6.34-10.66/8.57±1.40	3.40-7.80/5.80±1.55
	♀♂	19	17.50-32.00/25.26±5.20	36.00-67.00/51.11±9.56	0.89-2.53/1.30±0.60	6.34-10.66/8.45±1.33	3.40-7.80/5.54±1.50
Northeast Monsoon	♀	6	17.50-31.80/22.60±5.59	36.00-61.00/46.33±10.44	2.36-2.62/2.48±0.95	6.83-9.50/8.13±0.97	3.50-7.40/4.87±1.49
	♂	15	14.00-38.00/24.54±7.02	21.00-82.00/48.87±16.49	0.84-1.11/0.98±0.86	7.31-10.0/8.30±0.92	2.80-11.44/5.60±2.22
	♀♂	21	14.00-38.00/23.98±6.56	21.00-82.00/48.14±14.80	0.84-2.62/1.40±0.70	6.83-10.0/8.25±0.91	2.80-11.44/5.39±2.03
All Monsoon	♀	27	14.00-38.00/23.72±6.20	21.00-82.00/47.74±9.41	0.84-2.62/1.52±0.70	5.56-10.66/8.16±1.20	3.00-7.40/4.25±1.01
	♂	54	16.00-31.80/21.43±3.96	33.00-61.00/43.74±7.80	0.83-2.67/1.43±0.71	5.50-10.37/7.50±1.22	2.10-11.44/5.24±1.96
	♀♂	81	14.00-38.00/22.97±5.65	21.00-82.00/46.40±9.69	0.84-2.67/1.48±0.70	5.50-10.66/7.98±1.24	2.10-11.44/4.91±1.77

Note: GW: Gonad weight; gutted weight: GtW.

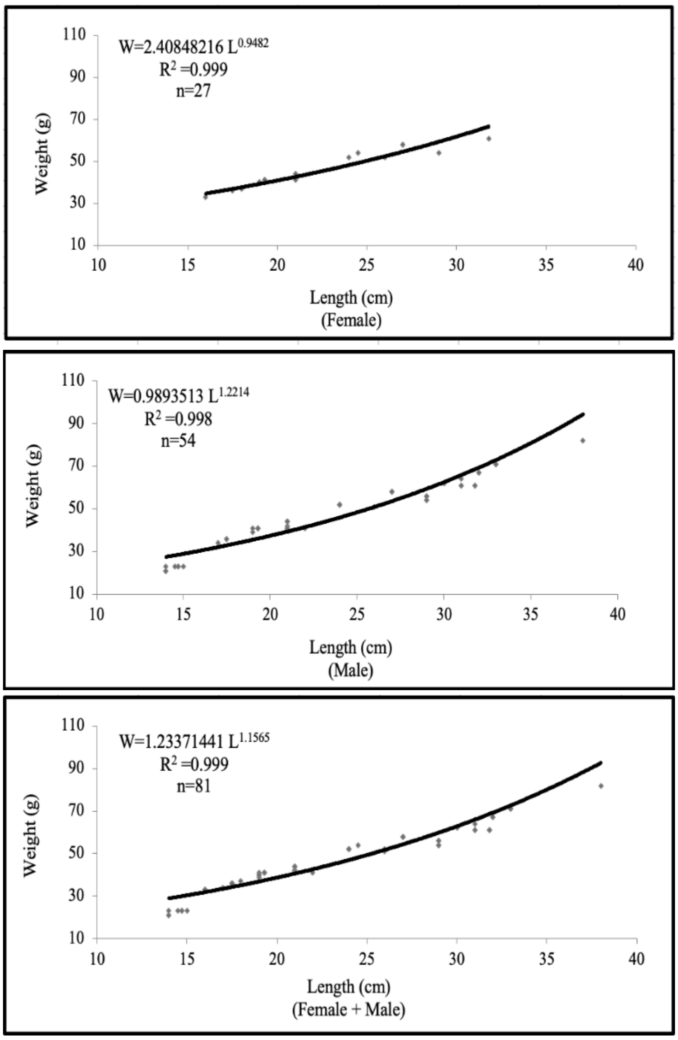

Figure 3. Length–weight relationships in female, male and all individual of sea cucumber

The condition factor and GSI distribution according to season and sex of sea cucumber are shown in Figure 4 and Figure 5, respectively.

The gutted weight–length and gutted weight–weight curves for females, males and all individuals according to sex of sea cucumber are drawn in Figure 6 and Figure 7, respectively.

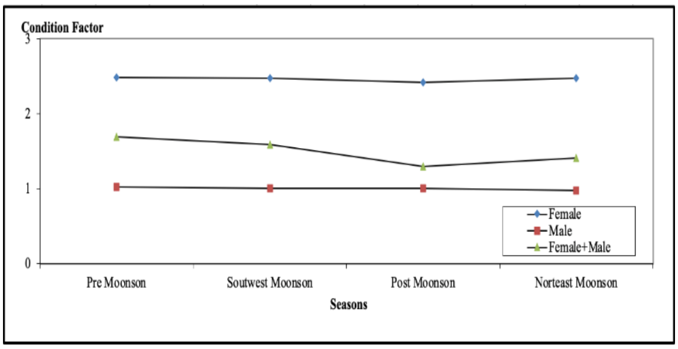

Figure 4. Condition factor according to season and sex of sea cucumber

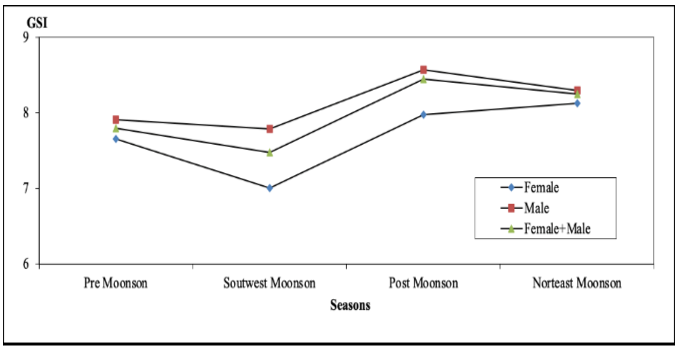

Figure 5. GSI according to season and sex of sea cucumber

Discussion

The comparison of the length weight relationships parameters, L_{max}, W_{max}, gutted weight (GtW), gonadosomatic index (GSI) and condition factor (C) of sea cucumber species showed in Table 2. The slope (b) of length weight relation values calculated for Buleji were higher than literature (Razek et al., 2010; Ahmed et al., 2018). The b value was lower than Siddique et al. (2014, 2015). The L_{max} value was lower than Ahmed et al. (2018) while W_{max} values was lower than Ahmed et al. (2018) and Siddique et al. (2014, 2015) (Table 2). The b values in fish is species specific and varies with sex, age, seasons, physiological conditions, growth increment and nutritional status of fish (Bagenal and Tesch, 1978). Variations in fish growth in terms of length and weight can be explained as an adaptive response to different ecological conditions (Balık et al., 2009).

In this latest study conducted in the Karachi coast, it was reported that sea cucumber species such as *H. atra* and *H. arenicola* show rapid and seasonal growth characteristics (Ahmed et al., 2020). Allometric growth has been determined when the data are examined. As a general assumption, species grow isometrically, but commercial sea cucumber does not show isometric growth. *H. arguinensis* allometric growth has also been reported (González-Wangüemert et al., 2016). Therefore, comparing growth rates from different commercial species should be carefully evaluated.

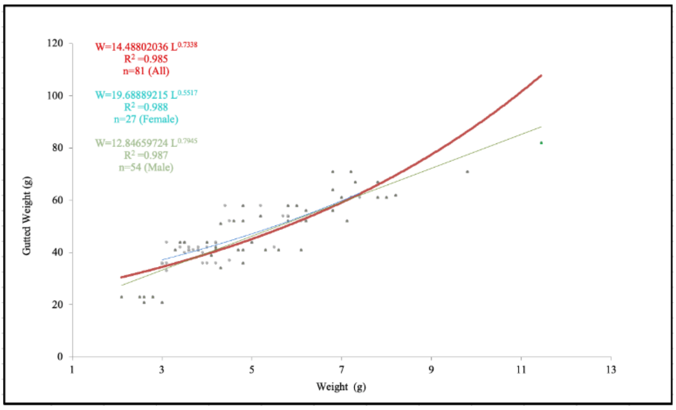

Figure 6. The gutted weight–length curves for females, males and all individuals

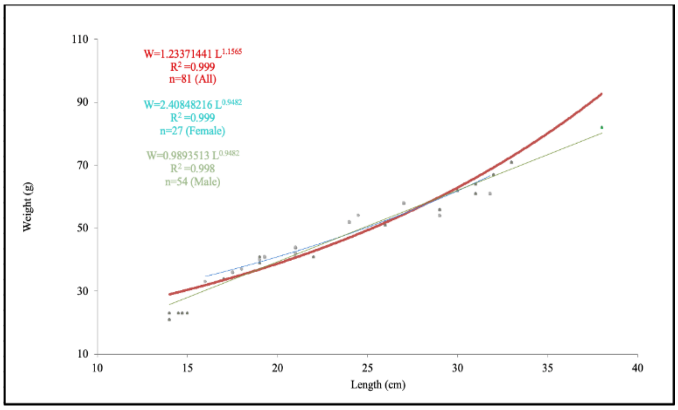

Figure 7. The gutted weight–weight curves for females, males and all individuals

Table 2. Comparison of sea cucumbers same parameters from different studies

Specimen	Sex	Location	a	b	R^2	Lmax (cm)	Wmax (g)	GtW	GSI	C	References
H. arenicola	♀♂	Manora	0.000	2.157	0.913	-	-	-	-	-	Siddique et al., 2014
	♀♂	Buleji	0.003	1.855	0.691	-	-	-	-	-	
H. arenicola	♀♂	Manora	0.001	2.137	0.886	41.6	375.0	21.70	8.80		Siddique et al., 2015
	♀♂	Buleji	0.003	1.813	0.687	37.6	352.0	12.50	6.30		
H. arenicola	♀♂	Sunehri/Buleji	0.760	1.370	0.880	48.0	136.0	-	-	-	Ahmed et al., 2018
H. arenicola	♀	Egypt	7.410	0.8473	0.9484	-	-	-	-	-	Razek et al., 2010
	♂		47.935	0.8372	0.8572	16.50	-	-	-	-	
H. arenicola	♀	Buleji	2.408	0.9482	0.999	38.00	82.00	4.25	8.16	1.52	This study
	♂		0.989	1.2214	0.998	31.80	61.00	5.24	7.52	1.43	
	♀♂		1.233	1.1565	0.999	38.00	82.00	4.91	7.95	1.48	

The mean values of length of sea cucumber of pre monsoon, southwest monsoon, post monsoon, northeast monsoon were 20.58±3.30 cm, 21.62±5.40 cm, 22.56±5.20 cm, and 23.98±6.56 cm, respectively. The mean of weight of sea cucumber of pre monsoon, southwest monsoon, northeast monsoon, post monsoon was 41.46±8.97 g, 44.03±13.30 g, 48.14±14.80 g, and 51.11±9.56 g, respectively. While the highest length average was observed in northeast monsoon, the highest weight average was determined in post monsoon. The lowest length and weight average were determined in pre monsoon (Table 1).

Gutted weight value of this study was lower than the value of Siddique et al. (2015). The results of gonadosomatic index value are in agreement with some earlier reports (Siddique et al., 2015). LWR and C parameters offer valuable information for understanding the biology and ecology of fish in terms of predicting the average weight corresponding to a known length group (Froese, 2006).

The lowest C value (1.30) was found in the post monsoon, while the largest C value (1.68) was found in the pre monsoon. The higher GSI value (8.45) was found in the post monsoon, while the lower GSI value (7.48) was seen in the South monsoon (Figure 4 and Figure 5).

Conclusion

This study provides knowledge on certain biological aspects of *Holothuria arenicola*, which can be utilized for effective management measures (monitoring and hunting) in order to avoid the over exploitation of this species. Studies about environmental variables and characteristics are necessary in order to complement the understanding of the length-weight relationships and condition of sea cucumber in Karachi coast.

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This study was written while at home quarantine during the COVID-19 pandemic.

Compliance with Ethical Standards

Authors' Contributions

QA, SB and QMA performed the research, analyzed the data and helped to draft the manuscript; QA and SB conceived and designed the work and wrote the manuscript. All authors contributed to 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.

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RESEARCH ARTICLE

Administration of different derivatives of *Oliveria decumbens* improves innate immunity of Nile tilapia (*Oreochromis niloticus*) without affecting fish growth and blood biochemical parameters

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ABSTRACT

The objectives of this study were to investigate the effects of *Oliveria decumbens* as a medicinal herb with reported immunostimulatory potential on the growth, immunity status and health of Nile tilapia (*Oreochromis niloticus*). In an eight- week trial, fish (45 ± 5 g) were randomly divided into 13 treatments as follows: in 10 treatments fish were fed on diets containing 0 (control), 0.01, 0.1 and 1% of *O. decumbens* extract and essential oil and their 1:1 combinations. Also, in three treatments fish received plant hydrolate at doses of 312.5, 625 and 1250 ppm as bath treatment. At the end of experiment, blood samples were taken for immunological and biochemical measurements. All treated fish showed comparable growth performance to those received control diet. The highest levels of respiratory burst activity were observed in extract 1% group. Similarly the serum lysozyme levels were significantly affected by essential oil and extract supplemented diets. Fish received plant hydrolate at dose 312.5 ppm showed the highest significant protein level. Also, the globulin levels were increased in group fed on diet containing extract 1%. Plant supplementation had no negative effects on the fish health biochemical indices including cholesterol, triglyceride, alkaline phosphatase and aminotransferase enzymes. In conclusion, the results of this study showed that application of *O. decumbens* derivatives improved the immunity of Nile tilapia without adverse effects on fish growth and health.

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Introduction

Variety of agents (viruses, bacteria, fungi and parasites) causes diseases in intensive aquaculture resulting in huge financial losses (Park and Choi, 2012; Dhayanithi et al., 2015). Antibiotics are commonly used in aquaculture industry to treat diseases, but they are criticized for the negative effects on fish and the environment. There has been a growing concern regarding antibiotic residue in human food and environment. Therefore, it is of great importance to find biodegradable, environmental friendly alternatives to replace antibiotics and other chemicals currently used in aquaculture sector for disease control (Tang et al., 2014).

Natural immunostimulants with the potentials to enhance the resistance against diseases by improving innate immune system are promising alternatives to antibiotics, vaccines and other artificial compounds (Divyagnaneswari et al., 2007; Van Hai, 2015). The effects of immunostimulants on aquatic organisms depend on multiple factors such as duration, dose and method of administration (Sakai, 1999; Divyagnaneswari et al., 2007). The immunostimulants usually use through oral, immersion or injection in aquatic animals (Sakai, 1999; Park and Choi, 2012).

It has been proven in previous studies that plant based immunostimulants improve the innate immune responses against bacterial, viral and parasitic diseases in freshwater and marine fishes and crustaceans (Tang et al., 2014). One of the most important advantages of using herbal immunostimulant in aquatic organisms is that they contain natural organic substances which do not harm fish, the environment or humans (Ardó et al., 2008; Van Hai, 2015; Brum et al., 2017).

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is the second major aquaculture species in the world and has been considered as the main candidate for developing aquaculture in unconventional water resources because of its tolerance to environmental changes (Grammer et al., 2012; Brum et al. 2017). Extensive studies have been carried out on the effects of medicinal plants' extracts and essential oils as immunostimulants in aquatic organisms (Harikrishnan et al., 2009; Zhang et al., 2009; Adel et al., 2015; Safari et al., 2016). Also, improving the immune status in tilapia fish using herbal products has already been reported (Divyagnaneswari et al., 2007; Park and Choi, 2012; Tang et al., 2014; Gabriel et al. 2015). *Oliveria decumbens* Vent (Apiaceae) is a native medicinal plant to Iran and adjacent countries. Effective antimicrobial properties have been traditionally considered for this plant and confirmed recently against different bacteria (Alizadeh Behbahani et al., 2018). Yet, there is no study available on the effects of *O. decumbens* on innate immunity of Nile tilapia. Incorporation of extract or essential oils in diet is the most common method for application of herbal products in fish (Awad and Awaad, 2017). Oral administration is the most common route to use herbal oils and extracts in aquaculture, although there are some reports on the bath treatment of fish with herbal oils and extracts (Yilmaz and Ergun, 2012), mostly for external pathogen treatments (Andrade et al., 2016; Cunha et al., 2018). Despite the wide use of plant hydrolate –an aqueous composition that obtained via distillation method during essential oil extraction and contained some polar compounds – as the most common derivative of medicinal plants in human, the effects of medicinal plants' hydrolate have not been investigated on aquatic animals, unless our most recent studies evaluating the antibacterial activity of *O. decumbens* hydrolate on Nile tilapia resistance against *Streptococcus iniae* (Vazirzadeh et al., 2019). The objectives of this study were to evaluate the effects of essential oil, extract and hydrolate of *O. decumbens* on the innate immune, growth performance and blood health biochemical parameters in Nile tilapia.

Material and Methods

Medicinal Plant

Plant was collected from Khonj, southern Iran and was authenticated by morphological features as *O. decumbens* by experts in medicinal plants at Horticulture department of Shiraz University. The aerial parts of the plant, including stems and flowers, were dried at room temperature with proper ventilation for 4 weeks, and kept in a dry and cool place till use.

Essential Oil and Hydrolate Extraction

The dried plant (20 g) was powdered and the essential oil and hydrolate were extracted simultaneously by a semi-industrial Clevenger (Namagol, Isfahan-Iran) with water (100 ml per each 20 g plant materials) and by steam distillation method (Acar et al., 2015). During distillation, steam supplied from a boiler was passed through the plant materials and removed the essential oil out. The steam was then cooled using a condenser and the essential oil (the upper phase lighter than water) was separated from aromatic water or hydrolate by using a separation funnel. Finally, each 20 g plant materials yielded to 1 g essential oil (equal to 5% of plant materials used) and 75 mL hydrolate. The essential oil was kept at -21ºC until use. The obtained hydrolate was kept away from sunlight in a closed container at room temperature until use (Karami et al., 2019).

Hydroethanolic Extract

Extract was prepared with maceration (soaking) method. In this procedure, 20 g herbal powder was mixed with 300 ml of water and ethanol (70%) with ratio of 1:15 (v:v). The mixture was stirred with a magnet for 1 h. After 72 h, the solvent was evaporated by rotary. The product was then dried and kept in refrigerator until use (Karami et al., 2019). The harvested rate of extract from plant materials was 20%.

Diet Preparation

Supplemented feeds were prepared according to Vazirzadeh et al. (2017). In order to prepare the experimental diets containing essential oil, the amount of feed per week was calculated for all treatments based on 3% body weight daily feeding. A formulated commercial feed (21 Beyza Mill Co, Shiraz, Iran) with 37% crude protein, 10% crude fat, less than 10% moisture and 4000 kcal/kg digestible energy in pellet form as basal diet was thoroughly mixed with 0.1, 1 and 10 g of essential oil dissolved in 30 g sunflower oil per kg of feed. For preparation of diets containing extract, 0.1, 1 and 10 g of extract was dissolved in 50 ml distilled water and sprayed on 1 kg of food. To prevent water leaching, all diets (including control diet without any additive) were coated by 50 ml/kg of 3% gelatin solution. The prepared diets were kept at 4°C until use.

Experimental Design

Tilapia (45±5 g) was procured from Yazd, Iran and transported to aquaculture facilities in Shiraz University. After two weeks of acclimation, the fish were divided into 13 treatments (in triplicates each of 20 individuals). Each replicate was kept in 75L glass aquarium. Treatments were as follows: fish at control group received only basal diet containing gelatin and oil (C); Extract groups fed on diet containing extract at 0.01, 0.1 and 1% (Ex); Essential oil groups fed on diet containing essential oil at 0.01, 0.1 and 1% (Es). In three treatments fish fed on diet containing Essential oil and Extract in 50:50 combination at doses 0.01, 0.1 and 1% (Es+Ex). In hydrolate groups, plant hydrolate was added to aquariums at doses of 312.5, 625 and 1250 ppm as bath treatment (Hy). In order to prevent the accumulation of hydrolate in the aquarium, after daily replacing of 50% water, half of the calculated doses were added to each aquarium.

Water Quality

The daily water change rate was 50%, which was done two times in the morning and evening. The water quality parameters including dissolved oxygen (5.5±0.4 g/L), temperature (24±2°C) and pH (7.5±0.2) were measured and maintained at optimal ranges for *O. niloticus*.

Growth Parameters

Growth indices were calculated using the following formulas:

$$WG\ (\%) = 100 \times \frac{Final\ fish\ weight - Initial\ fish\ weight}{Initial\ fish\ weight}$$

$$FCR = \frac{Feed\ intake}{Weight\ gain}$$

$$SGR = 100 \times \frac{\ln(final\ fish\ weight) - \ln(initial\ fish\ weight)}{Experimental\ days}$$

In these formulas, *WG* is the weight gain, *FCR* is the feed conversion ratio, and *SGR* is the special growth rate.

Sampling Method

Fish were sampled by day 60. Three fish per aquarium were taken. Fish were anesthetized with clove powder (150 g/l) (Vazirzadeh et al., 2019) and bled using non-heparinized syringes. Sera were separated by centrifuging (K241R, Centurion Scientific Ltd England) at 5000 rpm and 4°C for 20 min and kept at -21°C for further analyses.

Respiratory Burst Activity

This assay was carried out following the previous method (Siwicki, 1993). Briefly, 50 µl of fresh blood was mixed with 50 µl of 0.2% NBT solution and incubated for 30 min at 25 °C. Then, 50 µl of solution was mixed with 1 ml of dimethylformamide and centrifuged at 12000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm using a spectrophotometer (PG Instruments Ltd, UK). Dimethylformamide was used as blank.

Lysozyme Activity

The levels of lysozyme activity were measured using a turbidiometric assay according to Ross et al (Neil et al. 2000) with minor modification. To prepare a bacterium suspension, 9 mg of *Micrococcus luteus* cell wall were dissolved in 30 ml phosphate buffer (pH= 7.4). Then 90 µl of this suspension were mixed with 10 µl of fish serum and the absorbance was read at 450 nm using microplate reader (Bio Tek TS 800, USA) at times 0 and 10. One unit of activity was defined as 0.001 min^{-1} decrease in the mixture absorbance at 450 nm.

Blood Biochemical Parameters

Triglyceride (TG), cholesterol (Chol), alkaline phosphatase (ALP), alanine aminotransferase (SGOT), aspartate aminotransferase (SGPT), total protein (Tpr), and albumin (alb) levels were measured using commercial kits (Pars Azmun, Iran) following the manufacturer's protocol. The serum globulin (Glb) was estimated by subtracting the amounts of protein and albumin.

Statistical Analyses

This experiment was carried out as a completely randomized design. Data were analyzed by One- way ANOVA in SAS 9.1.4 software (SAS institute, NC). Before ANOVA analysis, the normality of data was checked by Shapiro-Wilk test. The mean squares of the treatments were compared by Tukey post-hoc test at the significant level of P <0.05. Data were presented as mean ± pooled standard error of mean (SEM).

Results

Growth Parameters

Table 1 shows the growth parameters of Nile tilapia after 60 days of treatment with different derivatives of *O. decumbens*. Although, there were some differences among treatments, the differences were not significant at P≤0.05 for WG%, SGR and FCR.

Table 1. Differences of growth parameters in Nile tilapia received different doses of essential oil, extract and hydrolate of *O. decumbens*

Treatments	Weight gain%	Growth parameters	
		SGR%	*FCR*
Control	86.18	3.08	0.97
Essential oil			
0.01%	83.14	3.02	0.81
0.1%	106.40	3.62	0.77
1%	90.88	3.23	0.85
Extract			
0.01%	139.88	4.35	0.71
0.1%	89.66	3.19	0.87
1%	122.71	3.96	0.77
Extract+ Essential oil			
0.01%	75.00	2.78	0.82
0.1%	133.37	4.23	0.60
1%	122.99	4.01	0.76
Hydrolate			
312.5 ppm	98.35	3.42	0.73
625 ppm	86.78	3.09	0.88
1250 ppm	79.19	2.92	0.90
P-Value	0.1119	0.1179	0.3356
Pooled sem	14.89	0.36	0.08

Note: Data are presented as means ± pooled S.E.M.

Immunological Parameters

Respiratory Burst Activity

The extract 1% group showed the highest level of respiratory burst activity, which was significantly different from control group at P≤0.05 (Figure 1).

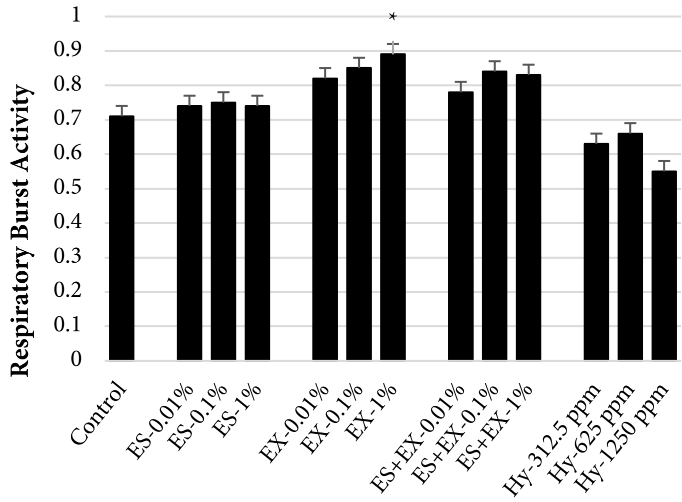

Figure 1. The effects of treatments (receiving different doses of essential oil, extract and hydrolate of *Oliveria decumbens*) on the respiratory burst activity (OD at 540 nm) in Nile tilapia. Treatments indicated by * are significantly different from control group at p ≤ 0.05. Es: essential oil; Ex: extract; Es +Ex; combination of essential oil and extract; Hy: hydrolate.

Lysozyme Activity

Different treatments significantly affected the lysozyme level in fish (P≤0.05). The level of lysozyme in fish received extract and essential oil or their combinations were significantly higher than that in control group (Figure 2).

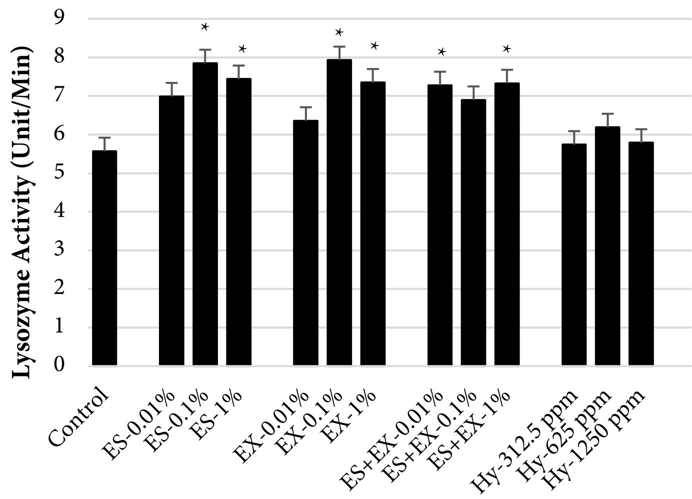

Figure 2. The effects of treatments (receiving different doses of essential oil, extract and hydrolate of *Oliveria decumbens*) on the lysozyme levels (U/min) in Nile tilapia. Treatments indicated by * are significantly different from control group at p ≤ 0.05. Es: essential oil; Ex: extract; Es +Ex; combination of essential oil and extract; Hy: hydrolate.

Total Ptotein (Tpr), Albumin (Alb) and Globulin (Glb)

The levels of Tpr, Alb and Glb are depicted in Figure 3. The highest level of Tpr (3.75 g / dl) was reported in hydrolate 312.5 ppm group, which was significantly different from control group. No significant differences were observed among treatments in case of Alb level. But, based on our findings, the effects of treatment were significant on the Glb levels (P≤0.05). The extract 1% receiving group had the highest level (2.7 g/dl) which was significantly higher than the control group (1.65 g/dl).

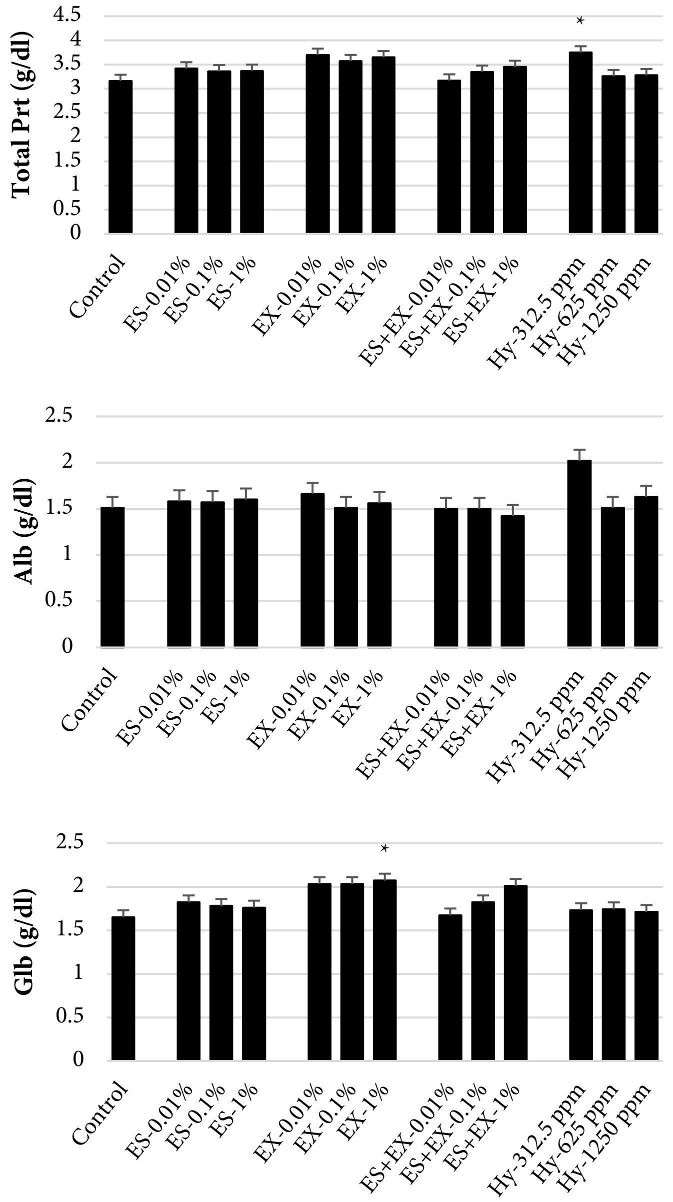

Figure 3. The effects of treatments (receiving different doses of essential oil, extract and hydrolate of *Oliveria decumbens*) on the serum total protein (g/dl), albumin (g/dl) and globulin levels (g/dl) in Nile tilapia. In case of serum total protein and globulin, treatments indicate by * symbol are significantly different from control group at P<0.05. No significant differences were observed for serum albumin levels. Es: essential oil; Ex: extract; Es +Ex; combination of essential oil and extract; Hy: hydrolate.

Triglyceride (TG) and Cholesterol (Chol)

The levels of TG and Chol are presented in Figure 4. Although, some fluctuations were observed among treatments, due to intra group individual variations, the differences were not significant at P≤0.05.

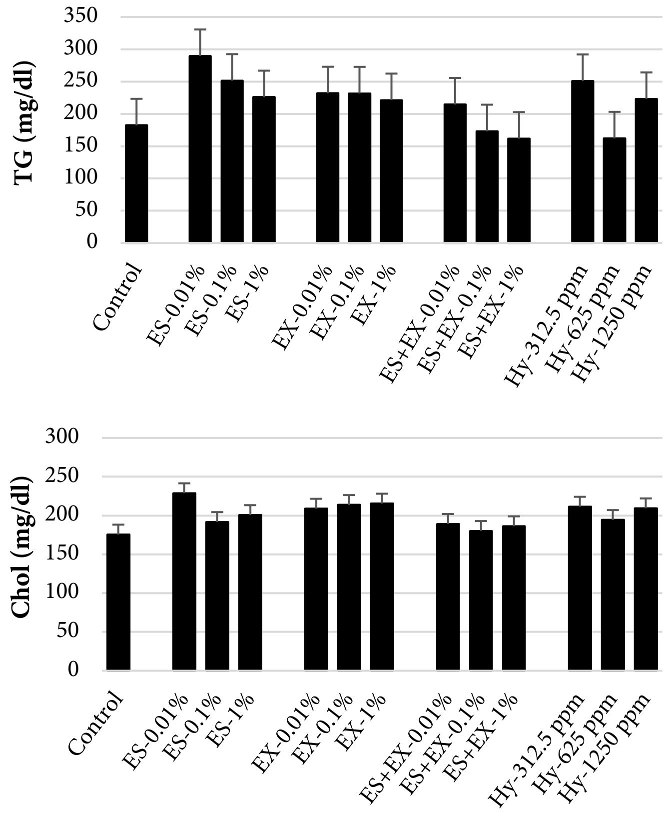

Figure 4. The effects of treatments (receiving different doses of essential oil, extract and hydrolate of *Oliveria decumbens*) on the serum triglyceride (g/dl) and cholesterol (g/dl) in Nile tilapia. No significant differences were observed among treatments (p > 0.05). Es: essential oil; Ex: extract; Es +Ex; combination of essential oil and extract; Hy: hydrolate.

Aspartate Aminotransferase (SGOT), Alanine Aminotransferase (SGPT) and Alkaline Phosphatase (Alp)

Figure 5 shows the results of changes in SGOT, SGPT and Alp levels in fish received different derivatives of *O. decumbens*. No significant differences were observed in either SGOT and SGPT values or Alp levels.

Discussion

Recently use of medicinal plants has emerged as an appropriate alternative to control diseases in aquatic organisms (Awad and Awaad, 2017; Wang et al., 2017) due to the adverse effects of chemicals and drugs used in aquaculture on fish as well as on the environment and human health.

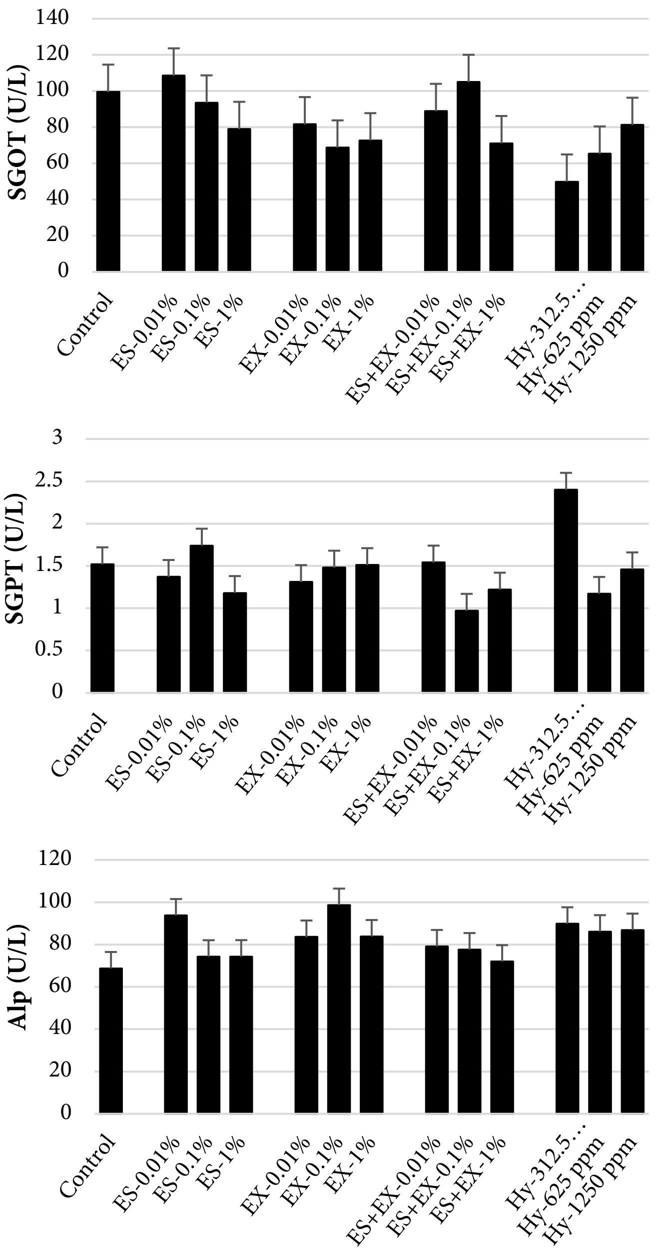

Figure 5. The effects of treatments (receiving different doses of essential oil, extract and hydrolate of *Oliveria decumbens*) on the serum alanine aminotransferase (U/l), aspartate aminotransferase (U/l) and alkaline phosphatase (U/l) levels in Nile tilapia. No significant differences were observed among treatments ($p > 0.05$). Es: essential oil; Ex: extract; Es +Ex; combination of essential oil and extract; Hy: hydrolate.

Extract, essential oil and hydrolate are the most common derivatives of medicinal plant used as traditional and green antimicrobial and immunomodulatory agents in humans and animals including fishes ((Esmaeili et al., 2018; Karami et al., 2019). In previous studies, extract and essential oils of a wide variety of plants were effectively used to improve immune responses in fishes (Ringø et al., 2012; Reverter et al., 2014). To the best of our knowledge, there was no study on the immunostimulatory effects of hydrolate of any plants in aquaculture. Therefore, the effects of hydrolate and two other common derivatives (essential oil and extract) of *O. decumbens* were evaluated on innate immunity of Nile tilapia in this study. The results of current study showed that treatment with extract, essential oil and hydrolate of *O. decumbens* had no adverse effects on the growth parameters of Nile tilapia. Similar finding has been reported in the use of different concentrations of *Myristica fragrans* on growth of juvenile greasy grouper (*Epinephelus tauvina*) (Sivaram et al., 2004). It has been reported that diets containing propolis did not affect the growth rate of gilthead seabream, *Sparus aurata* (Cuesta et al., 2005). Also, *Mucor circinelloides* diet did not make a difference in the growth of gilthead seabream in six weeks (Rodríguez et al., 2004). In another study, plantain (*Plantago asiatica*, fish mint (*Houttuynia cordata*) and field mint (*Mentha haplocalyx*) did not make a significant difference in growth factors of cobia (*Rachycentron canadum*) in comparison with that of the control group (Wu et al., 2016). Based on the present study, *O. decumbens* had no negative effects on growth parameters in fish. Therefore, *O. decumbens* can be safely used in tilapia aquaculture.

In this study, the high level of respiratory burst activity in the *O. decumbens* extract was indicative of innate immune response stimulation. It is believed that the respiratory burst activity inhibits pathogenic activity through producing toxic reactive oxygen species (Kumar et al., 2013). In line with our results, the respiratory burst activity increased in Nile tilapia fed with *Viscum album coloratum* plant extract (Park and Choi, 2012). Confirming to the results of our current study, an increase in respiratory burst activity was reported by diets containing 5 and 10% mangrove *Rhizophora apiculata* extract in clownfish *Amphiprion sebae* (Dhayanithi et al., 2015). Also, the extract of devil-pepper *Rauvolfia tetraphylla* at 5 and 10% resulted in increased respiratory burst activity in carp, *Labeo rohita* (Yogeshwari et al., 2015). Similar results were reported in the rainbow trout (*Oncorhynchus mykiss*) fed on 0.1 and 0.5 g/kg *Nigella sativa* (Celik Altunoglu et al., 2017). Overall, there was no direct positive correlation between the effects of immunostimulants and their doses. A higher dose of an immune stimulant may lead to the immune system suppression and therefore, deterioration of fish health status (Divyagnaneswari et al., 2007). Thus, it is likely that 1250 ppm dose of hydrolate has a negative effect on the immune system, and lowered the respiratory burst activity. Our finding was in line with the studies by (Giri et al., 2015) and (Gabriel et al., 2015) who reported the suppression of the immune system by high doses of *aloe vera* (4%) and *Pedalium murex* medicinal plant with the diet in several fishes.

As an important enzyme to cope with pathogens such as viruses, bacteria, and parasites, lysozyme was measured in tilapia in this study (Saurabh and Sahoo, 2008). It is well understood that the level of lysozyme elevates in serum during the infections (Celik Altunoglu et al., 2017). Lysozyme fights against bacteria by lysing their cell walls. According to the results of this present study, supplementing feed with extract and essential oil of *O. decumbens* led to higher levels of serum lysozyme in comparison to fish in control group. The increment in blood lysozyme levels is associated with a higher phagocytosis activity of leukocytes (Kumar et al. 2013). Altogether, these results are in line with previous findings suggesting the effectiveness of herbal immunostimulants in enhancement of blood lysozyme levels (Zhou et al., 2012; Kumar et al., 2013; Zanuzzo et al., 2015; Celik Altunoglu et al., 2017).

Total serum protein is one of the major factors in the innate immune responses of fish (Acar et al., 2015) and they are divided into albumin and globulin groups. Within the latter group, gamma globulins are the source of nearly all immunologically active proteins in the blood (Kumar et al., 2013). Increasing levels of protein, albumin, and globulin in the serum should lead to a stronger immune response in fish (Gabriel et al., 2015). Nile tilapia fed on *O. decumbens* for 60 days had the lowest level of protein in the control group and the highest for the hydrolate group at dose of 312.5 ppm. Several studies have reported the increase in serum total protein levels in fish after receiving herbal immunostimulants (Dügenci et al., 2003; Bilen et al., 2011; Awad and Awaad 2017). For example, feeding smoke tree *Cotinus coggyria* for 9 weeks increased the serum protein levels in rainbow trout compared to control group (Bilen et al., 2011). Also, the extract of loquat *Eriobotrya japonica* at doses of 1 and 2% increased serum total protein levels in longtooth grouper (*Epinephelus bruneus*) at the second week of treatment (Harikrishnan et al., 2011). Albumin is an essential component for maintaining osmotic pressure, the robustness of the immune system, and as a carrier in plasma (Talpur and Ikhwanuddin, 2012). In the present study, no significant differences were observed among serum albumin levels of treatments. Similar to our result, the use of lemon *citrus limon* essential oil in *O. mossambicus* did not have any effect on serum albumin levels (Baba et al., 2016). Also, common nettle *Urtica dioica urtica* powder at doses of 3, 6 and 12% did not alter the serum albumin levels in beluga sturgeon (*Huso huso*) after 8 weeks (Binaii et al., 2014). Also, in our previous study, the essential oil of *Ducrosia anethifolia* did not affect the amount of albumin in rainbow trout received three doses of 0.001, 0.01 and 0.1% (Vazirzadeh et al., 2017). Globulins are important proteins that play an essential role in the innate immune response against stress and infection (Talpur and Ikhwanuddin, 2012; Vazirzadeh et al., 2017). It also leads to a stronger immune response in fish (Binaii et al., 2014) and is essential for the stability of the immune system (Kumar et al., 2013). The results of this study showed an increase in globulin levels in 1% extract treatment compared to control group. In line with this study, an increase in serum globulin levels in rainbow trout has been reported after feeding common nettle (Binaii et al., 2014), fennel flower *Nigella sativa* and common nettle (Awad et al., 2013). Also, Serum globulin increased in *E. bruneus* received 1 and 2% of loquat extract, compared to control group (Harikrishnan et al., 2010).

Our previous study (Vazirzadeh et al., 2019) and earlier studies on the active compositions of *O. decumbens* by GC-MS spectrometry analysis showed γ-terpinene, myristicin, thymol, ρ-cymene and carvacrol as the most frequent compounds of plant (Amin et al., 2005; Hajimehdipoor et al., 2010; Esmaeili et al., 2018). All these compounds have significant immunostimulatory and antimicrobial activities which justify the immunostimulatory results obtained by present study. Although, the mechanism of immunostimulatory effects of plants have not been clearly discussed yet, it is believed that plants improve the innate immune system of fish via strengthening cellular and molecular defense system (Harikrishnan et al., 2010). Also, our previous study showed a remarkable *in vitro* antibacterial activity for essential oil and a significant *in vivo* resistance following challenge by *Streptococcosis iniae* in Nile tilapia for hydrolate at 312.5 ppm of *O. decumbens* (Vazirzadeh et al., 2019).

The results of this current study showed no significant differences in the levels of TG, Chol, and ALP in Nile tilapia fed on diets containing different compounds of *O. decumbens*. TG, Chol and ALP are main intravascular health indicators in vertebrates (Chatzifotis et al., 2011). Among them, Chol is one of the main components of cell membranes and precursors for steroid hormones, and bile acids and TG are stearic compounds of glycerol and fatty acids which have an important role in detecting and tracking lipoprotein disorders (Dadras et al., 2016). Measurement of ALP is an indicator for diagnosis of diseases and problems in bone, liver and gallbladder. High levels of ALP can be associated with disease (Wang and Sun 2016). Comparable to the results of this study, there are reports corroborating innocuousness of different herbal compounds on the level of aforementioned factors (Acar et al., 2015; Yeganeh et al., 2015; Baba et al. 2016). Thus, *O. decumbens* has no negative effects on liver and heart tissues and cardiovascular activity. The increment of the parameters over times have also no concerns because all fall in the normal range for fish under experimental condition and raise of the parameters relates to the age and weight of fish (Binaii et al., 2014; Reyes-Becerril et al., 2014).

SGOT and SGPT enzymes are important indicators for the diagnosis of hepatotoxicity in the pancreas (Dadras et al., 2016). Based on the results of this study, there was no significant difference in the level of hepatic enzyme of SGOT and SGPT in different treatments. Therefore, the findings indicated that the *O. decumbens* does not contain detectable toxic compounds with negative effects on the liver tissue and consequently on the activity of hepatic enzymes as confirmed by GC-Mass spectrometry analysis. In line with our results, in beluga sturgeon , SGOT and SGPT levels were not affected by the different levels of the nettle as immunostimulant (Binaii et al., 2014). One concern in using herbs or their derivative is the possibility of liver and tissue damages due to activity of toxic compounds (Dadras et al., 2016). For example, a study by (Vasudeva Rao et al., 2006) reported an increase in liver enzymes of *Labeo rohita* treated with diet containing *Achyranthes aspera* – an Indian medicinal plant- which indicates a negative effect of the plant on fish liver tissue. Therefore, evaluation of liver enzymes as indicators of fish hepatic health is necessary to ensure further about safety of using herbal compounds.

Conclusion

Overall, the results of this study showed that different derivatives of *O. decumbens* in the forms of essential oil, extract and hydrolate improved innate immune parameters status without any negative effects on growth and biochemical parameters. The possibility to use hydrolate form of herbal compounds as an immunostimulant in fish was also confirmed for the first time in this study. The use of different compounds of *O. decumbens* as an environment-friendly immunostimulant to improve the innate immune system and to cope with diseases is recommended in Nile tilapia aquaculture.

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Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Compliance with Ethical Standards

Authors' Contributions

AV conceived and designed the experiment. SJ and AV prepared the diets, performed the trial and collected the experiments data. SJ and AV carried out all immunological and other required analyses. MA carried out biochemical analyses. AK provided herbal composition analyses. AV and SJ analyzed and interpret the data. AV wrote the draft of the manuscript. All authors critically reviewed the manuscript for intellectual content and gave final approval for the version to be published.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The experiments and fish handling were conducted based on the Institutional Animal Care and Ethics Committee of the Shiraz University regulations with minimal suffering of experimental animals.

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RESEARCH ARTICLE

Determination of spatial and temporal changes in surface water quality of Filyos River (Turkey) using principal component analysis and cluster analysis

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ABSTRACT

Monitoring water quality is one of the high priorities for the protection of water resources. Many different approaches are used to analyse and interpret the variables that determine the variance of water quality observed in various sources. Statistical methods, especially multivariate statistical techniques, constitute an important part of these approaches. In this study, ten water quality parameters, which were measured for twelve months from seven stations determined on Filyos River, were evaluated by carrying out principal component analysis (PCA) and cluster analysis (CA) from multivariate statistical methods. In addition, dominant quality parameters designating the quality of the water source were determined. According to PCA results, 4 principal components contained the key variables and accounted for 69.49% of total variance of surface water quality from Filyos River. Dominant water quality parameters were observed to be temperature, EC, DO and pH. While the study revealed that the river is exposed to agricultural pollution alongside with the water quality character generated by the climatic conditions, it also suggested that multivariate statistical methods are useful tools in evaluating complex data sets such as water quality data, and monitoring the quality of water resources.

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Introduction

Monitoring water quality is one of the highest priorities of environmental conservation policy (Simeonov et al., 2002). Supplying water of high quality for purposes such as irrigation, drinking water, etc., and controlling and minimising problems caused by pollution are the principal objectives. Thus, nowadays, surface water quality constitutes one of the most significant determinants in resource management.

For this reason, monitoring water quality is a must in terms of water source management. Moreover, employing accurate methods in monitoring quality parameters is just as important.

Water quality can be defined as characterization of some parameters which represent a water composition in a specific place and time. Raw data are usually vast, meaning mostly they will not be distributed normally, they will be auto correlated or co-linear. Thus, multivariate analysis methods such as discriminant analysis, factor analysis, cluster analysis and principal component analysis are widely used in understanding spatial and temporal dissimilarities in water quality (Zeng and Rasmussen, 2005; Shrestha and Kazama, 2007)

Principal component analysis (PCA) is a data analysis method that is often used to decrease the number of variables of a large number of interrelated variables and also to keep as much variation (information) as possible. PCA is used to calculate an uncorrelated set of variables (pc's or factors). These factors are put in an order so that most of the variations existing in original variables are retained.

Cluster analysis, on the other hand, is used to group objects within a class according to their similarities, and among different classes according to their dissimilarities (Panda et al. 2006). These similarities and dissimilarities are determined based on Euclidean and Manhattan distance measures (Kaufman and Rousseeuw, 1990).

Based on the fundamentals discussed above, in this study, we implemented principal component analysis (PCA) and cluster analysis for identifying practical pollution indicators in order to reveal agricultural and domestic pollution in Filyos River located in Western Black Sea Basin, Turkey. In addition, we also intended to provide a basis for future work on developing realistic tools that could help local decision-makers on the suitable management of the surface water quality in the river basin.

Material and Methods

Study Area

Filyos River is located on the southern west coast of Black Sea, Turkey. As shown in Figure 1, it flows through West Black Sea River Basin and disembogues into Black Sea at Filyos district of Zonguldak province. It has a drainage area of 13300 km^2, has two main branches: Yenice and Devrek streams, and has a length of 312 km (Kucukali, 2008;Sönmez et al., 2018). Gökçebey and Çaycuma districts are located by the river and their populations are on the rise. Araç and Gerede streams are among its main branches. Recent industrial investments in the region such as paper and cement factories has changed economic structure while, economy of the local community was depending on forestry and agriculture formerly (Seker et al., 2005). Sönmez and Kale (2020) reported that the annual streamflow of the river tended to decrease particularly caused by climatic changes.

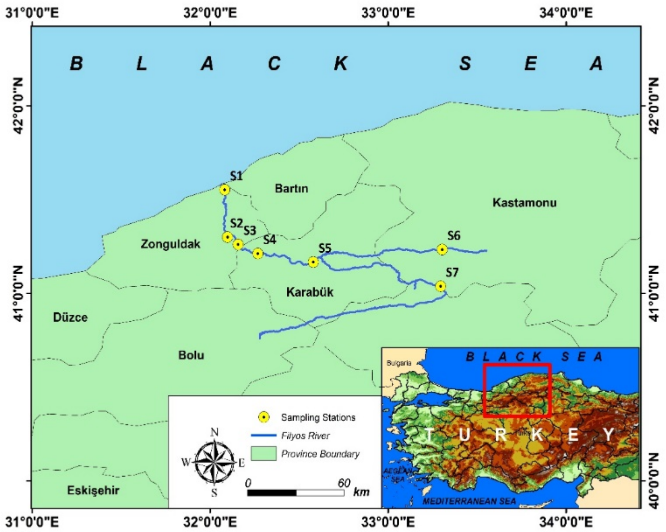

Figure 1. Sampling stations and study area

Delta of the river is wilderness and terrestrial ecosystem is quite rich and diverse as a consequence of topographical features of the region in which the river is located (Kucukali, 2014). Locations of the stations are indicated on the map (Figure 1) and a handheld GPS device was used in obtaining coordinates (Table 1).

Sampling Design and Collection

Samplings were carried out in duplicate and monthly from seven stations on Filyos River, Turkey between 2014 December and 2015 December. Samples were collected by using Nansen bottle and filtered through membrane filter with a 0.45 µm pore size, and stored in polyethylene bottles. Both polyethylene and Nansen bottles were rinsed with ambience water beforehand (Alam et al., 2001). Dissolved oxygen, turbidity, conductivity, pH and temperature parameters were measured with multiparameter *in situ* during sampling. On the other hand, spectrophotometric techniques were used while determining COD, BOD, phosphate, nitrite, nitrate and ammonium parameters in the laboratory (APHA, 2012).

Principal Component Analysis (PCA)

Principal component analysis (PCA) method explains the variance structure expressed by variables via correlations with new variables that are the components of original variables and are not intercorrelated. The number of base components is either equal to the number of original variables or less. When principal components are present, correlation matrix or variance-covariance matrix of the original variables is used. The basic components help analysing the dimension and deducing. Basic components are calculated based on the given formula in Equation 1 (Kuo et al. 2008; Mehat et al., 2014).

$$Y_{mk} = \sum_{i=1}^{n} X_m(i).V_{ik} \quad (1)$$

Contribution of chemical and physical characteristics factor related to the base components are calculated by the square of the eigenvalue vectors. Weight values (wk) of the chemical characteristic variables are expressed as mean of the contributions of chemical characteristic factors acquired by squaring each eigenvalue vector (Mehat et al., 2014).

Cluster Analysis

Cluster analysis (CA) is used for exposition of multidimensional and large datasets as it is often seen in environmental data (Cieszynska et al., 2012). CA is a useful tool for grouping water samples resulting in high external (between clusters) heterogeneity and high interval (within clusters) homogeneity (Shrestha and Kazama, 2007). A widely used approach in analysing similarity between dataset and the sample is hierarchical agglomerative clustering (McKenna, 2003). Euclidean distance is a distance coefficient that is used to determine the similarity between two samples and a distance which can be represented by the difference between analytical values from both of the samples (Otto, 1998). Cluster analysis result is often presented as a tree like diagram (dendogram), that demonstrates the summary of clustering procedure with a considerable reduction in dimensionality of original data (Shrestha and Kazama, 2007).

In our study, hierarchical agglomerative CA was used on normalized data set through Ward's method, and measure of similarity was Euclidean distances.

Results and Discussion

Descriptive Statistics and Correlations

Basic statistics were performed in order to give initial information related to water quality data. Details of the descriptive statistics of the water quality variables measured in twelve months are presented in Table 1.

Table 1. Descriptive statistics of study area

Variables	Mean	Median	Mode	Std. Deviation	Minimum	Maximum
pH	8.54	8.5	8.44	0.17	7.88	9
DO	9.57	9.59	8.21	1.17	7.73	11.83
EC	452.8	439.12	412.5	110.99	203.12	761
Temperature	14.71	13.38	6.97	6.19	4.85	26.02
Nitrite	0.06	0.02	0.01	0.1	0.01	0.75
Nitrate	0.72	0.6	0.3	0.62	0	4.95
Phosphate	0.8	0.43	0.26	0.8	0.04	2.92
Ammonium	0.38	0.17	0.02	0.62	0.02	3.2
COD	9.14	8.21	1.79	5.45	1.79	25.31
BOD	10.69	10.21	8.89	5.15	0.5	21.24

Descriptive statistics indicate that most of the parameters have high standard deviation and high change interval. Therefore, it we can say that water quality in Filyos River has temporal and spatial dependence due to ongoing natural and anthropogenic processes in the basin (Gonzalez et al., 2014). When average values of water quality parameters compared with Water Pollution Control Regulation of Turkey, it is found that Filyos River partially suffers from oPO_4^{3-} , NH_4^+ and NO_2^- pollution. This situation indicates that Filyos River has high organic pollution since nitrogen compounds in surface waters are usually related to organic pollution (Yang et al., 2007). Also, severe oPO_4^{3-} pollution shows the impact of agricultural and domestic effluents (Wu, 2005).

According to correlation coefficients (Table 2), there were direct and statistically significant correlations between DO and BOD (0.515), EC and Ammonium (0.471), Nitrite and Nitrate (0.358), Phosphate and BOD (0.351), and Temperature and Nitrite (0.282). We can say that an increase in any of these variables positively affects the other. On the other hand, negative significant correlations were found between temperature and DO (-0.915), Temperature and BOD (-0.600), Nitrite and DO (-0.328), Ph and BOD (-0.283), and

Ammonium and BOD (-0.218). It was shown in many studies that the DO level in water is inversely proportional to the temperature (Sönmez et al., 2008; Wang et al., 2013). There are also various studies which reported similar results regarding correlations with other parameters (Özgüler, 2001; Boyacıoğlu et al., 2005; Ustaoğlu and Tepe, 2018).

Table 2. Correlations between dependent variables

Variables	pH	DO	EC	Temperature	Nitrite	Nitrate	Phosphate	Ammonium	COD	BOD
pH	1	-.082	-.048	.141	-.114	-.105	-.153	.091	-.163	-.283**
DO		1	-.073	-.915**	-.328**	-.039	.013	-.073	-.049	.515**
EC			1	.070	.214	.176	-.052	.471**	-.021	-.108
Temperature				1	.282**	.012	-.062	.056	.073	-.600**
Nitrite					1	.358**	-.052	.209	-.146	-.257*
Nitrate						1	.137	.005	-.063	.040
Phosphate							1	-.185	-.111	.351**
Ammonium								1	-.166	-.218*
COD									1	.108
BOD										1

Table 3. Principal component analysis results

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	2.717	27.174	27.174	2.717	27.174	27.174	2.606
2	1.627	16.271	43.445	1.627	16.271	43.445	1.778
3	1.461	14.609	58.054	1.461	14.609	58.054	1.514
4	1.144	11.439	69.493	1.144	11.439	69.493	1.275
5	.899	8.991	78.484				
6	.765	7.653	86.137				
7	.502	5.022	91.159				
8	.447	4.470	95.629				
9	.361	3.612	99.242				
10	.076	.758	100.000				

Principal Component Analysis

Eigenvalues of each principal component are given in Table 3. The components with an eigenvalue greater than 1 were considered as significant. Eigenvalues of principal components were found to be 2.717, 1.627, 1.461, 1.144, 0.899, 0.765, 0.502, 0.447, 0.361 and 0.076, respectively. First four components explained 69.49% of total variation in the data set while, 27.174% of total variance was explained by the first factor; 16.271% by the second factor; 14.609% by the third factor; and 11.439% by the fourth factor. Remaining six components were found to explain 30.51% of total variation, while these were found to be insignificant.

Contribution of the chemical characteristics corresponding to the principal components is shown by the eigenvalue vectors. Contributions of the chemical characters calculated by squaring each eigenvalue vector are given in Table 4. For instance, the contribution weight of water parameters was 0.973 for the first, -0.950 for the second, 0.786 for the third, and -0,632 for the fourth component. In our study, first four main components explained 69.49% of the total variance. An eigenvalue provides information on measure of the significance of a factor. In addition, eigenvalues greater than 1 are accepted as significant (Kim and Mueller, 1987; Muangthong and Shrestha, 2015). According to Liu et al. (2003), loadings greater than 0.75 are considered as strong, loadings between 0.50-0.75 as moderate and loadings smaller than 0.30-0.50 as weak.

Table 4. Eigenvectors of the principal component analysis and contribution of each individual chemical characteristic for the principal component

Parameters	Component			
	1	2	3	4
pH			-.373	-.632
DO	-.950			
EC		.786		
Temperature	.973			
Nitrite	.372	.321	.543	
Nitrate			.711	
Phosphate		-.442	.611	
Ammonium		.813		
COD			-.311	.850
BOD	-.702			

In the first component, the negative correlation of temperature with DO and BOD points out the natural process (Kükrer and Mutlu, 2019). Temperature values are inversely proportional to the solubility of oxygen in the water (Shrestha and Kazama, 2007; Atea, et al., 2017; Abdelali et al., 2018).

The second component constitutes 16.27% of the total variance. In this component, EC and Ammonium were found to have a strong positive correlation. EC values reveals the presence of electrolytic contaminants and dissolved salts. However, it does not give information regarding the specific ion composition (Adekunle et al., 2007). Positive strong ammonium value revealed the presence of nutrient pollution caused by agricultural effluent (Sing et al., 2005). Positive values of Nitrate, Nitrite and Phosphate in the third component also showed the effect of agricultural activities on the water source. In the fourth component, pH exhibited negative results while, COD exhibited positive results. Mostly, biological and chemical reactions depend on pH value. In addition, it determines the metal ion solubility thus, effecting aquatic natural life (Hamed, 2019).

Figure 2 shows the graphical spatial representation of provided factors for chemical parameters. In this graph, the grouping of parameters and their correlation with the maintained factors can be observed.

Cluster Analysis

K-means algorithm is implemented in order to procure generalized cluster characteristics by using dominant parameters according to optimum number of clusters that is determined by FCM in the previous step. First, medians of clusters are found, thereafter the clusters are formed by assigning each object from the dataset to the nearest cluster medians. Dissimilarities of each object in the dataset from these centers of the clusters are evaluated by Euclidean distance. Cluster centers are chosen according to minimum distance. For validation and interpretation of clusters, silhouette is used (Kaufman and Rousseeuw, 1990).

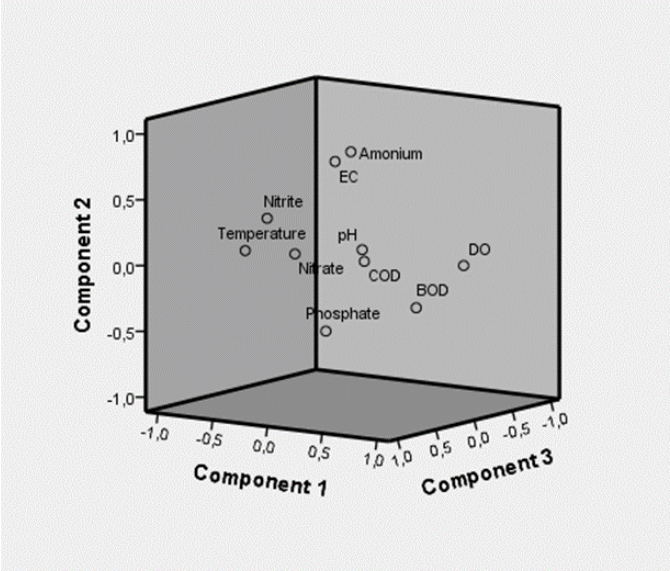

Figure 2. Component plot in rotated space

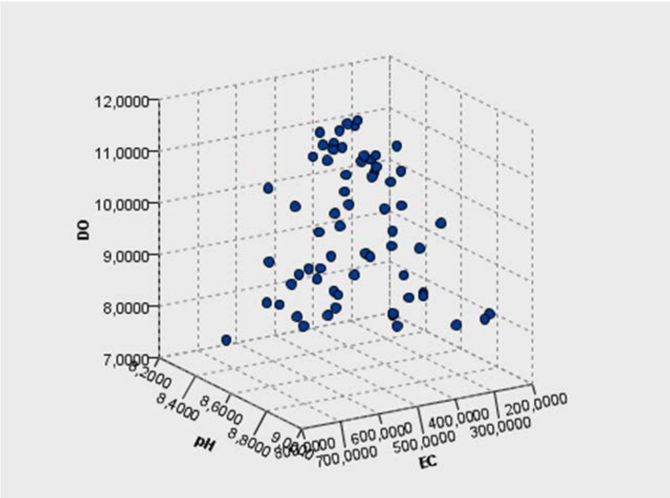

Figure 3. Lower-dimensional projection of the predictor space, which contains a total of 10 predictors

Since the similarities between DO and BOD, and EC and Ammonium were very strong, these variables formed a group at a distance of 1 and 2 units, respectively. Nitrite and Nitrate were also very similar parameters. However, their distance was 6 units in the dendogram. While similarity distance of oxygen and phosphate was 13 units; ammonium was included to the group of nitrite, nitrate and pH at 14 units. At a distance of 14 units, pH and Temperature were similar, whereas Phosphate is merged with the group. BOD and COD came together at a distance of 20 units and phosphate was similar to nitrite at a distance of 25 units. The findings obtained in the cluster analysis results are in line with various similar studies (Kilic and Yucel, 2019; Hamed, 2019; Kükrer and Mutlu, 2019). Our results characterized the pollution parameters in Filyos River and their relationship with each other.

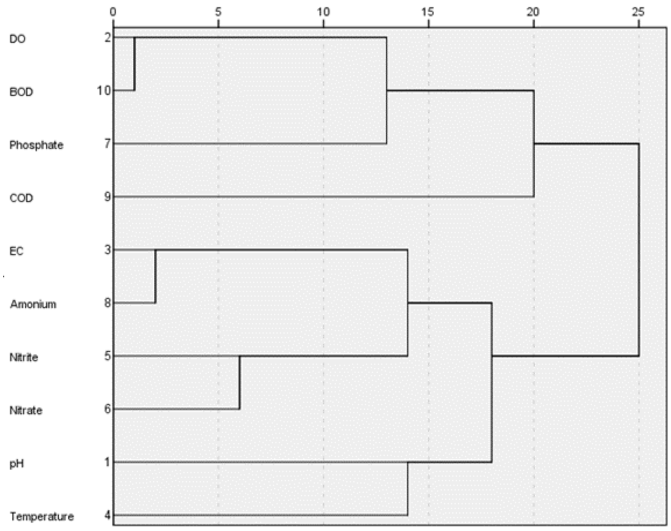

Figure 4. Dendrogram using average linkage (between groups)

Conclusion

In this study, multivariate statistical techniques were used to identify spatial and temporal changes in water quality of Filyos River. Principal component analysis revealed that four principal components were able to explain 69.49% of the variability. The dominant water quality parameters were found to be temperature, DO, pH and EC. These indicators have shown that the river is under climatic and environmental pressure, especially when the flow rate is low. In addition, Ammonium, Nitrate, Nitrite and Phosphate parameters, which are in positive interaction under these dominant components, showed that Filyos River suffers from agricultural pollutant sources. Results of basic correlation, factor and cluster analyses applied to 10 physico-chemical water parameters measured from Filyos River for 12 months have substantially supported each other and have emerged to reveal both spatial and temporal pollution characteristics of the river. Moreover, it was shown that multivariate statistical techniques are effective in the investigation of water quality datasets. To be able to succeed effective water resources management, similar works should be conducted frequently in large water resources.

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Compliance with Ethical Standards

Authors' Contributions

AYS: Collection of data, analysis and writing of the article. EY: Analysis and article writing MY: Statistical analysis. GA: Analysis of samples and article writing

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.

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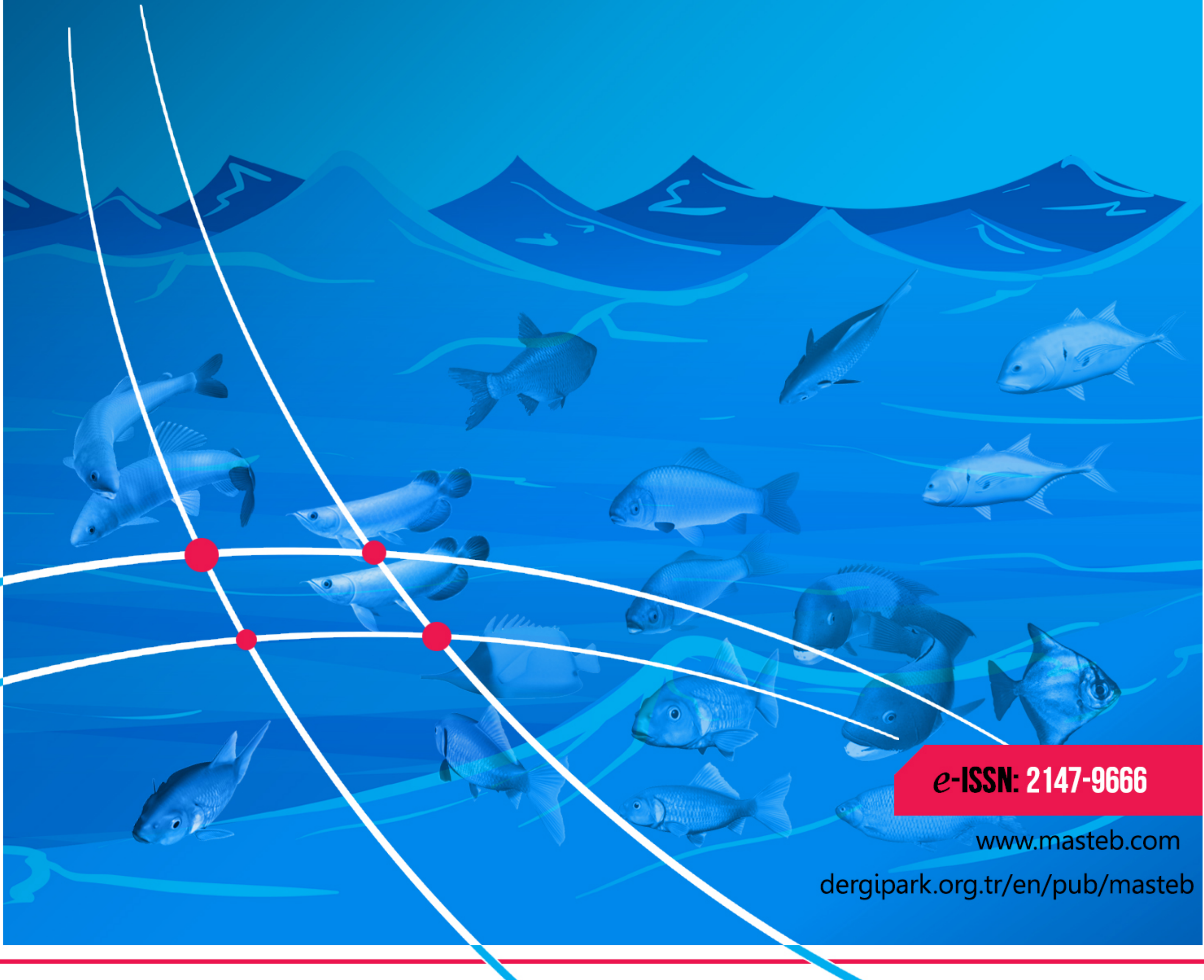

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