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TABLE OF CONTENTS

TABLE OF CONTENTS

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SHORT COMMUNICATION

The maximum size and age of *Umbrina cirrosa* (Linnaeus, 1758) in the World

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ABSTRACT

On April 17, 2020, one male specimen of the shi drum, *Umbrina cirrosa* (Linnaeus, 1758) was measured as 104 cm in total length, weighed 11080 g, and 18 years old. The *U. cirrosa* were sampled at 2 m depth by using trammel net in Ordu at the southern Black Sea. Up to date, this length, weight and age are a new record for the maximum size of *U. cirrosa*. These measurements make it the largest individual in the world.

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Introduction

The shi drum, *Umbrina cirrosa* (Linnaeus 1758), is a member of the Sciaenidae family (Fischer et al., 1987). It is a demersal fish largely spread from Eastern Atlantic to the Mediterranean and the Black Sea living within a depth range of 0 to 200 m (Fischer et al., 1987; Bizsel et al., 2020).

Fischer et al., (1987) stated that they are usually between 30 and 80 cm, but they can grow up to 100 cm. Chao & Trewavas (1990) measured a 40 cm of average length with a maximum size of 73 cm. According to FishBase (2020), this species can grow up to a maximum size of 73 cm and 3.1 kg. Aydın & Sözer (2020) reported the maximum sizes given for all Turkish coastal waters that the total length and weight of shi drum were 94 cm and 7051.1 g respectively and 5 years old. The maximum observed length is a useful tool for a rapid evaluation of growth rates in the absence of basic data (Legendre & Albaret, 1991; Froese & Binohlan, 2000). In this study, the maximum total length, maximum weight and maximum age are given as a new record data for *U. cirrosa* in the world.

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Material and Methods

Sampling was carried out on April 17, 2020 in the Fatsa district of Ordu province of the southern Black Sea Region (41° 02’ 11.80” N–37° 29’ 48.85” E) (Figure 1). *U. cirrosa* individual was caught by a trammel net (mesh size: 70 mm inner panel-240 mm outer panels) that was installed for commercial purposes at a depth of 2 m. Total length (TL) and weight (W) were measured to the nearest 0.1 cm and 0.1 g, respectively. Fischer et al. (1987) catalog book was used for identifying of the species. Right sagitta otolith was used for the age determination and otolith dimension. Sagittal otolith dimension was measured to the nearest 0.01 mm with digital caliper. The otolith was sectioned, sanded, and polished to enhance the visibility of growth zones. It was embedded into polyester moulds and then sectioned using an ISOMET Low Speed Saw. Age was read by a stereoscopic zoom microscope under reflected light against a black background (Morales-Nin, 1987; Jenke, 2002).

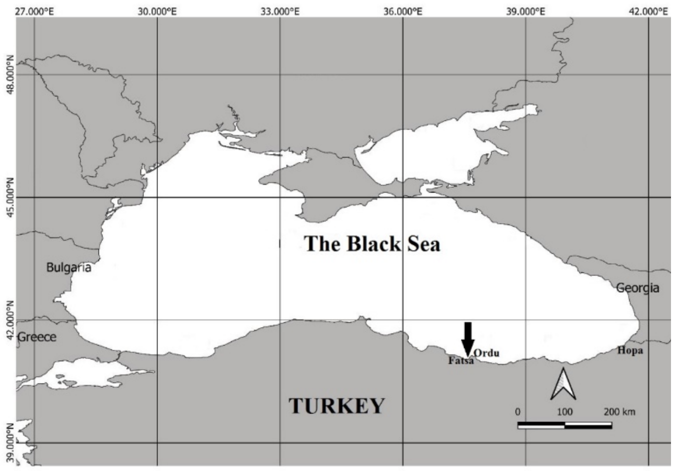

Figure 1. Sampling location of *Umbrina cirrosa* specimen

Results and Discussion

A male specimen of *U. cirrosa* was measured as 104 cm in total length (TL), weighed 11080 g. (gonad weight: 60 g) (Figure 2) and found to be 18 years old (Figure 3).

Figure 2. The largest and oldest individual sampled in the world

Right sagitta otolith dimensions were 25.10 mm length, 14.49 mm width and 9.7 mm thickness.

The maximum size for *U. cirrosa* was reported as 100 cm by Fischer et al., (1987), as 73 cm by FishBase (2020) and in the latest study this value was given as 94 cm by Aydın & Sözer (2020). The new maximum length (104 cm) and weight (11080 g) recorded in this study are the largest length and weight for this species in the World. The maximum length and weight of *Umbrina cirrosa* from different studies are given in Table 1

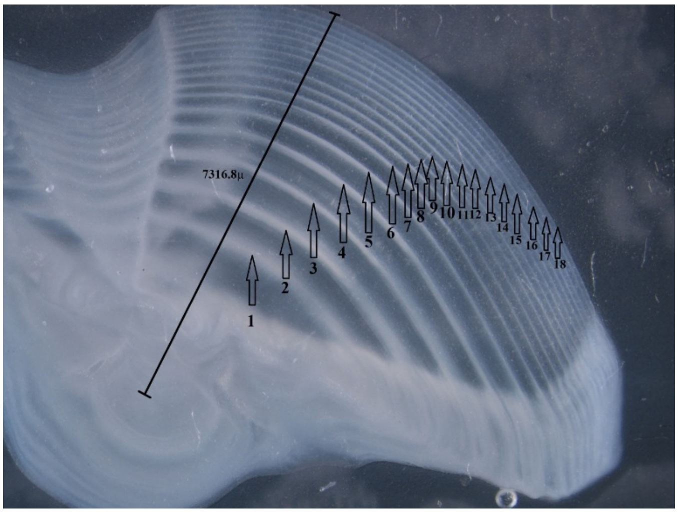

Figure 3. A thin stained otolith section of *Umbrina cirrosa* (total length 104 cm, weight 11080 g) aged 18 years old.

The age was determined to be 18 years old. The age was reported as 3 years old for 67 cm total fish length by Arneri et al. (1998), 5 years old for 97cm by Aydın & Sözer (2020). In this study, the largest size and oldest individual recorded in the world up to date.

Table 1. The maximum length and weight of *Umbrina cirrosa* from different studies

L_{max} (cm)	W_{max} (g)	Region	References
66.5	2915.0	Eastern Adriatic Sea	Dulčić & Kraljević (1996)
24.7	-	Porto-Lagos Northern Aegean (Greece)	Koutrakis & Tsikliras (2003)
47.0	-	Northern Adriatic	Dulcic & Glamuniza (2006)
49.5	1281.0	Adriatic Sea	Bolognini et al. (2013)
42	817.0	Sinop (Black Sea)	Bat et al. (2018)
26.8	214.04	Mersin Bay (Mediterranean)	Başusta et al. (2019)
68.8	2600.0	Aegean Sea	Cengiz & Paruğ (2020).
94	7051.1	Southern Black Sea (Hopa)	Aydın & Sözer (2020)
104	11080.0	Southern Black Sea (Fatsa)	**Present study**

In fisheries science maximum length and maximum age are important parameters that are applied directly or indirectly in most of the stock assessment models (Pauly, 1980; Welcomme, 1999; Froese & Binohlan, 2000; Borges, 2001; Cengiz et al., 2019; Özdemir et al., 2019). Therefore, it is important to regularly update the maximum size of commercially important species (Navarro et al., 2012; Özdemir et al., 2019). In this study, new maximum data (length, weight, age) is introduced to the literature for *U. cirrosa* species.

Compliance with Ethical Standards

Conflict of Interest

The author declares that they have no conflict of interest.

Ethical Approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the author.

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RESEARCH ARTICLE

Length-weight relationships for fourteen fish species collected by bottom trawl from the Eastern Black Sea coast, Turkey

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ABSTRACT

Length-weight relationships (LWR) were described for fourteen demersal and pelagic fish species; whiting (*Merlangius merlangus*), red mullet (*Mullus barbatus*), picarel (*Spicara maena*), scorpion fish (*Scorpaena porcus*), anchovy (*Engraulis encrasicolus*), sprat (*Sprattus sprattus*), horse mackerel (*Trachurus mediterraneus*), bluefish (*Pomatomus saltatrix*), *turbot (Scophthalmus maximus),* thornback ray (*Raja clavata*), shore rockling (*Gaidropsarus mediterraneus*) round goby (*Neogobius melanostomus*), black goby (*Gobius niger*) and stargazer (*Uranoscopus scaber*) caught with bottom trawl (12 mm mesh size) from the Eastern Black Sea. Samples were caught in depths from 10 m up to 60 m between April 2017 and March 2018 at monthly intervals. The minimum and maximum lengths and weights, length-weight relationships, parameters of a and b, ± 95% CI of b, r^2, growth type (isometric or allometric) of samples, and statistical analyses of the relationship were determined. Estimates for parameter b of the length–weight relationship ranged between 2.44 and 3.54.

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Introduction

The Black Sea is the world's largest land-locked inland sea (Bakan & Büyükgüngör, 2000). It has been exposed to environmental fluctuations and strong anthropogenic stresses (Bologa, 2001). In the Eastern part of the Black sea, the fishing grounds are quite different and the big rivers (Bzyb, Kodori, Inguri of Rio and Çoruh River) flows into the Black Sea from Georgia, which is close to the sampling area of the present study. The rivers change the physico-chemical properties parallel to the food spectrum of the environment (Berkün et al., 2010). This may play an important role in determining the nutrient composition, quantity and quality of the environment.

The length-weight relationship (LWR) has great importance in fish biology, physiology, ecology and fishery assessment (Gonçalves et al., 1997; Silva et al., 2013).

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Furthermore, the LWR allows fish condition to be estimated. The condition factor is frequently used in the analysis of ontogenetic changes (Safran, 1992) and for between-regions life-history comparisons (Weatherley & Gill, 1987). Length and weight parameters are also highly crucial for fisheries science, and stock assessment studies. It gives information about the growth type of fish, whether it is isometric or allometric (Ricker, 1975; Erzini, 1994). Previous studies about the length-weight relationships for fish species in the Black Sea coast of Turkey were performed by many researchers (Demirhan & Can, 2007; Kalaycı et al., 2007; Ak et al., 2009; Yankova et al., 2009; Özdemir & Duyar, 2013; Kasapoğlu & Düzgüneş, 2013; Çalık & Sağlam, 2017; Samsun et al., 2017). This study aims to provide data on the length-weight relationship for the 14 fish species captured by bottom trawl from the coastal waters of the Eastern Black Sea, Turkey.

Material and Methods

Study Area and Fish Sampling

The fishing operations were performed with the special permission of the Ministry of Agriculture and Forestry with the R/V Karadeniz Araştırma belongs to the Fishery Faculty of Recep Tayyip Erdoğan University due to the restriction of trawl fisheries in the study area. Depths of the surveys were started from 10 m up to 60 m and the operations were done between April 2017 and March 2018 (monthly) off the Rize coast in the south-eastern Black Sea. Samples were obtained by hauling an experimental bottom trawl net (12 mm mesh size) at a constant speed of 2.5-3 knots. Fishing took place within an area defined by the following coordinates: 40°59'29''N/40°19'52''E; 40°59'57''N/40°18'50''E; 40°01'32''N/40°22'53''E; 41°02'10''N/40°22'04''E (Figure 1).

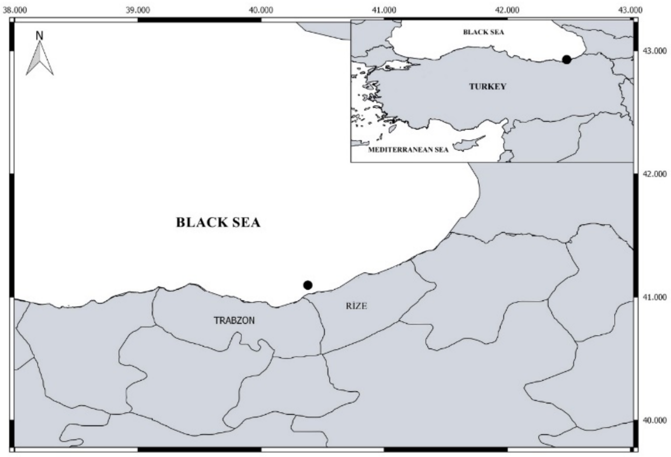

Figure 1. Study area (Iyidere coast)

Length- Weight Relationship (LWR)

All the yield was classified according to the fish species and identified. If the same species of fish were in a small amount all of the samples was measured. For fish species that were at a high number, the sub-sampling method was applied in order to measure the length and weight values. The fresh samples' total length (TL; cm) was recorded to the nearest 0.1 cm and total weight (W; g) was measured to the nearest 0.01 g. The length and weight relationship of fish were calculated using the exponential relationship (Ricker, 1973) (Equation 1) using the least-squares method:

$$W = a \times TL^b \tag{1}$$

where a is the intercept and b is the slope. The association degree between variables of W (total weight; g) and TL (total length; cm) was calculated by the determination coefficient (r^2). Additionally, 95% confidence limits of parameter b were estimated. The Student's t-test was used for comparison of the slopes (Zar, 1996). When the parameter 'b' is statistically equal to 3, the growth is called isometric, but the growth is positive allometric when the 'b' value is more than 3 and negative allometric when the 'b' value is less than 3 (Dutta et al., 2012).

Results and Discussion

In this research, length-weight relationships for 14 species were examined: *Sprattus sprattus* (Linnaeus, 1758*), Engraulis encrasicolus* (Linnaeus, 1758), *Scorpaena porcus* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868), *Spicara maena* (Linnaeus, 1758), *Pomatomus saltatrix* (Linnaeus, 1766)*, Merlangius merlangus* (Linnaeus, 1758), *Mullus barbatus* Linnaeus, 1758, *Gaidropsarus mediterraneus* (Linnaeus, 1758), *Raja clavata* Linnaeus, 1758*, Neogobius melanostomus* (Pallas, 1814), *Uranoscopus scaber* Linnaeus, 1758 and *Gobius niger* Linnaeus, 1758. For each species, the sample size, length ranges (minimum- maximum and average), parameters of length-weight relationships (a and b), 95% confidence intervals of b and the coefficient of determination (r^2) and growth type were given in Table 1. According to the results of this study, the "a" values ranged from 0.0013 to 0.169 while the "b" values varied between 2.4454 and 3.5474. The coefficients (r^2) ranged from 0.89 (*E. encrasicolus*) to 0.99 (*S. porcus*).

In this study, 7591 fish belonging to 14 families were examined. The most sampled species were *M. barbatus* (47%), *M. merlangus* (23.2%), *S. sprattus* (12.5%) respectively. Length-weight relationships for 14 species presented here were discussed deeply with previous studies from the Black Sea, Marmara, Aegean, Mediterranean and Adriatic Seas (Table 2).

Table 1. Length-weight relationships of 14 fish species caught from Eastern Black Sea, Turkey

Species	N	$L_{min-max}$	$W_{min.-max.}$	a	b	S.E. of b	95% CL of b	r^2
S. sprattus	780	5.7-16.6	0.79-33.54	0.0047	3.06	0.0012	3.13-2.99	0.90
E. encrasicolus	83	6.6-11.2	1.2-5.53	0.0043	3.04	0.0138	3.27-2.81	0.89
S. porcus	219	5.5-25.9	3.03-49.58	0.0145	3.11	0.0004	3.15-3.07	0.99
T. mediterraneus	581	7.3-18.4	2.26-51.18	0.0027	3.42	0.0019	3.51-3.33	0.91
S. maena	162	9.1-19.1	7.14-65.42	0.0081	3.08	0.0013	3.15-3.01	0.97
P. saltatrix	14	14.4-22	25.51-88.29	0.0118	2.89	0.0253	3.24-2.55	0.96
M. merlangus	1444	5.7-24.9	1.13-111.49	0.0063	3.04	0.0001	3.06-3.02	0.97
M. barbatus	2930	5.2-23.6	1.15-129.21	0.005	3.23	0.00007	3.25-3.21	0.98
S. maximus	18	22-69	400-6540	0.169	2.44	0.0117	2.67-2.21	0.96
R. clavata	478	24-97	40-6290	0.0027	3.20	0.0006	3.24-3.15	0.97
G. mediterraneus	22	14.2-26.6	15.87-134.95	0.0013	3.54	0.0130	3.78-3.31	0.97
N. melanostomus	169	9-24.6	8.83-250.34	0.0069	3.24	0.00091	3.30-3.18	0.98
G. niger	427	5.7-13.5	1.91-24.78	0.0112	2.97	0.0014	3.05-2.90	0.93
U. scaber	264	4.8-24.2	2.31-263.45	0.0178	2.96	0.00094	3.02-2.90	0.97

Table 2. Length-weight relationship parameters of 14 fish species estimated from other areas

Species	N	L_{min}-L_{max}	W_{min}-W_{max}	a	b	r^2	Region	References
S. sprattus	5087	5.6-12.6	3.34-47.37	0.008	2.86	0.88	Black Sea	Kalaycı et al., 2007
	134	4.3-7.9	0.37-3.18	0.004	3.35*	0.90	Aegean Sea	Moutopoulos & Stergiou, 2002
	423	5.6-10.7	1.08-8.14	0.006	2.92	0.91	East Black Sea	Kasapoğlu & Düzgüneş, 2013
	15016	5.5-12.5	2.54-9.41	0.002	3.46*	0.98	West Black Sea	Panayatova, 2001
	599	5.9-10.9	1.4-8.1	0.007	2.92	0.94	Black Sea	Özdemir & Duyar, 2013
	3060	6-11.5	1.32-7.99	0.01	2.70	0.95	West Black Sea	Yankova et al., 2011
E. encrasicolus	10062	5.5-14.5	0.9-17.4	0.008	2.86	0.89	Black Sea	Samsun et al., 2017
	4027	10.3-15.7	8.3-24.5	0.024	2.51	0.99	West Black Sea	Yankova et al., 2011
	696	8-13.6	3.5-16.4	0.018	2.62	0.88	Black Sea	Özdemir & Duyar, 2013
	1588	5.9-14.6	1.1-18.1	0.012	2.77	0.94	East Black Sea	Kasapoğlu & Düzgüneş, 2013
	575	8-14.7	2.85-19.14	0.017	2.60	0.85	Black Sea	Kalaycı et al., 2007
S. porcus	50	8.5-21	13-165	0.025	2.89	0.97	East Black Sea	Çalık & Sağlam, 2017
	351	5-34.2	2.1-406.1	0.009	3.27*	0.88	East Black Sea	Ak et al., 2009
	980	6.1-35.5	7-640	0.018	3.02*	0.97	Mediterranean	Morey et al., 2003
	98	8.2-26.4	-	0.012	3.18*	0.98	Aegean Sea	Karachle & Stergio, 2008
	15	17.3-21.4	84.2-186.02	0.006	3.34*	0.94	Marmara Sea	Bök et al., 2011
	136	8.5-29.2	13-508	0.017	3.03*	0.98	Black Sea	Kalaycı et al., 2007
T. mediterraneus	1432	7-18.4	4.5-55	0.005	3.17*	0.92	West Black Sea	Yankova et al., 2011
	526	9.4-15.3	4.6-25.2	0.003	3.3*	0.9	Black Sea	Özdemir & Duyar, 2013
	17	25.5-34.5	129-320	0.000	2.72	0.97	Adriaatic	Dulčić & Kraljević, 1996
	344	12-34.2	16.8-306.8	0.029	2.60	0.93	Mediterranean	Torres et al., 2012
	191	17.3-34.1	-	0.014	2.82	0.92	Aegean Sea	Moutopoulos & Stergiou, 2002

Note: * Studies showing similarities with this study

Table 2 (Continued). Length-weight relationship parameters of 14 fish species estimated from other areas

Species	N	L_{min}-L_{max}	W_{min}-W_{max}	a	b	r^2	Region	References
S. maena	528	8.3-24.2	3.51-29.4	0.009	3.00*	0.86	East Black Sea	Ak et al., 2009
	52	4.2-20.1	0.6-86	0.011	2.86	0.98	Mediterranean	Morey et al., 2003
	176	7.5-16.9	5.12-52.64	0.028	2.59	0.92	Mediterranean	Sangun et al., 2007
	118	7-18.5	-	0.009	2.99	0.96	Aegean Sea	Karachle & Stergio, 2008
	403	5.9-17.7	3.53-78.30	0.089	3.08	0.86	Marmara Sea	Bök et al., 2011
	83	11.2-20	14.24-87.67	0.006	3.15*	0.96	Black Sea	Kalaycı et al., 2007
P. saltatrix	820	16.1-27.5	32.5-227.9	0.005	3.25	0.95	Black Sea	Samsun et al., 2017
	14	11.6-21.2	12-131	0.003	3.34	0.96	East Black Sea	Ak et al., 2009
	207	12.2-24	15.4-127.2	0.005	3.25	0.98	Black Sea	Özdemir & Duyar, 2013
	25	12.5-20.2	16-75.2	0.009	3.01	0.87	East Black Sea	Kasapoğlu and Düzgüneş, 2013
	143	13.2-21.7	23.21-88.19	0.013	2.86*	0.92	Black Sea	Kalaycı et al., 2007
M. merlangus	140	10-27	9-118	0.013	2.77	0.91	East Black Sea	Çalık & Sağlam, 2017
	943	6.7-29.5	2.15-241.2	0.004	3.16*	0.98	East Black Sea	Ak et al., 2009
	44	14.1-29.1	-	0.004	3.18*	0.98	Aegean Sea	Karachle & Stergio, 2008
	166	7.6-24.2	2.7-121.40	0.004	3.14*	0.94	Marmara Sea	Bök et al., 2011
	1282	7.2-42.5	2-593	0.005	3.15*	0.98	Adriatic Sea	Bolognini et al., 2013
	3715	5.5-22.5	1.05-80.9	0.004	3.15*	0.99	West Balck Sea	Yankova et al., 2011
	432	6.8-14.6	-	0.005	3.24*	0.97	East Black Sea	Demirhan & Can, 2007
M. barbatus	22	17.3-24.7	60-180	0	3.12*	0	Adriaatic Sea	Dulčić & Kraljević, 1996
	76	12.5-22.3	-	0.004	3.27*	0.94	Aegean Sea	Karakulak et al., 2006
	2693	5.3-19	1.20-73-40	0.007	3.12*	0.96	East Black Sea	Kasapoğlu & Düzgüneş, 2013
	99	10-15.7	-	0.0049	3.32*	0.91	Marmara Sea	Bök et al., 2011
	714	6.1-21.9	2.08-161.14	0.007	3.13*	0.99	East Black Sea	Ak et al., 2009
	451	8.2-22	4.96-106.26	0.0032	3.06*	0.94	Mediterranean	Sangun et al., 2007
	432	6.8-18	-	0.0051	3.24*	0.97	East Black Sea	Demirhan & Can, 2007
S. maximus	16	37.5-70.5	925-7865	0.0113	3.11	0.93	East Black Sea	Çalık & Sağlam, 2017
	63	10--61	14.6-4494.4	0.007	3.24	0.98	Black Sea	Ak et al., 2009
	155	25-79	-	0.011	3.10	0.99	Adriatic Sea	Arneri et al., 2001
	97	32.5-80	444.2-9456	0.0069	3.37	0.93	Black Sea	Özdemir & Duyar, 2013
	50	44-71.7	1390-5960	0.001	3.27	0.84	West Black Sea	Yankova et al., 2011
R. clavata	24	56-79	1200-5500	0.001	2.30	0.96	West Black Sea	Yankova et al., 2011
	31	20.50-99	28.86-2614	0.0016	3.29*	0.93	Aegean Sea	Filiz & Mater, 2002
	52	34.3-95	170-5450	0.001	3.42*	0.91	East Black Sea	Demirhan et al., 2005
	27	10.7-95.2	-	0.0019	3.24*	0.99	East Black Sea	Demirhan & Can, 2007
G. mediterraneus	172	6.5-32	2.24-313.52	0.0114	3.08*	0.96	East Black Sea	Kasapoğlu & Düzgüneş, 2013
	8	4.2-20.7	-	0.0006	3.01*	0.99	Marmara Sea	Keskin & Gaygusuz, 2010
	56	8.2-14.3	1.3-11.73	0.003	3.18*	0.98	Marmara Sea	Bök et al., 2011
	15	8.5-14.5	-	0.0069	2.86	0.97	Aegean Sea	Karachle & Stergio, 2008
	164	4.5-23.6	0.25-95.30	0.0029	3.28*	0.98	West Black Sea	Van et al., 2019

Note: * Studies showing similarities with this study

Table 2 (Continued). Length-weight relationship parameters of 14 fish species estimated from other areas

Species	N	L_{min}-L_{max}	W_{min}-W_{max}	a	b	r^2	Region	References
N. melanostomus	99	8.6-19.1	-	0.0047	3.39	0.95	East Black Sea	Demirhan & Can, 2007
	172	6.5-32.0	2.24-313.52	0.0114	3.088	0.96	East Black Sea	Kasapoğlu & Düzgüneş, 2013
	3910	13.6-19.2	37.5-113	0.006	3.346	0.98	West Black Sea	Yankova et al.,2009
	73	9.1-35	8.58-381.42	0.01	3.033	0.89	East Black Sea	Ak et al., 2009
	758	3.6-13.3	-	0.0112	3.08	0.97	Caspian Sea	Abdoli et al., 2009
G. niger	113	7.6-13.2	-	0.0113	3*	0.91	East Black Sea	Demirhan & Can, 2007
	227	8.0-25.3	5.37-168.7	0.0166	2.86	0.96	Black Sea	Kalaycı et al., 2007
	286	6.9-19	3.49-33.3	0.0115	2.98	0.88	Marmara Sea	Bök et al., 2011
	447	7.7-16.5	-	0.0075	3.15*	0.97	Aegean Sea	Özaydın et al., 2007
	225	36-92	-	0.0123	2.97	0.96	Mediterranean	Verdiell-cubedo et al., 2006
U. scaber	30	12.4-28.4	-	0.007	3.22	0.98	Aegean Sea	Moutopoulos & Stergiou, 2002
	620	1.8-56.4	1.01-551.51	0.008	3.22	0.81	East Black Sea	Ak et al., 2009
	92	5.2-24.7	2.15-307.96	0.0103	3.15	0.99	Mediterranean	Sangun et al., 2007
	82	10.7-24.6	21.1-378.24	0.0109	3.15	0.96	Marmara Sea	Bök et al., 2011
	346	5.2-21.9	2-182.5	0.0167	3	0.99	East Black Sea	Demirhan et al., 2005

Note: * Studies showing similarities with this study

In the Black Sea *S. sprattus* and *E. encrasicolus* are not the target species of bottom trawling. However, these species were detected in the samplings. These common pelagic species migrate from offshore in the beginning of spring (Polat & Ergün, 2008). So this could be the reason for the presence of these species in the catch composition of the present study. It is remarked by Karakulak et al. (2006) that differences of *b* values for the same species are due to the differences in sampling methods, namely, the number of specimens and the differences in the length ranges of the species. These variations can also be arisen because of temporal variations of the sampling sites. In addition, in our study, it has been seen that the length range of the species is limited and mostly smaller fishes are dominant. On the coast of the Black Sea, an excessive fishing pressure exists (Knudsen et al., 2010). The high fishing mortality brings some changes to the biology of the species, such as a decrease in total length and first sexual maturity length (Jennings et al., 1999). Consequently, studies revealing variations in fish biology should be conducted continuously to monitor the recent situation of fish stocks (Ricker, 1975; Weatherley & Gill, 1987; Yankova et al., 2011). There is no doubt that fatness and shape of the species are initially stated according to the change on *b* values, but some factors such as food (size, quantity, and quality), salinity, sex, temperature, time of year, stage of maturity can change parameters of the length-weight relationship (Ricker, 1973).

Conclusion

The outcomes of this study have significant importance to make comparison with other studies. The results obtained will make a considerable contribution to the knowledge of fish populations in this special area. It is expected that fishermen and scientists take advantage of this study for future studies, especially about heavily exploited populations, as well as those under stock recovery plans or other management and conservation programs.

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Compliance with Ethical Standards

Authors' Contributions

Author GD designed the study, HO wrote the first draft of the manuscript, performed and managed statistical analyses. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

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

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RESEARCH ARTICLE

Phycocyanin extraction from frozen and freeze-dried biomass of *Pseudanabaena* sp. by using mild cell disruption methods

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ABSTRACT

Phycocyanin is a precious, natural, blue coloured pigment-protein complex that has commercial value and wide application in cosmetics, food, and pharmaceutical industries. In the present study, we performed various cell disruption methods (ultrasonication, homogenization, freeze/thaw and $CaCl_2$ extraction) for phycocyanin extraction from different forms of biomass of a thermophilic *Pseudanabaena* sp. that has a high potential to produce high-quality phycocyanin. Using potassium phosphate buffer and ultrasonic bath method, we achieved the highest phycocyanin yield (345 mgPC.g$^{-biomass}$) from freeze-dried biomass and we obtained increased yield as the duration of application increases. Phycocyanin yields were calculated as 345 mgPC.g$^{-biomass}$, 255 mgPC.g$^{-biomass}$ and 220 mgPC.g$^{-biomass}$ for 5, 10 and 15 min, respectively. In this study, cell disruption methods have determined significantly more effective on freeze-dried biomass rather than frozen biomass. Phycocyanin content of freeze-dried biomass was analysed after six months of storage and dramatic decrement was observed in the phycocyanin content of the cells.

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Introduction

Pseudanabaena species are non-heterocystous cyanobacteria which are characterized by trichomes and cells that are smaller than 2-4 µm in size (Tamburaci, 2009). Some species of *Pseudanabaena* possess chromatic adaptation mechanism that allows microorganisms to regulate their pigment composition depending on the exposed light quality (intensity, colour etc.) that results in quick adaptation to their environment (Acinas et al., 2009). In the literature, phycobiliprotein production capacity of *Pseudanabaena* sp. has been widely investigated in the aspect of phycoerythrin, and in some studies, the phycocyanin production capacity of the species was also reported (Tamburaci, 2009; Khan et al., 2019).

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Phycobiliproteins are highly fluorescent pigment-protein complexes that are produced in cyanobacteria species as an accessory pigment of chlorophyll. Having distinct spectral characteristics and bioactive properties (anti-inflammatory, anti-viral, anti-oxidant, etc.) are the main features that make phycobiliproteins widely-applicable natural substances in cosmetics, food, and pharmaceutical industries (Puzorjov & McCormick, 2020). Phycocyanin (PC), Phycoerythrin (PE), and Allophycocyanin (APC) are the subtypes of phycobiliproteins that have distinct spectral characters and commercial value depending on their purity and stability.

Phycocyanin is a blue-coloured water-soluble natural pigment that shows the major absorption peak at 620 nm. It is highly produced by cyanobacterium *Arthrospira* sp. (commercial name Spirulina) (Eriksen, 2008) and widely applied as a natural colourant instead of carcinogenic chemical colour additives (İlter et al., 2018). It has remarkable bioactive properties such as antioxidant, anti-inflammatory, hepatoprotective, and ROS scavenging, and depending on the purity ratio and stability, finds applications in different fields. The current PC industry highly depends on *Arthrospira* sp. since this species produce a high amount of PC and the cultivation process is not laborious. However, PC extracted from *Arthrospira* sp. has considerable drawbacks in terms of stability that impact its potential applications, especially when increased temperature and pH are desired (Liang et al., 2018, Liang et al., 2019; Klepacz-Smółka et al., 2020).

As PC has become commercially important product, efficient, cost-effective, environmentally friendly extraction of it has been emerged as a new challenge. All microalgae and cyanobacteria species have a rigid, tightly organized cell wall structure making liberation of intracellular molecules a great challenge while preserving their functionality and structure. Therefore, the applied cell disruption method highly influences the final product, its application, and the market value. For this reason, selecting the proper cell disruption method should be done considering the cell wall structure of the microorganism and the quality of the desired product. In terms of phycobiliproteins, since these compounds are sensitive to light and temperature, gentle disruption of the cells is vital to promote the liberation of proteins from the cell while preserving the functionality and bioactivity of the molecules (Phong et al., 2018).

Among various cell disruption methods, a feasible one for protein extraction should be selected considering the mildness of the method. Harsh conditions such as high pressure, high temperature and pH level should be avoided since they might cause structural changes in protein and later loss of activity. For this reason, mild cell disruption methods are mostly preferred for protein extraction from biomass. Bead milling, ultrasonication, enzymatic disruption, and ionic liquids are the main categories of existing mild cell disruption technologies (Phong et al., 2018). In the aspect of engineering, each method has its own advantages and limitations that should be taken into consideration in terms of cost and sustainability (Günerken et al., 2015).

This study was primarily aimed to determine the most effective PC extraction method for two different forms of the biomass of thermophilic *Pseudanabaena* sp. and to reveal the potential using of *Pseudanabaena* sp. as source of PC. For this purpose, frozen and freeze-dried biomasses were utilized, and selected mild cell disruption methods were compared in terms of PC extraction yields.

Materials and Methods

Materials and Chemicals

Pseudanabaena EGE MACC 40 was used as a biomass source and supplied from the Microalgae Culture Collection of Ege University (Ege Macc). Originally, the cyanobacterium was isolated from the thermal source in Turkey, Denizli-Sarayköy (Tamburaci, 2009).

BG 11 medium (Behle, 2019) with the addition of 22 g/L sea salt was used as a growth medium and at the end of the production, cells were harvested by centrifuge (6000 rpm, 5 min) and washed with distilled water. Following harvesting, half of the biomass was freeze-dried while the remaining was kept at -20°C. Freeze-dried biomass was stored at 4°C, preserving from humidity and light.

0.1 M potassium phosphate buffer (pH 7) was used as an extraction buffer. Buffer was prepared following the recipe of online tool (Centre for Proteome Research, Liverpool).

Extraction Procedure

Different phycocyanin extraction methods were performed by using freeze-dried and frozen biomass of *Pseudanabaena* sp.; all experiments were conducted by using 0.1 M potassium phosphate buffer (pH 7). Frozen biomass was thawed in 25°C prior to experiments. The freeze/thaw process was repeated twice. To explore the best cell disruption using frozen biomass, all samples were prepared resuspending 0.02 g (paste biomass) cell in 5 ml of 0.1 M potassium phosphate buffer (pH 7). Five different cell disruption methods were performed as freeze/thaw, homogenization (10 min, 8000 rpm), ultrasonic bath (HYDRA, 10 min, 45 kHz), ultrasonic probe (BADELIN, 2 min and 4 min, 9 cycles, 50% P, 20 kHz), and chemical extraction ($CaCl_2$ extraction). After each method, samples were centrifuged at 3500 rpm for 5 min and supernatant were subjected to spectroscopic measurement (ULTROSPEC 1100 PRO). $CaCl_2$ extraction was performed as described by (İlter et al., 2018).

Extraction from freeze-dried biomass was performed using the 0.02 g (freeze-dried biomass) of cell and 5 ml of 0.1 M potassium phosphate buffer (pH 7). Ultrasonic bath (5 min, 10 min, and 15 min, 50%P), ultrasonic probe (1 min and 4 min, 50% P, 15-sec cycle), and chemical extraction ($CaCl_2$ extraction) methods were implemented for cell disruption. After each method, the sample was centrifuged, and the supernatant was collected for spectroscopic measurements. Chlorophyll-*a* analyses were performed as described by Mishra et al. (2012). Briefly, the pellet was collected and extracted with acetone (80%) incubating 1 h in dark at 4°C. Following, the supernatant was measured, and chlorophyll-*a* amount was calculated using the equation given below (Eq. 1).

Phycocyanin (PC), Allophycocyanin (APC) and Phycoerythrin (PE) concentrations were calculated approximately by using Bennet & Bogobad's equations (Eq. 2, 3, 4) (Bennett & Bogobad, 1973) where A indicates the measured absorbance values at given wavelengths.

$$Chlorophyll - a(\mu g/ml) = 12.7(A_{663}) - 2.1(A_{645}) \quad (1)$$

$$PC(mg\ /ml) = [A_{620} - 0.474\ A_{652}]/5.34 \quad (2)$$

$$APC(mg/ml) = [A_{652} - 0.208A_{620}]/5.09 \quad (3)$$

$$PE(mg\ /ml) = [A_{568} - 2.41PC - 0.849APC]/9.62 \quad (4)$$

Results

Phycocyanin extraction from frozen biomass was performed by implementing different mild cell disruption methods including ultrasonication, homogenization, and freeze/thaw, and $CaCl_2$ extraction (not shown) (Table 1). Calculated PC yields showed that the ultrasonic probe was the most effective method for PC extraction from frozen biomass while the freeze/thaw method had almost no visible effect on the cells. PC extraction yields were calculated as 43.75 $mgPC.g^{-biomass}$ and 2.375 $mgPC.g^{-biomass}$ for ultrasonic probe and freeze/thaw methods, respectively.

As the most efficient method was determined as an ultrasonic probe, results showed that duration of application is an important parameter in terms of obtained PC content. 4 min ultrasonic probe application resulted in 25% increased PC content compared to 2 min application.

Microscopic images and colour values of extracts (Figure 1 and Figure 2) further confirmed the degree of cell disruption in the samples. According to microscopy images, after homogenization and freeze/thaw applications there was no cell disruption in the samples and the supernatants were colourless while slightly disrupted cells could be observed after 15 min ultrasonic bath. Images supported that the ultrasonic probe method was the most efficient one, therefore almost all cells were disrupted in the sample. The supernatant of the sample after overnight extraction with $CaCl_2$ was colourless and no further measurement was performed for the sample.

Table 1. Phycocyanin content and phycocyanin yield of frozen biomass of *Pseudanabaena* sp. obtained by performing different cell disruption methods

Frozen Biomass	***PC (mg/ml)***	***PC Yield (mg/g_{biom})***
Freeze/Thaw	0.0095	2.375
Ultrasonic Bath (15 min)	0.0339	8.475
Homogenization (10 min)	0.0178	4.45
Sonic Probe (2 min)	0.130	32.5
Sonic Probe (4 min)	0.175	43.75

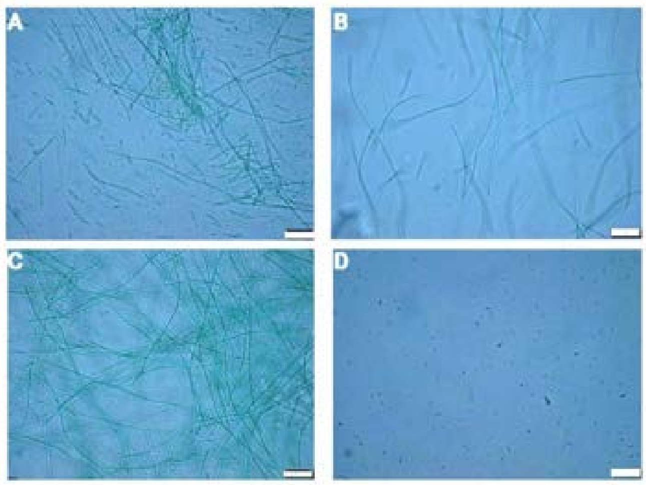

Figure 1. Microscopic images (10x) of frozen biomass of *Pseudanabaena* sp. after cell disruption by A) Ultrasonic bath method; B) Homogenization method; C) Freeze/thaw method; D) Ultrasonic Probe method. The morphology of the untreated cells is expected to be closer to the C.

PC extraction using freeze-dried biomass was performed by ultrasonication (ultrasonic bath and ultrasonic probe) and $CaCl_2$ extraction methods (Table 2). The highest PC yield was obtained by using an ultrasonic bath even though this method was not efficient for fresh cells, the best results were obtained by this method for freeze-dried cells. Figure 3 shows a comparison of obtained PC amounts from frozen and freeze-dried cells by ultrasonic method. The ultrasonic bath was performed for 5, 10, and 15 min, and compared to other methods, obtained PC amounts were significantly higher and increased by a longer duration of application. PC yields were calculated as 345 $mgPC.g^{-biomass}$, 255 $mgPC.g^{-biomass}$ and 220 $mgPC.g^{-biomass}$ for 5, 10 and 15 min, respectively.

Figure 2. The supernatant of frozen biomass collected after performing cell disruption by freeze/thaw, ultrasonic probe, homogenization, and ultrasonic bath methods.

Table 2. Phycocyanin content and Chlorophyll-*a* content of freeze-dried biomass of *Pseudanabaena* sp. obtained by performing different cell disruption methods. n/a: not available

Freeze-dried Biomass	PC (mg/ml)	Chl-*a* (mg/ml)	PC Yield (mg/g_{biom})
Sonic Probe (1 min)	0.24	0.0015	120.36
Sonic Probe (4 min)	0.22	0.0040	110.16
$CaCl_2$	0.21	0.014	109.64
Ultrasonic Bath (15 min)	0.69	n/a	345
Ultrasonic Bath (10 min)	0.51	n/a	255
Ultrasonic Bath (5 min)	0.44	n/a	220

The ultrasonic probe was performed for 1 min and 4 min. The results showed that obtained PC amounts following 1 min and 4 min ultrasonic probe and $CaCl_2$ extraction were the same while chlorophyll-*a* contents were varied. The highest chlorophyll-*a* content was observed following cell disruption by using an ultrasonic probe (4 min) while the lowest was observed after 1 min. Chlorophyll-*a* contents were observable by the colour values of the samples. After 1 min ultrasonication, the sample was brilliant blue while after 4 min it was green (Figure 4). According to the results, for freeze-dried cells, a longer duration of application (ultrasonic probe) caused an increment in undesired chlorophyll-*a* content while obtained PC amount was the same. After $CaCl_2$ extraction, the same amount of PC was obtained while 10-fold higher chlorophyll-*a* content was found in the sample.

PC content of freeze-dried cells was analysed after five and six months following the lyophilization. After five months, PC concentration was calculated as 1.5-fold lower while it was dramatically decreased after the fifth month.

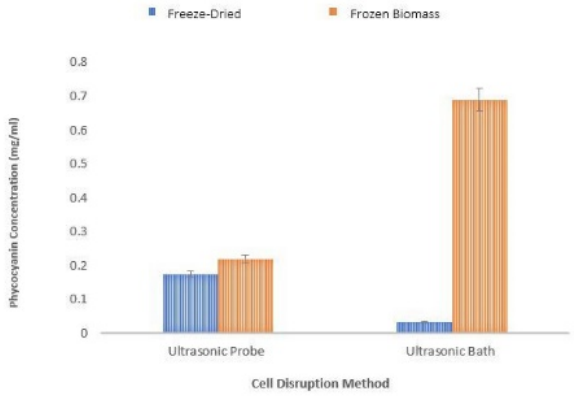

Figure 3. Phycocyanin concentration obtained by ultrasonic bath (15 min) and ultrasonic probe (4 min) methods from different forms (freeze-dried and frozen) of biomass of *Pseudanabaena* sp.

Figure 4. Raw protein extracts prepared from freeze-dried biomass of *Pseudanabaena* sp. A) The supernatant collected after cell disruption by ultrasonic probe (4 min) method; B) The supernatant collected after cell disruption by ultrasonic probe (1 min)

Discussion

In the present study, protein extraction was performed by using different forms of biomass (frozen (wet) and freeze-dried (dried)) and various cell disruption techniques were performed to reveal the most efficient PC extraction method. The maximum yield of PC was obtained by using freeze-dried biomass and the ultrasonic bath method. Obtained yield value showed that *Pseudanabaena* sp. produced a remarkable amount of PC considering the commonly used *Arthrospira* species that have yield values ranging from 100 to 380 $mgPC.g^{-biomass}$ (Leu et al., 2013; Lima et al., 2018; Prates et al., 2018; Hsieh-Lo et al., 2019).

Calculated PC concentration and yields for frozen biomass were critically lower than freeze-dried biomass (Table1 and Table 2). These findings show that extraction yield and obtained amount of PC were highly affected by the form of biomass. Cell disruption methods were more efficient on freeze-dried cells. These results are considered to be associated with the water content of the cells that affects cell wall strength and enhances cell disruption ratio. Pan-utai & Iamtham (2019) reported varying extraction yields in *Arthrospira platensis* and purity values depending on the method of drying, and freeze-drying is the most suitable method to maintain cell composition. In the literature, freeze-drying method is recommended to preserve the heat-sensitive content of cells without damaging the cell wall (Güroy et al. 2017; Tavanandi & Raghavarao, 2019).

In the present study, the PC content of freeze-dried biomass was analysed after five months and revealed significant decrease. This situation may be related to the storage conditions of the samples. Throughout this period, samples were exposed to light and humidity, even for a short time, during sample preparation for experiments.

The freeze/thaw method and homogenization for phycobiliprotein extraction from frozen and freeze-dried biomass had no effect on cells, according to the literature, freeze/thaw method is widely used for phycobiliprotein extraction from fresh biomass. Cano-Europa et al. (2010), used the freeze/thaw method for phycobiliprotein extraction in *Pseudanabaena tenuis,* and they reported 2% phycocyanin and 89% phycoerythrin content. In the present study, freeze/thaw method was not enough to achieve cell disruption therefore, it was used to increase the efficiency of combined methods. Moraes et al. (2011) reported similar results in their comparative study with *A. platensis*, they obtained the lowest extraction yield after freeze/thaw and homogenization.

Ultrasonication method (ultrasonic bath and ultrasonic probe) was applied as a mild cell disruption method which is widely used for protein extraction. This method causes cell disruption exposing the cells to the high-intensity ultrasonic waves that create mechanical stress and ultimately allow releasing cell content into the extraction medium (Safi et al., 2014; Phong et al., 2018). During ultrasonication process, keeping sample cool to prevent overheating is highly important to maintain the functionality of the proteins (Lee et al., 2012). Therefore, sonication processes were performed using ice and water.

The biomass in frozen samples; the best result was obtained by ultrasonic probe and results showed that duration of application is an important parameter in terms of PC content. Increased duration of application resulted in 25% increased phycocyanin content for the ultrasonic probe. Furuki et al. (2003) reported increased PC content by applying longer ultrasonication, and they indicated that the proper application time is an important factor in terms of protein functionality.

The biomass in freeze-dried samples; the ultrasonic bath was the most effective method even though the same observation was not valid for frozen biomass. The PC extraction yield was remarkably increased compared to other methods. Considering other mild cell disruption methods, ultrasonication method promotes pore enlargement by cavitation and it disrupts cell walls more efficiently (Vinatoru, 2001). Similar to ultrasonic probe, longer duration of application of ultrasonic bath caused an increment in obtained PC amount without degradation. Longer ultrasonic bath application resulted in a 2-fold higher PC concentration.

Varying chlorophyll-*a* contents were observed depending on the duration of application of the ultrasonic probe. Chlorophyll-*a* contents were observable by the colour values of the samples. After 1 min ultrasonication, the sample was brilliant blue while after 4 min ultrasonication it was green. The same situation was detected by İlter et al. (2018), who reported that the highest PC content from *Spirulina* sp. was obtained after ultrasonication and the increment in green colour was observed which is attributed to chlorophyll.

Conclusion

In the current study, PC extraction yield was significantly affected by the form of the biomass. All applied cell disruption methods showed better results for the freeze-dried biomass in terms of yield, and the most efficient method was determined as the ultrasonic bath. Results revealed that *Pseudanabaena* sp. can produce a significant amount of PC compared to commonly used species and its thermophilic nature may be advantageous in the aspect of stability (Ferraro et al., 2020).

Compliance With Ethical Standards

Authors' Contributions:

This study was performed under supervision of MCD and all experiments were designed and performed by BK and ZD. Manuscript was written by BK and 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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RESEARCH ARTICLE

Control of ship roll and yaw angles during turning motion

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ABSTRACT

The aim of this paper is to determine factors that are exposed ships to assorted hydrodynamic forces originating from internal and external influences. The adverse effect of rolling caused by turning motion should be reduced with anti-rolling systems, to ensure safe navigation. If this effect is not eliminated, it may prevent the ship from keeping its course safely. In this study, fin stabilizer system the computational analysis of the roll motion formed by the turning motion and wave effect is included in fin stabilizer system. The rudder motion that allows the ship to have the desired maneuvering angle; calculations of the fin system, which decreases the excessive roll moment, and the roll motion caused by these two effects are examined using MATLAB software. The analysis period has been determined as 300 seconds, which is the time to reach the desired turning angle of the ship. Linear quadratic tracking (LQT) algorithm has been used to solve partially unknown continuous time problems such as roll motion in this study.

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Nomenclature

u_0	Surge velocity	N	Rigid-body Coriolis/centripetal matrix
v	Sway velocity	τ	Control vector
w	Heave rate	w_e	Wave encounter frequency
p	Roll rate	x_g	x- position the center of gravity
q	Pitch rate	y_g	y- position the center of gravity
r	Yaw velocity	z_g	z- position the center of gravity
$\emptyset$	Roll angle	N_i	Linear damping coefficient
θ	Pitch angle	$N_{\dot{i}}$	Hydrodynamic added mass
ψ	Yaw angle	Y_i	Force
e	East position	K_i	Moment
M	Added mass matrix		

Introduction

It is estimated that around 90% of the world trade volume is achieved by sea transportation. The volume of the ship fleet increases in parallel with this expansion (Lu et al., 2017). It is a common concern that ships are always strived to navigate at a certain speed to minimize spending time and fuel consumption. Various studies are carried out to remove the obstacles that arise while increasing energy efficiency (Lihua et al., 2018). One of the issues to be considered about safe navigation is the control of roll movement. Therefore, roll motion during the

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course is prominent in terms of the quality of navigation. Nonlinear mathematical models based on solid mechanics theory are used to estimate the roll movements of the ships with high accuracy (Hou et al., 2018). The hydrodynamic motion occurring on ships are damped by three different physical features in general; wave separation, vortexes on appendages and viscous friction on the wetted hull (Belenky et al., 2012).

The problem of energy optimization is one of the striking subjects that most of the researchers working on ships in recent years. As a result of the ship being exposed to severe rolling movements, the propulsive force and forwarding speed will decrease, and eventually some of the energy will be lost. Therefore, in this study, the roll angle reduction is taken into consideration to improve safety and energy efficiency during the cruise period. In past studies viscous and wave, resistance decreases when the vessel has a certain rolling angle range. In addition to ensuring safety at sea, increasing thrust and reducing fuel consumption can also lead to cost savings. (Yu et al., 2015). Under the influence of risen energy prices, the International Maritime Organization introduced the EEDI (Energy Efficiency Design Index), which required improved energy efficiency and emission reductions (GHG-WG, 2009). IMO has developed intact stability criteria on such fundamental principles as metacentric height (GM) and righting lever (GZ) in 1993. Then, the International Code on Intact Stability (IS Code), which entered into force on July 1, 2010, has been adopted in 2008. The mandatory requirements and recommended provisions relating to stability significantly influence the ship design parameters and overall ship safety (IMO, 2019). Considering the criteria of efficiency and legal necessities, it can be said that there are numerous related studies on this issue (Liu & Jin, 2013).

As a result of excessive roll motion, seafarers on the ship, vessel construction and transported load are adversely affected. Due to this reason, many studies have been conducted to damp the roll motion by utilizing such stabilization systems as anti-rolling tanks, bilge keels, rudders, gyro or fin stabilizers (Perez & Blanke, 2012). These systems have different characteristics since each of them has an individual triggering factor. For instance, the excessing of 10 knots, fin stabilizers are proper to damp the roll motion. The retractable fin stabilizers are hydrodynamically the most preferable way to ensure anti-rolling moment. On the contrary, as the pitching moment is relatively high than the rolling moment of a ship, a fin stabilizer is not able to handle trim angle (Kim & Kim, 2011).

There are several studies in the literature on the modelling and simulation of the rolling motion of a ship. Particularly, the roll motion problem has been presented a four-dimensional path integration approach in random beam seas (Chai et al., 2015). Falzarano et al. (2015) examined roll damping methods and summarized the viscous roll damping prediction model in the most complex and less understood roll motion from the degree of freedom of the ship.

Irkal et al. (2016) assert that using a bilge keel is the first thing for reducing roll movements of a ship-shaped floating body when excessive heeling moments come to be a problem. Roll damping predictions both with and without a bilge keel have been demonstrated by Computational Fluid Dynamics (CFD) method and the results of CFD have been verified with a wave flume experiment (Irkal et al., 2016). Wassermann et al. (2016) have been tried to compare the advantages and disadvantages of three techniques on rolling such decay of motion, harmonic excited motion and harmonic forced motion. Chai et al. (2016) have been investigated the dynamic stability of a ship by probabilistic methods for random seas since nonlinear rolling movement is a serious threat to shipping stability. Markov diffusion theory has been applied to come out with the stochastic features of rolling motion originated from random sea wave loads. Hou & Zou (2016) have been predicted rolling fluctuations of a floating structure at an irregular sea state, it highly depends on accurate estimation of unknown damping and restoring moment coefficients in nonlinear roll motion equations. The coefficients of roll motion equations of a floating body have been suggested by using Support Vector Regression (SVR) method and random reduction technique. The roll damping coefficients have calculated with a method based on the three-dimensional CFD approach and compared with the experiment results in the study of Zhou et al. (2015). Overcoming the resistance and the yaw deviation, which needs running energy and appendage resistance due to the fin/rudder stabilization system, the total energy consumption has been analyzed by Yu et al. (2015). For this purpose, to achieve the roll reduction, the multi-objective genetic algorithm (MOGA) method based on the self-organizing PID controller has been utilized for the optimization of performance. Ship speed is significantly reduced due to the additional resistance caused by wave motion. The use of actuators to control the rudder of the ship can retrieve this speed loss. Liu et al. (2016) have been exerted a combined system using the sway–yaw motion and the roll dynamics to the reduction of loss speed.

The idea of using fin as a stabilization device consists of the dynamic ship model and fin hydrodynamic theory. The roll moment is observed in the turning process until the vessel reach desired yaw angle. In this study, it is examined that how the fin stabilizers are able to degrade the roll motion of a container ship, which is under the influence of turning in a random beam sea. In cases where the self-restoring moment acting on the hull is insufficient; the fin stabilizer is subsequently activated to restrict the heeling angle at an acceptable range. The ship has been modelled with one fin at each side of the hull. The attack angle values of each fin are equal during the maneuver period, but their directions must be reversed to achieve a worthwhile restoring moment. For this reason, in this research, instead of using two equal restoring moments with opposite directions, magnitudes of each moment have been acted on the desired direction. The interaction of yaw, rudder and roll angles are modelled in the MATLAB environment. The restoring moment provided by the fin stabilizer is counteracted by the rolling moment in the vessel body in the absence of the fin.

The remainder of this study is organized as the linear mathematical model of the case ship, random sea state and stabilizer system description. The specifications of the vessel and selected fin stabilizer system are introduced in part 2 and the parameters of the study have been determined here. Part 3 details the results of modelled system, together with simulation statistics. The final part consists of some inferences and is derived from the recommendations.

Materials and Methods

A ship moves at sea with 6 degrees of freedom. The planar motions are defined as surge, sway and heave which are formed by external effects. The remaining three motions are rotational movements in the x, y and z axes and are expressed as roll, pitch and yaw respectively in Figure 1.

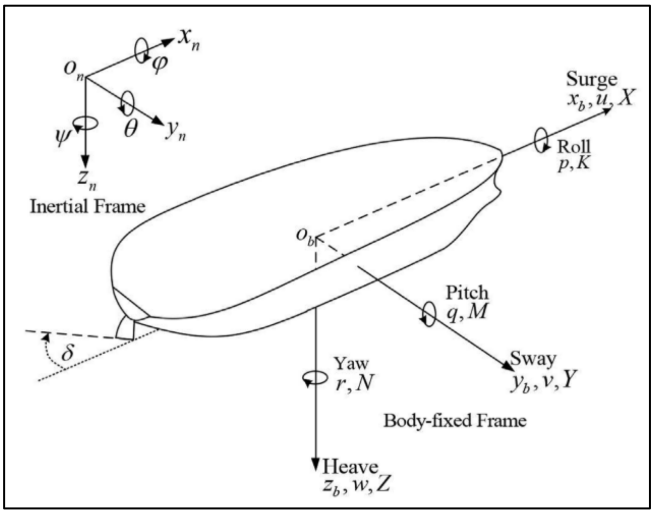

Figure 1. 6-DOF ship motion (Huang et al., 2018)

It is well known that roll motion has a direct and negative impact on ship stability as scrutinized in previous studies. The main research subject of this study is to reduce this destructive effect with the help of auxiliary equipment which is known as a fin stabilizer. In this part, the roll motion of a container ship during its maneuver period is examined and the damping of the excessive roll movements of the ship are caused by waves is provided by the fin stabilization mechanism (Huang et al., 2018). In this study, the disturbance effect that causes dynamic movements is accepted as the wave force and the wind force is ignored.

Mathematical Model of Nondimensional Maneuvering and Autopilot

The electronic control systems added on ships by the developing technology should have sufficient capacity to ensure safe and secure operations. One of these applications, auto-pilot design of the ships is conducted by such methods as PID-control, LQ design techniques, feedback linearization and backstopping designs. The purpose of each method is to be able to predict the course dynamics easily with high accuracy and to make accurate responses. In case the illustration of differences of ship dynamics, experimental and real ship body results are used together (Le et al., 2004).

The variables such as u velocity used in the calculations as the compound $u = \sqrt{{u_0}^2 + v^2}$ of sway and surge velocities of a vessel need to be normalized in the autopilot system design equations. This study aims to examine the motion at a set turning maneuver of a container ship for 3-DOF (Fossen, 2011).

Nonlinear control systems are used to solve problems that arise in various fields of science and technology. In the maritime transportation areas, it is utilized to determine the course and position of a ship. Due to the nonlinear equation's linearization process is applied to complex problems. In ship motions, linearization of nonlinear equations is solved by one of the controllers called the MIMO (Multi-Input Multi-Output) model which is an adaptive control approach (Bańka et al., 2013).

The linearization of the ship speed equation which is shown in equation 1 is carried out with the assumption that the external factors do not affect the motion of the ship in the direction of surge and heave, but only affect sway direction. The Linear Model of Van Amerongen and Van Cappelle MIMO model is described below:

$$M\dot{v} + N(u_0)V + G\eta = \tau \quad (1)$$

where u_0=constant, $V = [v, p, r]^T$ and $\eta = [e, \phi, \psi]^T$ are the states of the ship, and τ is the control vector of the system (Perez et al., 2006).

$$M = \begin{bmatrix} m - Y_{\dot{v}} & -mz_g - Y_{\dot{p}} & mx_g - Y_{\dot{r}} \\ -mz_g - K_{\dot{v}} & I_x - K_{\dot{p}} & (-I_{xz} - N_{\dot{p}}) = 0 \\ mx_g - N_{\dot{v}} & (-I_{xz} - N_{\dot{p}}) = 0 & I_z - N_{\dot{r}} \end{bmatrix} \quad (2)$$

When port and starboard of a ship to be regarded as symmetrical weight distribution, $I_{xy}=I_{yz}=0$ and $y_g=0$ equations are obtained. For this reason, it is compatible to choose the origin of the ship $r_g = [x_g, 0, z_g]^T$ so $I_{xz}=0$ with corresponding added inertia $K_{\dot{r}} = N_{\dot{p}} = 0$ (Blanke & Christensen, 1993).

$N(u_0)$ is generated by the linearization with the acceptance of symmetrical weight distribution and $V = [u_o, 0, 0]^T$ (Bańka et al., 2015).

$$N = \begin{bmatrix} -Y_v & -Y_p & mu_0 - Y_r \\ -K_v & -K_p & -mz_g u_0 - K_r \\ -N_v & -N_p & mx_g u_0 - N_r \end{bmatrix} \quad (3)$$

The restoring forces and moments for a ship as a part of equation 1 are seen below:

$$G = diag\{0, W\overline{GM_T}, 0\} \quad (4)$$

where, $W = mg$ is the weight of displaced water and $\overline{GM_T}$ is the transverse metacentre height (Perez et al., 2006):

The linear state-space model is described as $x = [v, p, r, \phi, \psi]$T matrix. The elements of A and B matrices are below:

$$\dot{x} = \overbrace{\begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} & 0 \\ a_{21} & a_{22} & a_{23} & a_{24} & 0 \\ a_{31} & a_{32} & a_{33} & a_{34} & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix}}^{A} . x + \overbrace{\begin{bmatrix} b_{11} & b_{12} & \dots & b_{1r} \\ b_{21} & b_{22} & \dots & b_{2r} \\ b_{31} & b_{32} & & b_{3r} \\ 0 & 0 & \dots & 0 \\ 0 & 0 & & 0 \end{bmatrix}}^{B} . u \quad (5)$$

where, the elements a_{ij} are found from equation 6:

$$\begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix} = -M^{-1}N(u_0); \begin{bmatrix} * & a_{14} & * \\ * & a_{24} & * \\ * & a_{34} & * \end{bmatrix} = -M^{-1}G \quad (6)$$

The elements of B matrix b_{ij} are found from $B = M^{-1}TK$. K is the diagonal matrix of force coefficients (Fraga & Liu, 2012). The main objective is controlling the course of the ship $\psi = \psi_d = constant$ and damping the roll motion ($p_d = \phi_d = 0$). As a result of the control of the vessel route, the natural frequency ω_{roll} and damping ratio ζ_{roll} are increased and thus the damping takes place. Besides, it is impossible to damp the roll ϕ and the heading ψ angle to a zero value with the help of a single rudder. Motion can be observed by performing a steady-state analysis of the compiled system. The output to be controlled is determined as follows:

$$y = [p, r, \phi, \psi]^T, \; y_d = [0, 0, 0, \psi_d]^T \quad (7)$$

For $y = C.x$, C is expressed as (Li et al., 2018):

$$C = \begin{bmatrix} 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad (8)$$

In Continuous-Time (CT) system problems, the Linear Quadratic Tracking (LQT) system and its common solution should be determined. For the solution of these problems, there is an assumption that the reference trajectory is asymptotically stable. When the reference trajectory goes to zero, the time goes to infinity. The linear Continuous-Time system is defined as:

$$\dot{x} = Ax + Bu \quad (9)$$

$$y = Cx \quad (10)$$

where $x \in R_n \times 1$ is a measurable system state vector, $y \in R_p \times 1$ is the system output, $u \in R_m \times 1$ is the control input, $A \in R_n \times n$ gives the drift dynamics of the system, $B \in R_n \times m$ is the input matrix and $C \in R_p \times n$ is the output matrix (Modares & Lewis, 2014).

With $x = [v, p, r, \phi, \psi]^T$ and $\phi = c_{roll}^T x$, $\psi = c_{yaw}^T x$, the solution to the LQT problem is given below (Huang et al., 2017):

$$u = G_1 x + G_2 y_d \quad (11)$$

where,

$$G_1 = -R^{-1}B^T P_\infty \quad (12)$$

$$G_2 = -R^{-1}B^T (A + BG_1)^{-T} C^T Q \quad (13)$$

Due to the variety of environmental conditions, ship movements cannot be expected to have the same amplitude and acceleration, so the reduction of the freedom degrees makes it easier to obtain a proper solution (Lozowicki, 2001).

The physical and the mathematical model components of the ship movement with one degree of freedom are based on the following assumptions (Zhang & Zou, 2011):

- The ship is symmetrical towards the port and starboard parts,
- All other degrees of freedom are neglected,
- The ship is accepted as a rigid body.

The above-defined model has been implemented on the container vessel to be able to design the mathematical model of ship movements easily. Under these constraints, the nonlinear state of the roll motion is expressed in equation 14.

$$(I+J)\ddot{\phi}+B_1\dot{\phi}+B_2\dot{\phi}|\dot{\phi}|+B_3\dot{\phi}^3+\Delta(c_1\phi+c_3\phi^3+c_5\phi^5+c_7\phi^7)=\omega_e^2\alpha_m I\cos(\omega_e t)-M_f \quad (14)$$

The mass moment of the inertia for roll motion is shown as I and the added mass moment of inertia for rolling is implied by J. B_1, B_2 and B_3 are roll damping coefficients, and c_1, c_3, c_5 and c_7 are expressed as coefficients for restoring force. Angle, angular velocity and angular acceleration of roll motion is represented as $\phi, \dot{\phi}, \ddot{\phi}$ respectively. Δ is the weight of the ship, wave encountering frequency is indicated by ω_e, α_m corresponds to the maximum wave slope, and M_f is for the fin control moment (Alarçin et al., 2014).

The ship's motion is mostly affected by the wave state. For the determination of the hydrodynamic forces on board, it is necessary to know that the wave characteristic. Equation 15 refers to the wave spectrum equation. χ is the angle of approach of the waves to the ship, w_e is the encounter frequency and ω is the wave frequency.

$$w_e = w - \frac{w^2 U}{g}\cos\chi \quad (15)$$

In Figure 2, U_0 implies the speed of the vessel and the generic wave model affecting the ship body is also shown. The used speed of the vessel is $U_0 = 7.3$ m/s, and the approaching wave angle $\chi=90^o$ (beam seas) in this analysis (Naik & Ross, 2017).

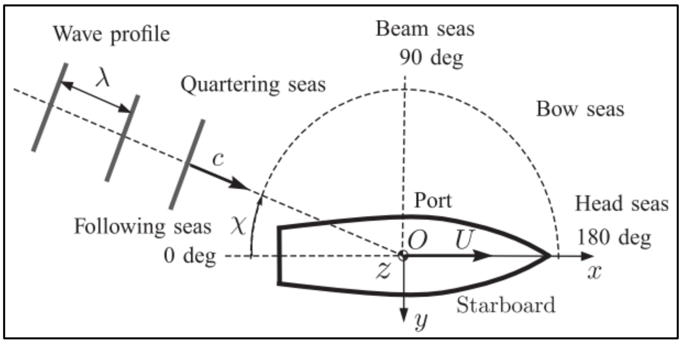

Figure 2. Wave demonstration

In this study, the dynamic behavior of roll motion, rudder and fin have been investigated under the wave effect for the turning trajectory. The fin stabilizer has 5-degree steps and the rudder has a 1-degree step with the purpose of damping the roll motion caused by the turning of the ship and correction of the course. The reason for separated steps is that these control systems consist of mechanical-electronic equipment and need continuous energy usage on the circuit. The control of the rudder and the fin is monitored by the LQT system. When it comes to the insufficient righting moment condition of the rudder, the fin stabilizer is activated with 5-degrees attack angles.

Ship Specifications

The ship model S-175, which is a container ship, is used in this study because of the possibility of finding experimental data and analyzing them in mathematical environment (Son & Nomoto, 1981). The main dimensions of the container ship and stabilizer system specifications are demonstrated in Table 1. The vessel has been modelled as a single-propeller and with a single rudder. To achieve the minimized roll motion, each side of the ship has been equipped with an identical fin.

The body plan of the ship, whose roll motions are examined, is shown in Figure 3.

Typically, the GZ curve is a comparison criterion to analyze for a ship stability is depending on the heel angle. When the ship has a positive GZ value, it tends to restore itself with the righting moment arm when hydrodynamically stimulated by any destabilizing force. Thus, the analysis of GZ-φ curve is an initial and essential step for a ship designer. When a capsize situation occurs due to the degree of freedom of nonlinear hydrodynamic forces, this analysis is halted (Naik & Ross, 2017). The GZ-φ curve of the case ship is indicated in Figure 4.

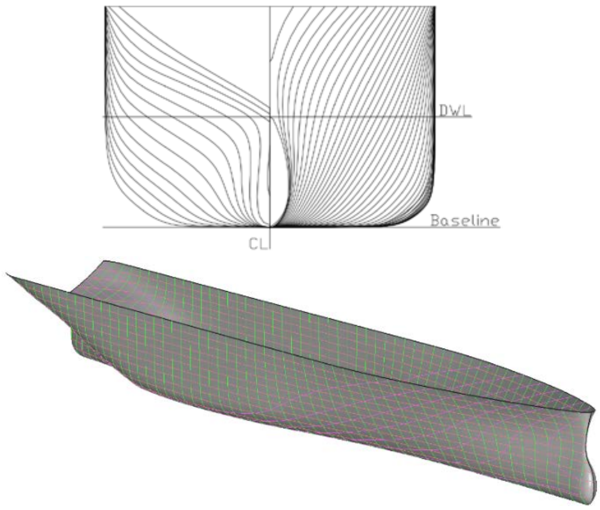

Figure 3. Cross sections (upper figure) and hull surface (lower figure) of the container ship

A fin is a similar construction with a rudder that is positioned usually perpendicular to the hull surface around the bilge. Nowadays, fins are widely used in oil tankers, warships, container ships, ferries and passenger ships. As shown in Figure 5, the fin geometry is positioned in the fluid material at a certain angle of attack. This positioning brings about the lifting force (L) to be formed on the wing (Demirel, 2013).

Table 1. Ship specifications

Main particular of ship		Fin stabilizer system		Rudder specifications	
Length (m)	175	Foil Section	NACA 0015	Rudder area (m^2)	33.03
Displacement (ton)	21752	Chord length (m)	1.5	Max rudder angle (deg)	30
Draft (m)	8.5	Span (m)	6.5	Rudder rate (deg/s)	2
Beam	25.4	Area (m^2)	1.4625	Quantity	1

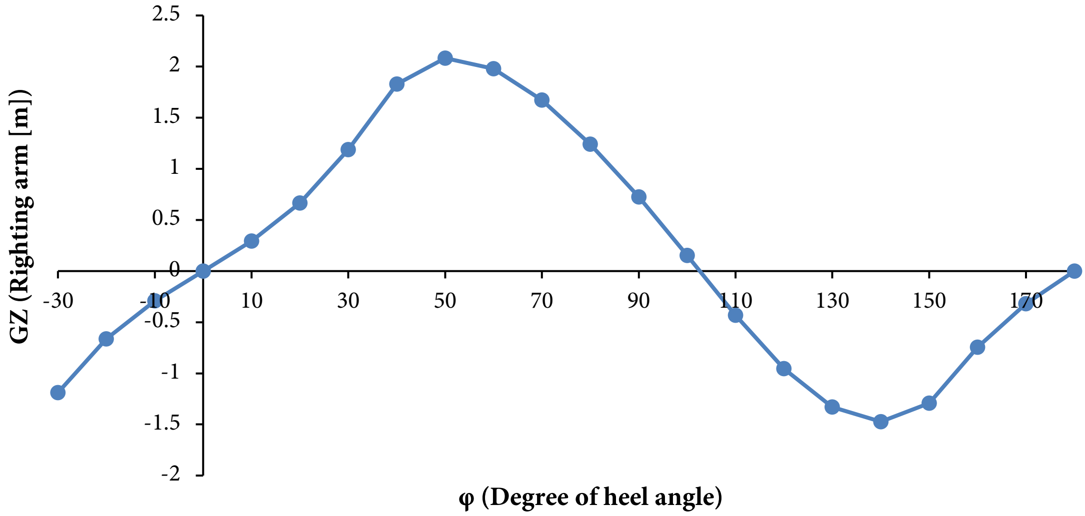

Figure 4. GZ-φ curve for the container ship

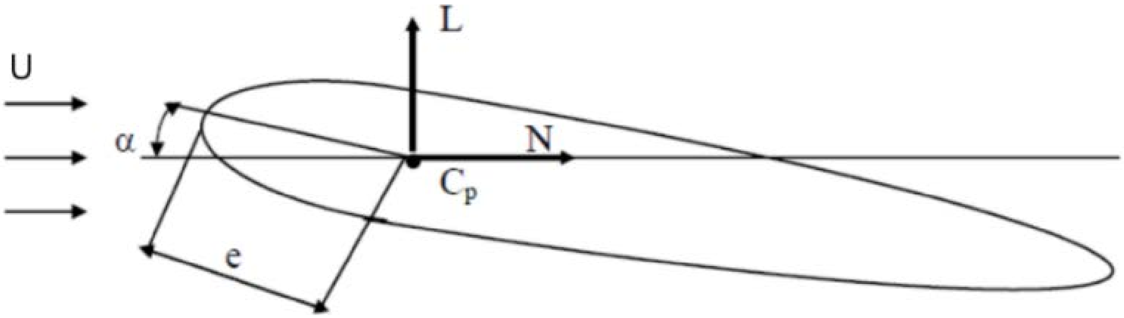

Figure 5. Fin geometry and forces

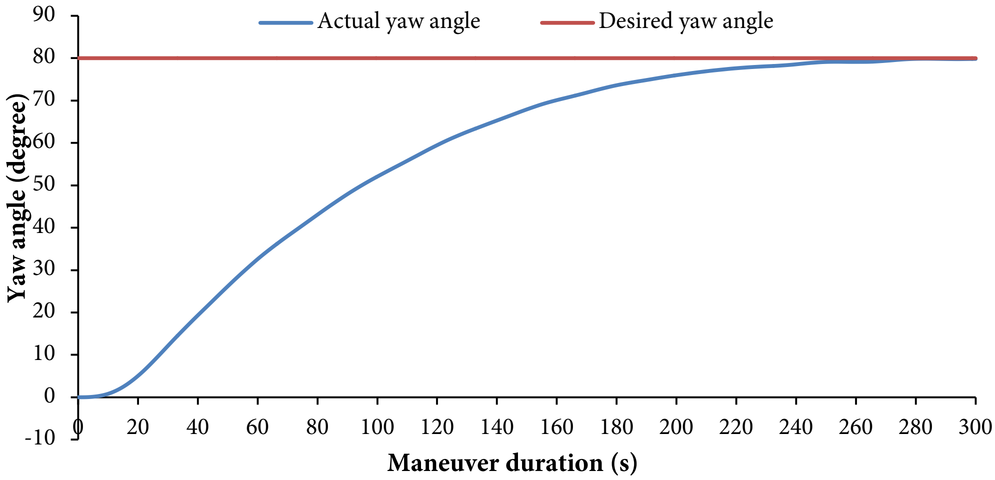

Figure 6. The changing of yaw angle during ship maneuver

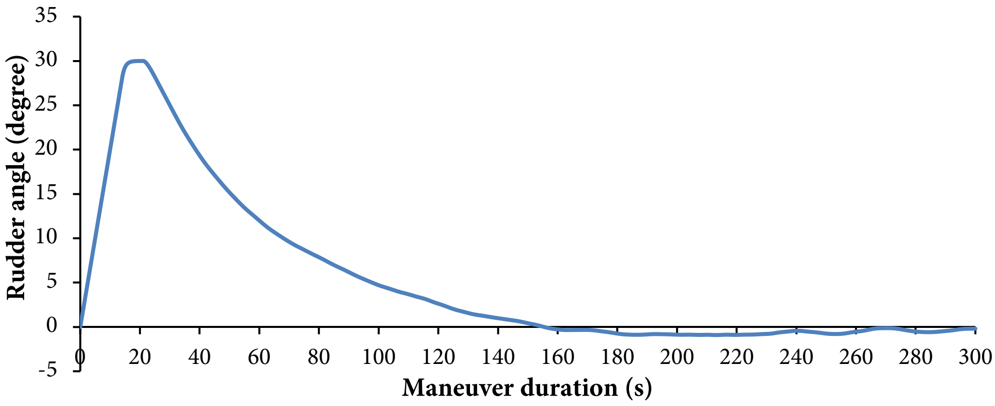

Figure 7. The changing of rudder angle during ship maneuver

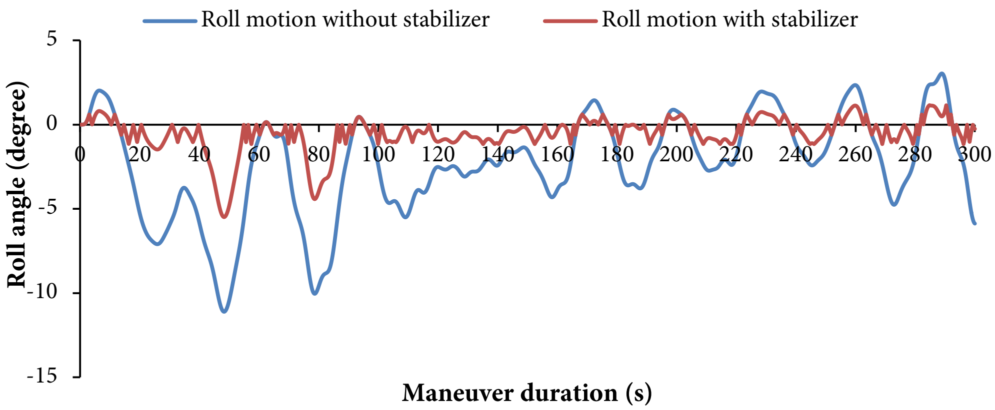

Figure 8. The changing of roll angle during ship maneuver

Results and Discussion

The movement and characteristics of the hull under the sea effect of the ships are related to the hydrodynamics, dynamics and forces. According to the Abkowitz (1969) method, hydrodynamic forces and moments acting on ship body are the unknown nonlinear functions of location, speed and acceleration in six degrees of freedom. Taylor series expansions are used in the calculations of hydrodynamic forces, but the high-order hydrodynamic coefficients are neglected in this expansion (Lihua et al., 2018).

Fin stabilizer has been used to dampen the effect of the forces and moments above mentioned. The geometric characteristics of the fin are shown in Table 1 above. The Mf fin in the equation 14 is the moment of control. In this study, fin lifting forces were calculated for 1025 kg/m^3 sea water density and 14 knot ship speed (Arslan, 2018). In the present study, the fin-stabilizer has been placed symmetrically on both sides of the vessel. The hydrofoil form with a low aspect ratio composes the shape of fins. The hydrodynamic behaviors on the fins can be neglected. In other words, except for the ship's roll motion, the fin systems have no hydrodynamic effect on the ship (Ghassemi et al., 2010).

The mathematical model used in the simulation was performed for the equation and boundary conditions described above. The yaw motion of the case ship to change course has been examined for 300 seconds. The reason that the time is limited to 300 seconds is that the vessel has reached the desired yaw angle of 80 degrees as shown in Figure 6. The motions after that point are about the environmental disturbances to divert the course of the ship. This new emerged problem is out of the scope of this study.

The yaw angle of the vessel for the desired maneuvering angle is modelled depending on time in the graph. The vessel is expected to rotate at 80 degrees for variable rudder angle values. As a result of the mathematical model about 240-250 s desired rotation has occurred.

For the ship to perform the action shown in Figure 6, the rudder must be controlled throughout the maneuver. In addition to asking the ship to provide the movement that will change the course, it is also expected to be a solution to the problem of roll damping. In the first stage, the equipment assigned to do this task is the rudder. The movement of the rudder along the 300-seconds route changing period is shown in Figure 7.

The rudder used for the desired maneuvering angle has a maximum rotation angle of 30 degrees in port and starboard directions. This feature varies from ship to ship and is in the maximum range of 30-35 degrees. The rudder of the vessel is constantly kept under the control to obtain desired maneuvering angle. In the beginning of the maneuver, it has been assumed that the ship does not have any deviation of its forward direction and that the rudder angle has 0 degree. The rudder angle reaches its maximum value in the 20th second after the maneuvering has been started and then it moves in the opposite direction after that point to about 160th second.

Figure 8 shows the change of the roll angle of the ship during the maneuver period is seen in the problem created by the ignoring of the effects of the wave except for turning direction. In Figure 8, two graphs are given together to be able to compare the roll angles obtained by without and with using a fin stabilizer.

In Figure 8, the higher amplitude drawing consists of two separate effects of the rudder. The first is that it provides the desired rotation angle, and the other is that the roll angle occurring during this rotation is minimal under the current conditions. Since the roll motion in the current conditions could not be reduced sufficiently by the rudder, the mitigation effect of the fin control system to the roll motion has been shown with decreased amplitude graph.

Conclusion

In this study, it has been investigated the hydrodynamic forces, which is affected by wave, and the rising of roll motion during turning motion is controlled by fin and rudder interaction using LQT controller. To get an easier solution under wave effect turning motion, 6-DOF ship motion has been reduced to 3-DOF. As a result of the assumption that the wave is approaching perpendicularly to the ship hull, these three neglected movements are heave, pitch and surge. However, while the ship has a constant forward speed, it is indicated by the formulas when the speed component deviates with the effect of the wave while turning. Also, in motion equations, that deviated speed has been considered. Under the specified boundary conditions, the following findings have been obtained from the results of the roll motion, rudder motion and yaw angle of the maneuvering vessel:

- Yaw angle simulation results show that desired turning motion has been got by a smooth course,
- The first 10 seconds of the turning motion have elapsed for reaction of inertia, for this reason the changing of yaw angle has a slight difference with initial condition,
- It has been seen that the rudder has been rotated by the maximum angle to the turning direction to overcome this inertia. Then, in order to ensure smooth change in the yaw angle, the rudder has been slightly rotated on the reverse direction,
- Maximum values of the roll angle are reduced to about half by the rudder and fin controller system also both motions have similar roll characteristics. This shows that LQT controller can be a practical choice for ship control systems.

Different controller systems should be applied to get an efficient ship operation as well as to conduct comfortable and safe ship operations during maneuver period. In future studies, this model can be developed and the applicability of alternative control systems to irregular.

Compliance With Ethical Standards

Authors' Contributions

BG: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Writing - review & editing,

KB: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Writing - review & editing,

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

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REVIEW ARTICLE

Population, aquaculture and transplantation applications of critically endangered species *Pinna nobilis* (Linnaeus 1758) in the Mediterranean Sea

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ABSTRACT

The population of fan mussel, *Pinna nobilis* across the Mediterranean Sea has been affected by factors such as overfishing, fisheries processes, environmental pollution, destruction of habitat, tourism, etc. Therefore, the species *P. nobilis* was taken under protection by the Decisions of the Council of Europe and Barcelona Convention. However, its mortality rates of 100% have been reported to be due to *Haplosporidium pinnae*, a parasite in different Mediterranean regions. The status of *P. nobilis* has thus been revised to be reduced from "Vulnerable" to "Critically Endangered" and the importance of all the studies on the species further increased. The aim of the study is to present the current status of *P. nobilis*, the native to the Mediterranean, by combining the relevant studies on ecology, aquacultural process (larvae, spat settlement and rearing), culture methods and transplantation. The present study has provided comprehensive knowledge on the current status *P. nobilis* population, aquaculture and transplantation activities. Except for studies to determine stocks, in particular, those on collecting young individuals from nature and planting and growing them in predetermined sites as well as their production through various cultures from their larval phase onwards are of great importance in terms of rehabilitation and sustenance of the damaged *P. nobilis* population. Therefore, alternative and potential habitats should be created thanks to transplantation and aquaculture. Marine protected areas should be determined to enable a healthy *P. nobilis* population to be sustained.

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Introduction

Fan mussels under the classis of Bivalvia are represented by the species of *Pinna nobilis* (Linnaeus 1758), *Pinna rudis* (Linnaeus 1758) and *Atrina fragilis* (Pennant 1777) in the Mediterranean basin. *A. fragilis* has distributed in southeast Africa, New Zealand, North Japan and East Coasts of Atlantic and *P. rudis* across East and West Atlantic, North American and South European shores while *P. nobilis* is native to the Mediterranean Sea. The species of the Pinnidae family are called fan mussel and noble pen shell. In recent years, *P. nobilis* population has significantly decreased along some Mediterranean seashores of France, Greece, Spain, Italy and Croatia (Vicente & Moreteau, 1991; Catanese et al., 2018). In other regions, its population density decreased from 17 individuals per 100m^2 (Richardson et al., 1999) to 1 individual per 100 m2 (Katsanevakis & Thessalou-Legaki, 2009). The future of the species has been established to be "Endangered" for the reasons below;

- various human activities have caused the destruction of coastal habitat,
- increased water pollution,
- damage due to fisheries activities,
- popular consumption of its delicious white muscular meat
- uses of pearly shells in weaving decorative and byssus threads into cloths,
- deep anchor dragging from boats to the destroy fan mussels to death,
- its usages as angling bait to lure and catch fish (Vincente & Moreteau, 1991; Hendriks et al., 2013; Deudero et al., 2015; Capó et al., 2015).

Considering the above all, *P. nobilis* has been listed as the species endangered in the Mediterranean ecosystem and thus taken under strict protection according to Annex II of the Barcelona Convention (SPA/BD Protocol 1995) and the European Council Directive 92/43/EEC (Annex IV). In addition, the central marine zone and southernmost coasts of the Iberian Peninsula (Spanish Western Mediterranean) showed a 100% mortality in all length groups of the population (Vázquez-Luis et al., 2017). The status of the species was therefore reconsidered to be increased from "Vulnerable" to "Critically Endangered" at national level by the Spanish Sectorial Environmental Conference on July 17/2017. Darriba (2017) was the first to report the presence of haplosporidan parasite as the earliest histopathology. *Haplosporidium pinnae*, a species of *Haplosporidium* parasite in particular has significantly damaged *P. nobilis* population over the last five years. Occasional western Mediterranean and the Tyrrhenian Sea in Italy have recently exhibited 100% mortalities (Catanese et al., 2018; Carella et al., 2019). Moreover, Katsanevakis et al. (2019) performed a study on mass mortalities of *P. nobilis* in and around the island of Lesvos in the Northern Aegean Sea However, many surviving individuals have been documented in France Port-Cros Archipelago Marine Protected Area (Ruitton & Lefebvre, 2021), Thau Lagoon (Foulquié et al., 2020), and the East of Corsica Diana Lagoon (Simide et al., 2019), in Spain Alfacts Bay (Ebro Delta) (Prado et al., 2020) and in Greek Kalloni Gulf and Laganas Bay (Zotou et al., 2020). Different stations in the Aegean Sea coasts of Turkey were recorded to exhibit 100 % mortalities (Acarli et al., 2020; Öndes et al., 2020) whereas the Aegean and Marmara mouths of Çanakkale Strait showed mortalities of 100% and 9.2% in 2020, respectively (Acarli et al., 2021a). Künili et al. (2021) mention that *H. pinnae* infected *P nobils* individuals and caused death in Çanakkale Strait. In addition, Cinar et al. (2021) indicated that at seven locations, a total of 191 *P. nobilis* individuals were discovered, with 88% of them being dead in the Marmara Sea. The authors claimed that the cause of mortality could be linked to an epidemic sickness or a catastrophic mucilage occurrence after November 2020. Recently, Acarli et al. (2021b) investigated the effects of the mucilage event on the population of critically endangered *Pinna nobilis* in Ocaklar Bay (Marmara Sea, Turkey) and the authors documented that the mortality rate was found 35.96% before the mucilage event while it was calculated as 16.12% after the mucilage event in the study area. Acarli et al. (2021b) asserted that the *P. nobilis* population could be resistant to extreme environmental stress and even juvenile individuals (smaller than 15 cm) were recruited in the study area during the mucilage event.

The intense amount of mucilage organic matter due to planktonic algal bloom was first reported in mid-autumn 2007 for the Marmara Sea (Aktan et al., 2008). However, mucilage was observed to be more intense in the Marmara Sea this year than in the previous years (Balkıs-Ozdelice et al., 2021). The planktonic algal bloom occurs as the result of higher seawater temperatures, agricultural activities, domestic and industrial waste discharges, and overfishing (Flander-Putrle & Malej, 2008; Savun-Hekimoğlu & Gazioğlu, 2021). Benthic organisms are the most affected by the mucilage sunken or accumulated on the bottom (Schiaparelli et al., 2007). It has been observed that *P. nobilis* covered of mucilage, one of the benthic organisms in the coast of Erdek-Ocaklar Bay in the south of

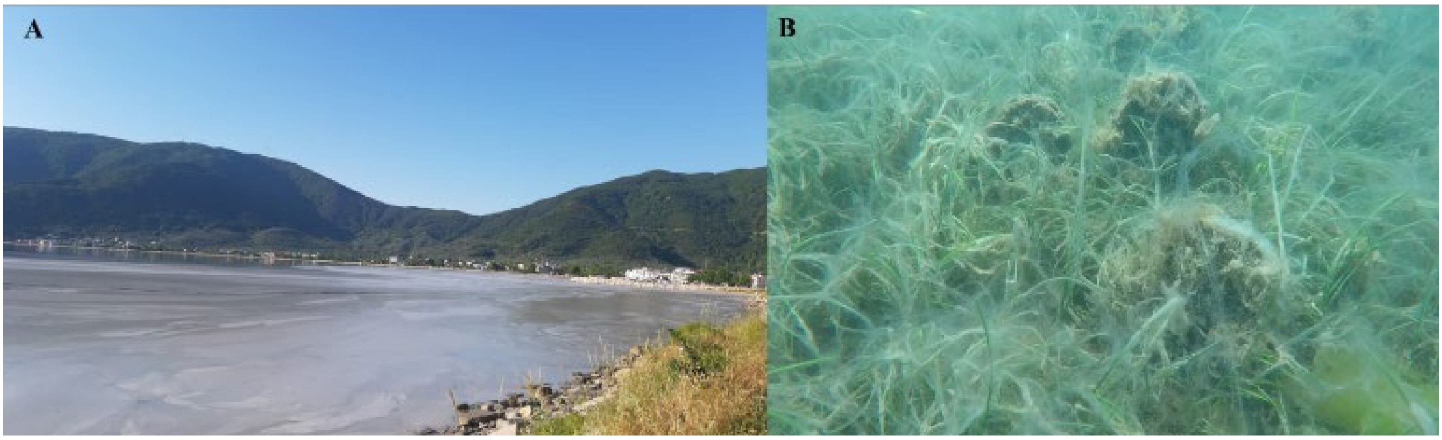

Figure 1. Mucilage on the sea surface of the Erdek-Ocaklar Bay in May, 2021 (A) and underwater view of mucilage on *P. nobilis* and *Posidonia oceonica* in the Erdek-Ocaklar Bay in June 2021 (B)

Marmara Sea in March 2021 (Figure 1), effects of which this event will later be understood more clearly.

P. nobilis hosts various species of microorganisms thanks to the width of its surface area and structure of calcium carbonate as well as its individual contributions (Acarli et al., 2010). The associated studies found that species of Bivalvia, Gastropoda, Ascidiacea, Polyplacophora, Echinodermata, Demospongiae, Gymnolaemata, Maxillopoda, Polychaeta, Cyanobacteria, Anthozoa and macroalgae were covered on the shells of the *P. nobilis* (Giacobbe, 2002; Addis et al., 2009; Acarli et al., 2010; Rabaoui, et al., 2015). The present study exhibited that any damages to *P. nobilis* population expose to macrobenthic species on its shell to danger as well.

The purpose of the present paper is (i) to summarize the basic information to recognize the species, (ii) to define the culture methods applied considering other pinna species and to reveal the importance of its aquaculture in conservation programs, and (iii) to define the role and importance of transplantation studies as part of rehabilitation programs.

General Characteristics of Fan Mussels

Morphology

Pinnids are of a triangular shape due to their adaptation to tying to the substrate via byssal threads (Figure 2). Generally, the shell has many functions, including serving as a skeleton for attaching muscles, protecting against predators and in burrowing species, and helping keep mud and sand of the mantle cavity (Gosling, 2003). It is composed of three layers: an outer horny periostracum and two calcified layers beneath it. The inner layer is consisting of aragonitic nacre, which is only found in the anterior part of the shell (García-March & Vicente, 2006). The ligament is located in the dorsal region with the mere function to hold the valves together. The ventral part of the shell has a small opening for the byssus to exit. Byssus consists of many filamentous fibrils that could grow up to 16 cm. Byssal extensions have sticky discs on each (de Gaulejac, 1993).

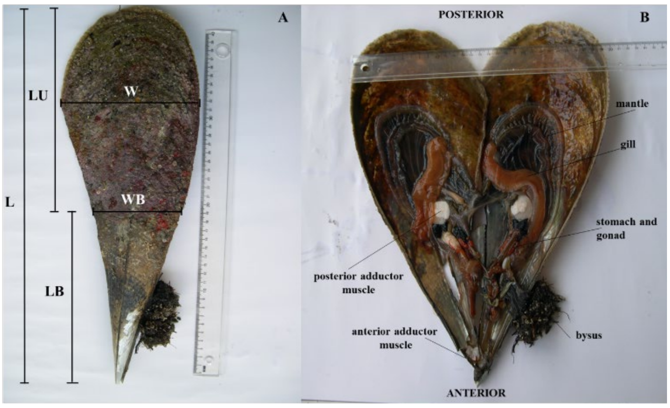

Figure 2. Morphometric parameters (A) and internal organs (B) of *P. nobilis* (*L*: total length; *LB*: bury in shell length; *LU*: unbury in shell length; W: width; *WB*: bury in shell width)

Members of the Pinnidae have two adductor muscles: the large posterior adductor is powerful and centrally located in the shell and the small anterior one is located at the umbonal vertex (Grave 1911; Coronel, 1981 in Escamilla-Montes et al., 2017). The posterior adductor and posterior foot retractor muscles are so close to each other that they appear to be one muscle (Czihak & Dierl, 1961 as cited in Basso et al., 2015). The mantle is not attached to the shell as occurs in other species (e.g., some Mytilidae) and therefore it is very retractable and without a pallial line (García-March, 2005).

The Pinnidae are characterized by waste channels, the ducts through the mantle in an antero-posterior direction from the palps to the of the mouth to the contact with the posterior-dorsal end of the gills, in which they combine with the inner lobe of the mantle edge to finally complete the division between the inhalant and exhaling chambers. These channels allow the removal of pseudo feces and other debris from the inhalant chamber and can be used to clean the cavity of sand and debris introduced by the waves during storms before closing the leaflets (García-March, 2005).

P. nobilis' functional morphology, in particular the unique pallial organ, the equally unparalleled buccal (previously pallial) gland, stomach and its contents are described (Morton & Pulijas, 2019). The function of the pallial organ has been controversial over time. It was previously believed to be a cleaning organ responsible for extracting the broken pieces of the shell in the pallial cavity (Yonge, 1953). However, later, it was discovered in *Atrina pectinata* that the organ proves thigmotropic and highly secretory, with a pH in its head of 2 to 4, and it is therefore supposed to have a defensive function, to protect the animal from the entry of epibionts and predator attacks (Liang & Morton, 1988). In addition, Morton & Pulijas (2019) found that the pallial gland produces sulphuric acid that engaged in prey capture and function as a shell cleansing pad.

Habitat

Fan mussels are generally brown, transparently beige to white and yellow in color and fanlike in shape. They are known to be able to live in the depth of 60 m regardless of variations in species and age. Umbonally attached to and partly embedded in the ground by their byssal threads (Figure 1), they inhabit areas of sandy, sandy pebbled and muddy seabed through seaweeds, *Posidonia oceanica* (Figure 3) (Tebble, 1966; García-March & Vicente, 2006).

Though variable in size, their lengths range from 20 to 40 cm (Fischer, 1987). *P. nobilis* is known to live until 45 years with a maximum (Rouanet et al., 2015) length of 120 cm (Zavodnik et al., 1991). It is necessary to count adductor muscle scar rings inside the shell to determine the age of an individual fan mussel. Because the first year-scar ring is invisible or too vague to notice, the age of the fan mussel is determined by adding 1 age to already counted scar rings (Richardson et al., 1999).

Nepinnotheres pinnotheres (Crustacea: Decapoda) (Linnaeus 1758) is known as pinna pea crab brown in color (Hayward & Ryland, 1995) (Figure 3), which is a parasitic species found in the mantle cavity of *P. nobilis* (Becker & Turkay, 2017). The adult female is significantly bigger than the mature male (Becker & Türkay, 2017). *N. pinnotheres* is believed to live within *P. nobilis* as a temporary refuge. Therefore, more than one male pinna pea crab has been encountered within the same *P. nobilis* individual (Rabaoui et al., 2008). Acarlı et al. (2019) reported that *N. pinnotheres* did not directly influence the intact *P. nobilis* since damages to the tissue of the infected fan mussels were in the process of the study. On the other hand, the same researchers noted that the physical condition of fan mussels might be threatened by adverse environmental conditions or invasion of infectious diseases such as mycobacterium and haplosporidian parasites.

Feeding

P. nobilis is a suspension feeder or filter feeder organism that feeds on suspended organic and inorganic matters, zooplankton, phytoplankton, bacteria, and viruses in the water column (Gosling, 2003; Davenport et al., 2011; Najdek et al., 2013; Trigos et al., 2014). Thus, it contributes to the improvement of water quality, playing a vital role in the ecology of the Mediterranean Sea (Natalotto et al., 2015). Gills are equipped with functions of feeding and respiration. Food particles captured by the mantle and gills and covered with

Figure 3. *Pinna nobilis* on the seagrass meadows (*Posidonia oceanica*) in the Karantina Island (İzmir Bay, Aegean Sea) in 2008

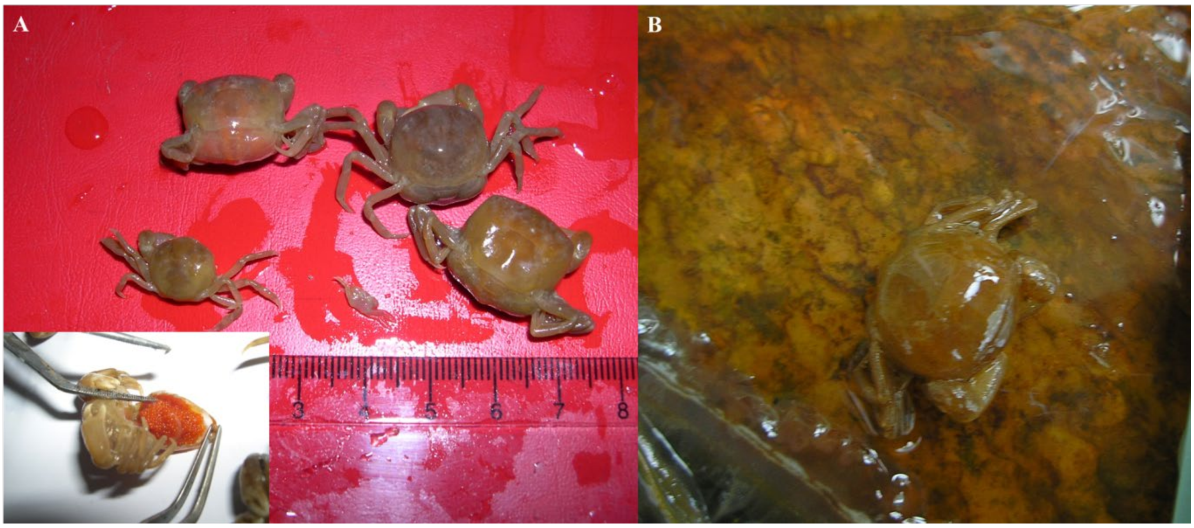

Figure 4. Photographs of *N. pinnotheres* (A) and *N. pinnotheres* on the *P. nobilis* inner shell (B)

mucus are transported by actions of cilia in the gills to labial palps through which they would be selected and processed. While discarded particles are moved to the sides of the palps, those suitable to be ingested are carried to the mouth, from which they pass through the stomach where they are digested by digestive enzymes. Indigestible part is passed through intestines and discharged out of the anus (de Gaulejac, 1993) (Figure 4).

Species of fan mussels do not have a siphon mechanism but a pallial cavity instead, in which inhalant and exhalant chambers are completely separated by lamellibranch gills. In the absence of siphons, the mantle cavity and large posterior extension of the shell serve the function of siphonage (Butler et al., 1993). The species of *P. nobilis* keep its valves open as a gaping process in order to filter the water around it, which shows that an individual fan mussel has been in the process of feeding and respiration thanks to its wide-open valves. However, insufficiently gaped or completely closed valves imply that filtering has decelerated or ceased. The rate of filtration and valve gaping is determined by the existence of factors such as particle concentration, temperature, salinity, and stress (Gosling, 2003). Butler et al. (1993) reported that the species of *P. nobilis* can inhabit oligotypic environment, clean water and little resuspension of sediment. Sediment loading which harms gill cilia can affect activities of feeding and respiration (Katsanevakis, 2005; García-March et al., 2008; Coppa et al., 2010). Garcia-March et al. (2008) found that the gaping activity of *P. nobilis* is directly influenced by current intensity and direction rather than factors such as temperature, dissolved oxygen, turbidity, and chlorophyll-*a*. The gaping activity of the population is usually synchronous and a periodic cycle from 21.9 to 24h of gaping activity occurs in all *P. nobilis* individuals during the year.

Aquaculture

Because species of fan mussels such as *Pinna bicolor*, *Pinna rugosa*, *Atrina pectinata* and *Atrina maura* are popularly consumed and marketed for high prices in Pacific and Asian countries (Cendejas et al., 1985), they are of commercially great potential. As a result, studies have been made on the aquaculture production of the species in order that its sustainable output should be obtained and natural stocks are protected.

As the species of *P. nobilis* has currently been under serious danger of extinction, in particular, the presence of aquaculture output, as well as control measures, is required in terms of protection of the species and regeneration of the stocks in the habitat. As a consequence of the diseases, stocks of *P. nobilis* have been on the brink of disappearance in most locations of the Mediterranean Sea. The fact that disease-free locations are determined where production is to be restarted requires the collection of spat from the environment and rearing larvae and thus juveniles.

Reproduction and Maturation

No studies have been encountered on determining the reproduction period of *P. rudis* and *A. fragilis* like *P. nobilis*. *P. bicolor* is gonochoristic (Roberts, 1984) whereas *P. nobilis* is of successively (alternately) hermaphrodite characteristic with its gonads developing through digestive organs. The color of gonads varies from light orange to dark brick hue, which is however not indicative of sexuality or any sexual phases (Figure 5) (Acarlı et al., 2018). Having matured, reproductive cells are

Figure 5. Gonadal appearance of *Pinna nobilis* (original)

water columns where the process of larval development is later completed (de Gaulejac, 1993). The reproductive cycle in fan mussels is influenced by endogenous factors such as energy reserves and genetic elements (Lee et al., 2015; Wang et al., 2017) and exogenous factors including variations in temperature, photoperiod and food quality and quantity (Baik, et al., 2001; Angel-Dapa et al., 2015; Qui et al., 2014; Gongora-Gomez et al., 2016a). Reproductive periods of *P. nobilis* in different regions are presented in Table 1. Histological studies show that the reproductive period of *P. nobilis* occurs when the temperature has been high. *P. nobilis* can be cited to be a summer breeder species according to the classification by Boolotian et al. (1962) considering spawning in terms of comparison of spawning periods (Table 1). These differences may occur due to depth, thermocline position, degrees of latitudes and other regionals (Hernandis et al., 2018).

Table 1. Spawning periods of *Pinna nobilis* in different regions

Locality	Spawning period	References
France	June-August (26°C)	de Gaulejac (1995)
Spain	May-August (20-26°C)	Deudero et al. (2017)
Turkey	May-September (24-26°C)	Acarli et al. (2018)

As for the collection of sexually mature individuals, it is necessary to choose periods when condition index is high and muscle index low (Acarli et al., 2015; Angel-Dapa et al., 2015; Acarli et al., 2018). Maeno et al. (2009) and Chung et al. (2006) reported that the maturation progress of fan mussel broodstocks in suspension culture resembled that of those in natural beds. Angel-Dapa et al. (2015) informed that gamete quality, in other words, broodstock condition and origin (depth, phases of tidal cycle), as well as environmental conditions, could have effects on larval survival and growth rates in the studies of larval production. Species of microalgae such as *Pavlova* sp. and *Chroococcus* sp. have been found to be effective nutrient sources on the maturation of adult individuals. 18: 1n-9 content is related to condition index and gonad index and can be used as the degree of maturation for gonads in them (Qiu et al., 2014). To enable the species under protection to sustain, rearing processes should be carried out. The first stage is therefore to determine the period of reproduction.

Production of Larva

When spawning has occurred, external fertilization of sperm and egg takes place and larval development from trochophore to pediveliger phase is planktonic with eventual settlement and metamorphosis (de Gaulejac & Vicente, 1990). Mature fan mussel individuals collected from nature are exposed to temperature shock (Angel-Dapa et al., 2015; Trigos et al., 2018), hydrogen peroxidation (McCoy & Chongpeepien, 1988) or UV (Yang et al., 2006) to obtain their gonadal cells.

The eggs having been incubated, larvae are fed on phytoplankton species of *Isochrysis galbana*, *Tetraselmis* sp., *Chaetoceros calcitrans*, *Pavlova lutheri* in such a way to be 5000cells/ml for about 5-6 weeks to continue until the juvenile phases (Kawahara et al., 2004). However, the length of the larval period could vary according to the species, larva and mature quality and food (Gosling, 2003). Ohashi et al. (2008) were the first to successfully study the process of growing larvae and juveniles of *A. pectinata* in indoor tanks, though the study showed that the rate of survival was not desirable upon fertilization, hatching and attachment. During the latter, the rate of survival and length of spats were 0.004 % and 514.1±25.9 µm, respectively.

Most of the studies on *P. nobilis* include those on population. Although a limited number of studies have been made involving juvenile production and growth, the first larval output process was performed by Trigos et al (2018), which involve the process from egg fertilization to pediveliger stage and tried different rearing tanks, larvae density, light conditions and food of amounts to determine the optimal condition for larval development. The results showed that 16 L tanks with a concentration of 2 larvae ml, 21°C constant temperature, 12/12 h photoperiod and feeding on an optimal mixture of 25 cells per µL of *Chaetoceros calcitrans* +33.3 cells per µL of *Pavlova lutheri* + 100 cells per µL of *Isochrysis galbana* seem to be the best conditions to rear *P. nobilis* larvae. The larva achieved the stage of pediveliger in 7 days with a length of 110 µm. However, it failed to metamorphose by the end of 22 days. The problem seems to be that Vibrio is suspected to have caused mortalities over 80 % during the first 2-9 days based on light conditions and food doses. The process of larval culture exhibited low survival rates and it was thus interpreted that hydrophobicity of pediveliger larvae resulted in their buoyancy on the water with eventual dehydration and/or starvation (Robles-Mungaray, 2004 as cited in Gómez Hernández, 2011) (Maeda-Martínez, 2008; Angel Dapa, 2015). Pediveliger larva has a thin and fragile shell and gradually stores lipids with the result that it has a positive buoyancy prior to metamorphosis (Maeda-Martínez, 2008). Successful completion of the larval stage with the metamorphosis of *P. nobilis* can only come true due to the formation and development of the culture hatchery protocol (temperature, salinity, density, feeding strategies, tank cleaning, water change, etc.).

Spat Collection

First young individuals obtained after the larval period are called spats with transparent shells and interior organelles visible to the naked eye (Figure 6). Yielding of spat collection is affected by environmental factors such as temperature, currents, water depth and type of collector material in particular (Narváez et al., 2000; Saucedo et al., 2005; Yıldız et al., 2005; Yildiz et al., 2013; Halla et al., 2018). A variety of natural and artificial collectors are used to obtain bivalve spats, including monofilament gillnets, PVC glass, onion bag, netlon, bivalve cultch (oyster, mussel, cardium, etc.), wood, fiberglass, car tires and tiles (Lök & Yolkolu, 1999; Helm & Bourne, 2004; Buitrago & Avarado, 2005; Velasco & Barros, 2010; Soria et al., 2015; Gregori et al., 2019). Although numerous collector designs and materials have been thus far tested, the productivity of a collector generally vary based on the species, the region and the duration of soaking the collector. Of such collectors, the most efficient are nylon filaments or onion bags made of polyethylene mesh for collecting fan mussels (Cendejas et al., 1985; Beer & Southgate, 2006: Kersting & Hendriks, 2019). The time of planting collector is generally important. Once it has been planted before reproduction, unwanted or untargeted species attached to the surface of the collector and the area to which species could attach would thus decrease. However, when it has been planted upon spawning period, its efficiency lowers then the rate of collecting target species drops as well (Lök & Acarli, 2006; Yıldız et al., 2010; Yiğitkurt et al., 2017). The most available period for *P. nobilis* to attach is the summer months when temperature and presence of nutrients are high (Acarli et al., 2011a; Theodorou et al., 2015). On the other hand, the rate of attachment close to the water surface was found to be much better than in the depth of 8 m (Kurtay et al., 2018). Therefore, pelagic bivalve larvae tend to swim up to surface water where food concentration is high (Bayne 1976). Bivalve larvae exhibit a negative phototropic behavior to attachment when they avoid light and prefer sheltered substrates in shadow areas (Baker, 1997; Saucedo et al., 2005; Lök & Acarli, 2006). In this context, Acarli et al. (2011a) observed that the spat settlement of *P. nobilis* was significantly higher within mesh collectors (92%) than their exterior.

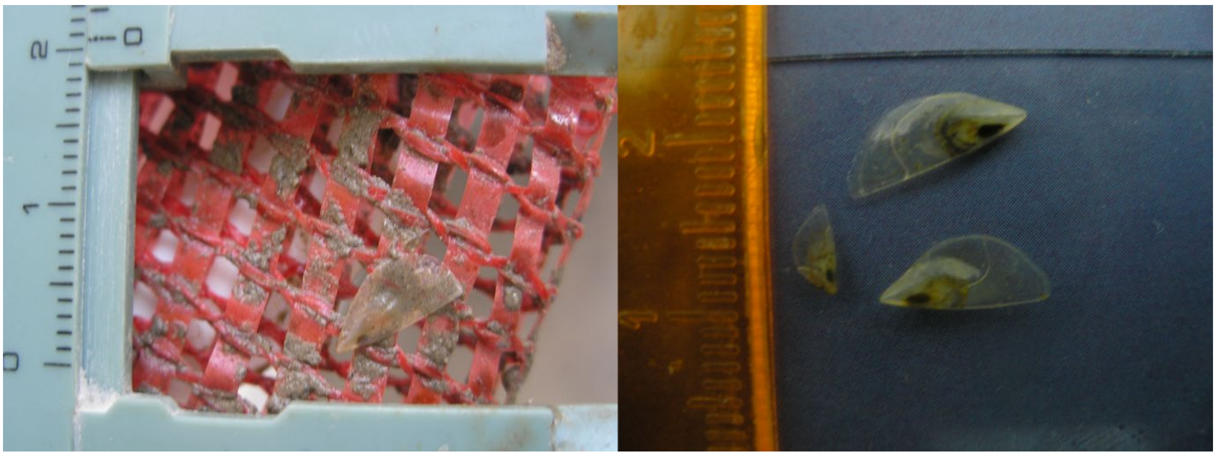

Figure 6. *P. nobilis* spat collection trials (Acarli et al., 2011a)

Culture Study

All other species of the Pinnidae family including *P. nobilis* and *P. bicolor* are those whose shells grow the fastest in all bivalve species (Richardson et al., 2004). The upper section of the shell in water grows faster than the lower part on the bottom. The ones to inhabit deep water show a slower growth but achieve greater length than those in shallow water (García-March et al., 2007). The growth of the Pinnidae family is influenced by food quality and quantity (Safi et al., 2007; Acarli et al., 2011b), temperature (Leyva-Valencia et al., 2001), salinity, reproduction (Narváez et al., 2000), pollutants (Góngora-Gómez et al., 2015), age and shells size (Demirci & Acarli, 2019). Of those, food supply and optimum temperature are considered to be the most important for bivalves without which sustainable growth is difficult or almost impossible. The relationship between shell length and the total weight *of P. nobilis* was described with the equation $W=0.003L^{3.6451}$ ($r^2=0.9625$), the result of which revealed that there was a positive allometric growth between shell length and total weight ($P<0.05$) (Acarlı et al., 2011b).

The juveniles are observed to grow faster as in other bivalve species but growth shows a deceleration later on both in culture and in situ growth studies (Katsanavakis, 2007; Aucoin & Himmelman, 2011; Demirci & Acarli, 2019). The rearing studies are generally based on the principle that the cultivation of juvenile individuals obtained from natural environments should be performed on suitable grounds. Culture trials and choice of system and field involve important criteria such as growth, mortality, meat yield, condition factor and biochemical composition of fan mussels (Arizpe, 1995). The process involves that the techniques of suspended culture and bottom culture are employed considering the area where individuals would be placed in the water column.

Suspension Culture

The purpose of the floating (long line or float raft) is to increase the amount of yield by means of excessive water volume and get rid of the pressure of predation present on the bottom as well, for which juveniles are placed in the materials such as boxes, tray pearl baskets, lantern nets, etc. hung down from the system above. On their bottom is placed sufficient amounts of sand into which fan mussels could be buried and planted (Wu & Shin, 1998) or instead rearing process can be performed without placing sand in the same materials cited above (Acarlı et al., 2011b) (Figure 7). Values of shell width, dry tissue weight and condition index for *P. bicolor* juveniles (30-40 mm shell width) in raft culture were found to be higher than those on the sea bottom (Wu & Shin, 1998). Koźul et al. (2011) reported that *P. nobilis* grew very rapidly and reached from 29 mm to 157 mm over a year. Kozul et al. (2013) also placed the cages with juveniles at three depths: 1 m, 3 m and 5 m to finally see that following the two-year growth period the average length was 244±22.9 mm at 1m, the specimens averaged 244±25.3 mm at 3 m and the average length was 231.1±22.5 mm at 5 m. The rearing processes within cages managed to solve some problems primarily caused by human activities for purpose of improving natural fan mussel populations. Beer & Sauthagate (2006) placed *P. bicolor* species in pocket panel nets to find that they grew from 75.5±1.19 mm to 175.5 ± 3.9 mm in hinge length by the end of an 80-week period when the deceleration of the growth was associated with the reproduction activity. There was a positive effect of the suspended culture system and pocket panel nets on survival with a 78 % rate at the end of the study. Because the suspended system generally makes it possible to work at a given distance from the ground, the pressure of predators on juveniles decreases while the rate of survival increases (Wu & Shin, 1998; Leal-Soto et al., 2011).

Figure 7. Suspension culture trials of *P. nobilis* (Acarli et al., 2011b)

Having collected *P. carnea* individuals, Velasco & Borrero (2004) stocked them at two different concentrations of 10% and 20% in pearl oyster rearing nets and left them in a 9 m depth. By the end of the study, the ones of 10 % and those of 20% concentrations grew to 167.5 mm and to 156.5 mm, respectively. There were no differences in survival rates between the two groups. Unlike other studies, early in the study, predator pressure (Cymattidae, Majidae, Portunidae and Xantidae) was cited and mortalities were encountered due to predation pressure as well as environmental factors. A suspended culture system requires basically higher investment in facilities, sophisticated equipment and labor force, which is of greater cost and thus supposed to be regularly controlled (Leal-Soto et al., 2011).

Bottom Culture

Post-settlement and juvenile mortality in benthic invertebrates are high because of predation (Aucoin & Himnelman, 2011). The ability to perform protected and unprotected systems on the bottom-related cultures is based on the availability of the predator population. The posterior portion of fan mussel species vertically rises over the bottom surface whereas its anterior part is buried into the bottom sediment, which could cause exposure to predators (fish, octopus, crabs, muricid, gastropods, etc.) (Yonge, 1953). Juveniles have relatively thin shells. If there is too much presence of predators likely to harm juveniles in the environment, they should be protected against predation. Kersting & Garcia-March (2017) reported that plastic frame-casing of individuals could result in the reduction of influences of currents and pressure of predators. After fan mussels have been planted into the bottom in the protected systems, net cages of suitable mesh size are placed to cover over in order to prevent predators from reaching there. Unprotected systems just involve the young to be planted into predetermined rearing spots without any protective cages.

A. maura were cultured in the sand bottom of 36 fan mussels/m^2 each in Ensenada Pabellones lagoon system (<10 m deep), Mexico, about which any protective systems were not cited at all. A one-year-culture study showed that the rate of survival was 91.66% with the shell length growing from 28.41±4.69 mm to 218.16±12.41 mm. The study also indicates that the presence of predators in the water column affects the survival rate. In this sense, it is important to choose the area in rearing trials as well. Some authors pointed out that the bottom culture of bivalves had lower growth rates and higher predation loss than the suspended culture Emerson et al., 1994; Barbeau et al., 1996; Lodeiros et al., 2002). However, the pressure of predators can be decreased by choosing an available region or some means to be used for protection. In this sense, it is important to choose the area in rearing trials as well. Some authors pointed out that the bottom culture of bivalves had lower growth rates and higher predation loss than the suspended culture (Emerson et al., 1994; Barbeau et al., 1996; Lodeiros et al., 2002). However, the pressure of predators can be decreased by choosing an available region or some means to be used for protection.

Suspended and Bottom Cultures

Studies on the combination of suspended and bottom cultures for fan mussels to be reared were reported by Miranda-Baeza (1995), and Cardoza-Velasco & Maeda-Martinez (1997). In addition, Leal-Soto et al. (2011) cultured *A. maura* juveniles by combining both production systems. The individuals were first placed into plastic trays covered over with 2 mm plastic mesh and suspended by long line systems. Shell lengths of 1300 juveniles were 15±5 mm early in the trial and kept in the system until they grew to a mean of 32.8±6.7 mm for two months. The survival rate in long line system was 90%. The juveniles were later placed on a location of 20 m^2 present in 4 m depth 100 m off the shore. Prior to the trial, the site bottom was cleared of rocks, pebbles and mosses. 140 individuals/m^2 were manually buried into the bottom and covered over by the cages of 2×1.5×0.2 m framed by a 0.5-inch construction steel bar. The cages were covered with a plastic net of 8mm mesh. The process of ground rearing was continued for 20 months with a mean shell length of 194.6±10.2 mm and a 70% survival rate.

Góngora-Gómez et al. (2016b) conducted an experimental study similar to the *A. maura* process, combining suspended and bottom cultures in Isla Los Redos in the southeast of California Gulf, Mexico for 15 months (February 2008-May 2009). Young individuals (n=2500) were subjected to the suspended culture and their mean shell height and total weight varied from 16.20±4.96 mm-and 0.3±0.2 g to 119.66±8.98 mm and 38.68±10.34 g, respectively. The survival rate was found to be 97%. They were then planted into sand bottom within the area surrounded by the protective cover framed with corral or fence and preserved until the harvest process for 11 months with a mean shell height of 220.48±12.41 mm, the total weight of 284.26±54.45 g and a survival rate of 88.72%.

P. nobilis individuals reared by the suspended system were buried into the bottom of 3m depth bottom around Karantina Island, Urla, Izmir in April 2008. Their total height (anterior-posterior) ranged from 125 mm to 185 mm. The survival rate was 100% after 6 months and 1 year (Figure 8) (Unpublished study of the author).

The reason for the combination of the two culture systems is that the survival rate is low based on the fragility of the newly settled individuals. However, their fragility decreases when they have achieved 100 mm length. In other words, the higher the size, the more resistance to predators would be (Velasco & Borrero, 2004). Moreover, juveniles less than 30 mm buried into the bottom could be uprooted by currents and/or easily killed by predators (Leal- Soto et al., 2011). It is generally an important parameter to often clean culture cages used in the suspended process and manually keep predators off in the water in terms of increasing rates of growth and survival. Grown individuals should be vertically buried into the lower layer to maximize their process of nourishment, which is better than suspended culture. Thanks to the above, they could adjust themselves to the process until they have been adults and function their reproduction activity healthily. It seems more appropriate to assess both culture systems in combination for *P. nobilis* to avoid risking any stages of development due to the security of accurate control over the process with minimum mortality in the process.

Transplantation

Transplantation is the method used to sustain and increase the production of endangered or threatened species of commercially valuable species under danger of extinction. The process has been usually performed for bivalve species such as *Crassostrea virginica* (Powell et al., 1997), European oyster *Ostrea edulis* (Çelik et al., 2013) and Gastropod species such as limpet *Patella ferruginea* (Espinosa et al., 2008), green abalone *Haliotis fulgens* (Guzman Del Proo et al., 2004). The conducted activity has also been used for fan mussels such as *Atrina maura* (Mendo et al., 2011), *Pinna bicolor* (Wu & Shin, 1998) and *Pinna rugosa* (Arizpe, 1995) which are of commercial importance and targeted to be cultivated. Achievement of the transplantation process can generally be affected by anthropogenic factors, stock density, size of individuals to be planted, the pressure of predation and environmental parameters (temperature, salinity, sediment structure, nutrient quality and quantity, and currents).

P. nobilis continues to decline where it inhabits. Therefore, transplantation could be performed as one of the remedies for the generation of *P. nobilis* to sustain, for which the first transplantation trial was conducted in the Adriatic Sea by

Table 2. Transplantation studies for *P. nobilis*

Location	Survival rate (%)	N	Depth (m)	Reference
North Eastern Coast of Sardinia	75	18		Caronni et al. (2007)
Le Brusc lagoon, Var, France	100	16		Trigos & Vicente (2016)
Lake Vouliagmeni (Korinthiakos Gulf, Greece)	95.6	45 (11-16 cm)	12	Katsanevakis (2016)
Capo Peloro Lagoon (Central Mediterranean, Italy	83	53	2	Bottari et al. (2017)

Figure 8. Individuals planted into bottom after rearing trial in the Karantina Island (İzmir Bay, Aegean Sea) in December 2006

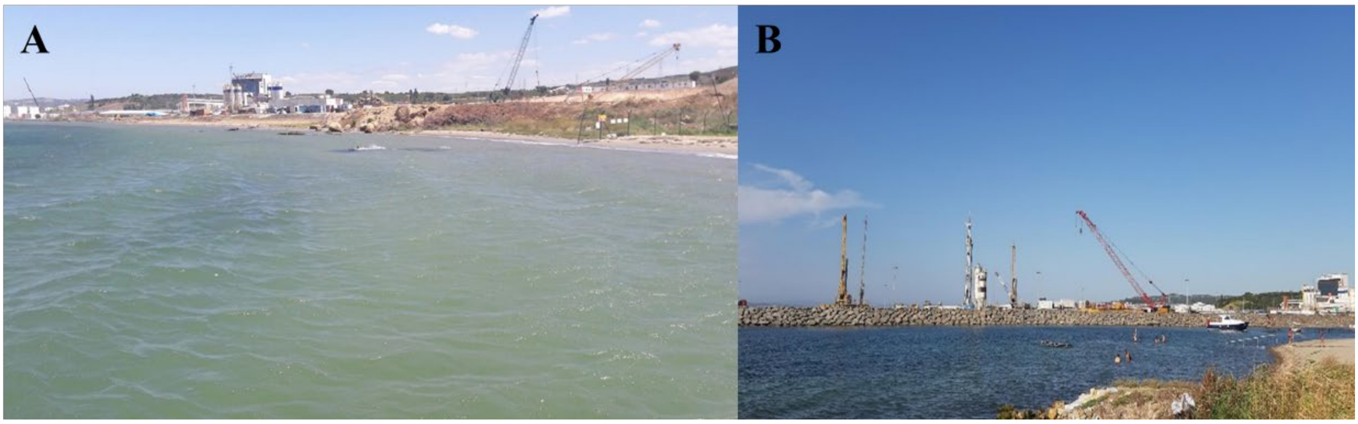

Figure 9. The location where *P. nobilis* collected from the Çanakkale Strait. Before (A) and after (B) the construction of the 1915 Çanakkale Bridge

Mihailinović (1955) for commercial purposes such as growing to market its shell, meat and byssus (Mihailinović, 1955 as cited in Trigos & Vicente, 2016), followed by the fact that Vicente et al. (1980), Hignette (1983). de Gaulejac & Vicente (1990) designed transplantation processes and performed small scale experiments. The importance of transplantation processes has further increased in the consequence of intense mortalities of *P. nobilis* species in several sections of the Mediterranean Sea (Table 2), which is therefore of additional emphasis for the rehabilitation of affected stocks. The related process is of additional importance in determining, monitoring and protecting surviving individuals in infected populations in order to pave the way for the studies to be made later. In other words, strong individuals are necessary to be able to survive. It would be a crucial issue to transplant generations that could be obtained from individuals able to survive and thus become resistant to diseases in natural and culture-based conditions where they could be rehabilitated and cultured in terms of their sustainability.

Transplantation Studies in Turkey

Present in the area of construction of 1915 Çanakkale Bridge was started in 2018 between the European and Asian sides of the Çanakkale Strait, live transportation of *P. nobilis* population was transported to a secure location (Figure 9) 14 *P. nobilis* individuals were found to exist in the construction of the European side of the strait and carried to the area 500 m away from where they would eventually be buried into a 1-1.5 m depth of the sea bed. 1040 individuals from the construction on the Asian side were transplanted to the area 300-350 m afar into which to bury them 4-4.5 m depth. Two months after the transplantation, submarine observation indicated 1% mortality. One year later, however, *P. nobilis* population was seen to be healthy with good adaptation to the transplantation site. Observatory scuba diving performed in 2020 showed high rates of mortality where transplantation was performed as well as in other sections of the strait. The fact that young individuals in the transplantation area on the Asian side are those estimated to be within the age range of 1-1.5 showed that the population completed the process of adaptation with the managed stress and resultant reproduction activity. However, the area may be currently exposed to the influence of the disease *H. pinnae.*

Recommendations for the Transplantation Operation of P. nobilis

The parameters to be considered during the transplantation can be listed as below;

- Depths, water conditions, individual density and sediment structure of the areas where fan mussels are distributed are to be taken into account.
- "Specific Protection Areas" have to be constituted in transplantation sites with the exception of those where fisheries activities are forbidden and anthropogenic factors are negligible,
- "Specific Sheltered Area" is supposed to be designed to decrease or eliminate the pressure of predation similar to Subaquatic Park Systems during the studies of rehabilitation,
- Caution and care should be exerted lest byssal threads could be broken off while removing *P. nobilis* individuals off the sea bed.
- Individuals ripped off the bottom should be carried without being shaken in seawater to the transplantation area,
- Individuals should be planted as vertically as possible in the plantation process.
- Individuals should be planted considering the previous depth traces of mud and sand or unburned depth.

Conclusion and Recommendations

P. nobilis and *P. rudis* were used as human consumption for meat and their shells and byssal threads for decorative purposes until 1992 and 1996, respectively. However, due to overfishing and the destruction of their habitats, their populations have been recorded to be significantly reduced. Considering the damage to the population of *P. nobilis*, the status of the species was established to be at critical levels. Accordingly, new plans of action should be constituted in terms of its sustainability.

By determining active disease agents (*Mycobacterium spp*. and *H. pinnae*), the interaction between pathogenesis and epizootiology namely, between host, parasite and environment should be explained, which is thus vital in determining methods to be followed for regeneration of the stocks and preventing diseases from spreading.

The rapid flow of information could be provided thanks to the formation of a network system involving local administrations, people, diving clubs and commercial and sportive fishermen along marine regions in terms of mapping distribution of *P. nobilis*. Following the establishment of healthy populations, in particular, the constituents within the network system above should be allowed to assume missions on information, protection and control. Therefore, a program of protecting and rehabilitating *P. nobilis* could be instituted within such an integrated system.

Surveys of field scanning helped to record masses of mortalities in most regions from Western Mediterranean shores to Aegean Sea. Similarly, a 100% rate of mortality was seen at the Aegean mouth of the strait whereas the entrance of the Marmara Sea exhibited a 9.2% rate of mortality, where there have yet to be any cases of the diseases. The disease-stricken populations are assumed to need a long time to recover, that is, the ability of resistant individuals to function in reproduction activity. It is therefore of great importance to determine, monitor and protect such populations in view of sustainability.

It is to be aimed those spats to be picked up by collectors from healthy populations in the Marmara Sea should be exposed to rearing and planting into new sediments when they have become 15 cm in length and thus forming new populations. However, it is quite necessary to keep the area of the newly planted individuals under protection and follow them up in terms of constituting the new population. Reviving mostly or completely damaged populations by replanting processes should be applied as a mere solution.

Knowledge of the reproductive biology of the species is very important. Development of programs for adult maturation is needed. What reduces the survival rate of larva, in particular, is its high hydrophobicity, to put clearly, its sticking onto water surface and walls of the tanks, which requires an emphasis on specific techniques. It seems very important that individuals preserved in the incubator under culture conditions until the phase of spats should be taken to progressive sheltered culture systems and reared on the surface and the bottom in terms of regeneration.

The related references should be taken into consideration in prospective research concerning the studies aimed at understanding comparative evolution, comparative genomics or genetic variation of *P. nobilis* to develop effective protective plans of the genome.

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

Size distribution, length-weight relationship, and catch per unit effort of frigate tuna, *Auxis thazard* (Lacepède, 1800) in Tawi-Tawi waters, southern Philippines, caught using multiple handline

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ABSTRACT

Frigate tuna *Auxis thazard* (Lacepède, 1800) is the most dominant species caught by the multiple handline in the coastal and offshore fishing grounds of Tawi-Tawi, southern Philippines. In this study, we investigated the size distribution, length-weight relationship, and catch per unit effort of frigate tuna (*A. thazard*) in Tawi-Tawi waters, southern Philippines, caught using multiple handline. A total of 383 frigate tuna fish were sampled with a size distribution (total length) ranging from 16.5 to 34 cm. The length-weight relationship of frigate tuna was $W = 8 \times 10^{-3} \times TL^{3.139}$, where the *b* value of 3.139 indicates a positive allometric growth pattern. The catch per unit effort of the used gear was 2.49±0.52 kg/hr.

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Introduction

Frigate tuna, *Auxis thazard* (Lacepède, 1800), is a pelagic-neritic tuna species that belongs to the Scombridae family, which is distributed globally both in tropical and subtropical waters (Liu, 2008; Tao et al., 2012). This species is highly migratory, thrives in the marine waters with 50 m depth with an optimum temperature ranging from 27 to 28 °C (Maguire et al., 2006; Herrera & Pierre, 2009). Previous studies of frigate tuna across many oceans and seas showed that the biological aspects of this species vary. For instance, the length sizes (total, standard, fork length) of frigate tuna (*A. thazard*) reported to

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be as low as 21 cm to as high as 40 cm (Noegroho et al., 2013; Tampubolon et al., 2016; Mudumala et al., 2018; Herath et al., 2019), with maturity stage starts at around 30 cm in length (Ghosh et al., 2012). Frigate tuna has a sex ratio of 1:1 sampled in the Eastern Indian Ocean (Noegroho et al., 2013). In Taiwan Strait, the dominant age of frigate tuna was two years (Tao et al., 2012). Numerous studies conducted in different parts of the world oceans revealed that this tuna species showed positive allometric growth (Ghosh et al., 2012; Tampubolon et al., 2016; Mariyasingarayan et al., 2018; Mudumala et al., 2018; Petukhova, 2019; Lelono & Bintoro, 2019; Herath et al., 2019).

Fishing this tuna species is important in many countries, especially in the purse seine fishery industry (Ahusan & Adam, 2011; Tao et al., 2012). Other fishing gears used to catch frigate tuna are gillnets, haul seine, ring net, beach seine, multiple handline, trolling, and pole and line (Haputhantri, 2016; Calicdan-Villarao et al., 2017; Mamalangkap et al., 2018; Sulistyaningsih et al., 2020).

Frigate tuna is one of the economically important tuna species caught in the Philippine waters. According to BFAR (2019), nearly 6% of municipal (approximately 54,000 metric tons) and commercial catch (approximately 58,000 metric tons) fish species in 2018 were contributed by frigate tuna. Tawi-Tawi waters are part of the Sulu Sea – one of the Philippines' richest fishing grounds and a home for most neritic and pelagic tunas, including frigate tuna (Mamalangkap et al., 2018).

Tawi-Tawi's local fisherfolks used ring net (*Kulibo*) to catch small pelagic fishes, usually done at night. In addition, small pump boat operators used multiple handline locally known as "bira-bira" to catch small tuna species such as frigate tuna, bullet tuna, eastern-little tuna, and other small pelagic tuna-like fishes. Using multiple handline to fish tuna has been practiced since the 1990s to the present, but with some innovation on the colors of artificial lures and the number of hooks of the gear to be used. Despite this tuna fishing practice, frigate tuna and other small tuna-like fish resources still sustain. However, to date, there is no available information about the size distribution, length-weight relationship, and catch per unit effort of frigate tuna in Tawi-Tawi. Hence, in this study, we determined the size distribution, length-weight relationship, and catch per unit effort of frigate tuna in Tawi-Tawi waters, southern Philippines, using multiple handline.

Materials and Methods

Study Site

The study was conducted in Tawi-Tawi waters, southern Philippines, particularly in Bongao municipal waters (Figure 1), from March 15, 2020 to April 27, 2020. Fishing operation, targeting frigate tuna, was done using multiple handline (Figure 2) and a motorized banca powered with Honda 13 HP gasoline engine.

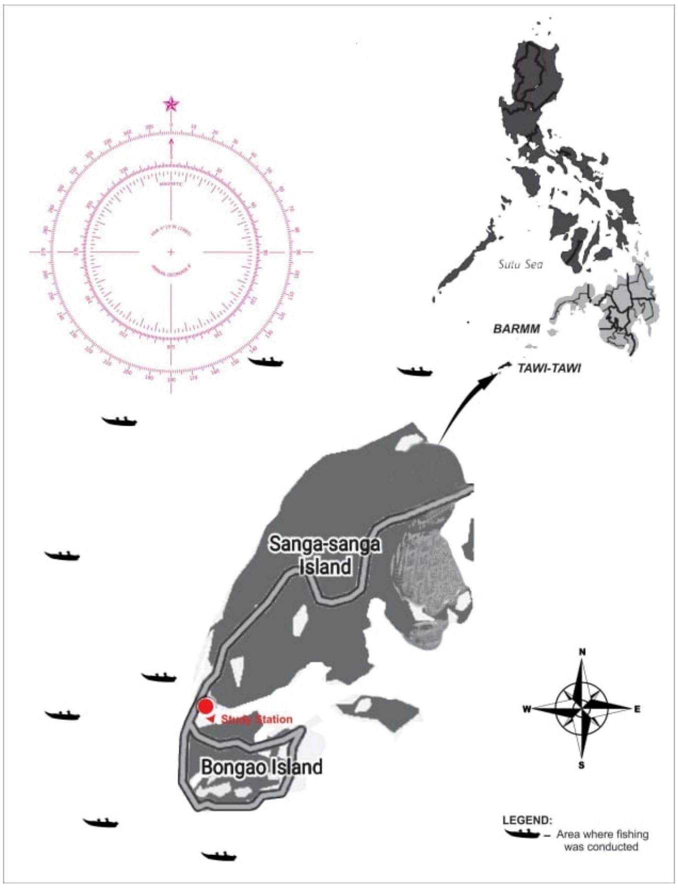

Figure 1. Map showing the study site

Figure 2. Multiple handline (bira-bira) used in the study

Size Distribution and Length-Weight Relationship

The total length and weight of frigate tuna were measured and weighed to the nearest 0.5 g/cm. Then, the relation between the number of catch and its corresponding length were investigated using the correlation analysis. For the length-weight relationship, the formula $W = aL^b$ was used to calculate the relationship between the body length of the fish and its body weight, where *W* is the fish weight, *L* is the total length of the fish, *a* is the intercept, and *b* is the slope of the linear regression (Petukhova, 2019).

Catch per Unit Effort (CPUE)

Catch per unit effort (CPUE) is defined by the total weight of fish caught divided by the total number of fishing hours. Researchers used two separate boats (corresponding to two fishers using the same fishing gear), fishing for two hours each trip, including time travel to the fishing ground, with a total of 18 fishing trips. The basis for the fishing time was based on the fishers' practice in the area. The total weight of the caught frigate tuna was recorded. Then, CPUE was calculated by diving the total fish caught with the total number of fishing hours.

Results and Discussion

A total of 383 frigate tuna were caught and sampled. Total length ranged from 16.5 to 34 cm, and body weight ranged from 45 to 451 g (Table 1).

Size Distribution and Length-Weight Relationship

The size distribution of frigate tuna categorized according to the number of fish caught to its total length (cm) is shown in Figure 3. The highest size category of frigate tuna was recorded on the size range of 26.5 to 30.5 cm with 227 fish, followed by the size range of 21.5 to 25.5 cm with 128 fish, and the size range of 16.5 to 20.5 cm with 16 fish. The lowest catch of the size category was 31.5 to 35.5 cm with 12 fish. The average total length of caught frigate tuna was 27.28±0.12 cm (Table 1). Our results are parallel to the study of Tampubolon et al. (2016), where they reported that the highest size distribution of frigate tuna ranged from 26 to 31 cm sampled from West Coast Sumatera, Eastern Indian Ocean. Also, the mean length of frigate tuna collected from the Northwest Coast of India was 32.35 cm (Mudumala et al., 2018). In Sri Lanka, the average standard length of frigate tuna ranged from 28 to 32 cm (Herath et al., 2019).

Table 1. Total length and weight of frigate tuna

Size Distribution	Descriptive Statistics		
	Minimum	*Maximum*	*Mean±SE*
Total length (cm)	16.5	34	27.28±0.12
Weight (g)	45	451	244.65±12.5

The length-weight relationship of frigate tuna was $W = 8 \times 10^{-3} \times TL^{3.139}$, wherein the *b* value was equal to 3.139, indicating positive allometry (b>3, *t*-test, *P* < 0.05), which implies that frigate tuna has a systematic pattern of growth such that the weight of the fish is proportional to its body length (Figure 4). This result is not so different from the study of Tampubolon et al. (2016), where the *b* value was 3.1489 of the same species in Indonesian waters. Also, Petukhova (2019) reported that the length-weight relationship of *A. thazard* was likewise showed positive allometry (*b* value of 3.240) collected from Northeast Atlantic. Besides, frigate tuna caught from the Northwest Coast of India showed positive allometry with an *r*-value of 0.9576 (Mudumala et al., 2018). Furthermore, positive allometric growth patterns (*b* values = 3.3385 and 3.48) were also reported for *A. thazard* in the South Coast of East Java waters, Indonesia (Lelono & Bintoro, 2019) and in the coastal waters of Sri Lanka (Herath et al., 2019), respectively. The correlation coefficient of determination "r^2" found in the present study was equal to 0.9005, indicating a strong correlation and highly statistically significant that the length of frigate tuna is proportional to its body length. Our result on the correlation coefficient of frigate tuna is relatively similar to the findings of Mariyasingarayan et al. (2018) collected from Parangipettai, Southeast Coast of India.

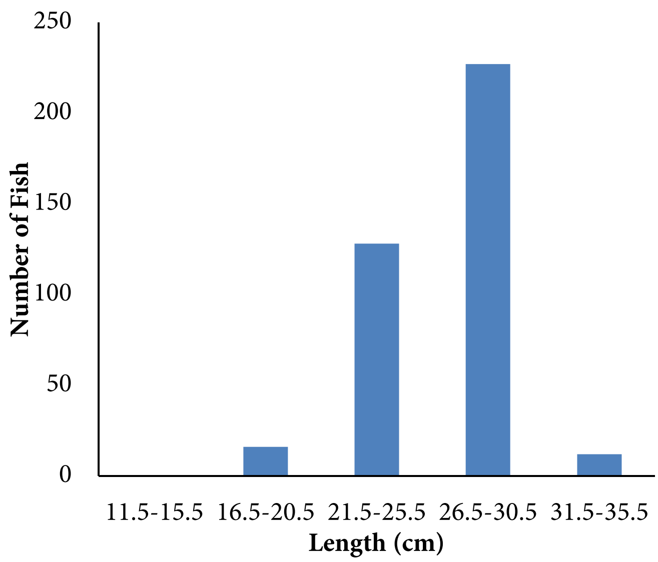

Figure 3. Size distribution of frigate tuna (*A. thazard*)

Catch per Unit Efforts (CPUE)

CPUE is one way of measuring the efficiency of particular fishing gear in terms of catch and likewise can be used as a fish abundance index (Mamalangkap et al., 2018). In this study, the average CPUE of frigate tuna for the whole fishing trip was 2.49±0.52 kg/hr. The CPUE of frigate tuna by the total weight of all the species is shown in Figure 5. The highest CPUE of frigate tuna was recorded on the second fishing trip with 8.87 kg/hr and followed by 5.44 kg/hr recorded on the first fishing trip. In Zambales, Philippines, the average annual (2003 to 2012) CPUE of multiple handline in catching tuna species, including frigate tuna, was 63 kg/hr (Yutuc et al., 2018).

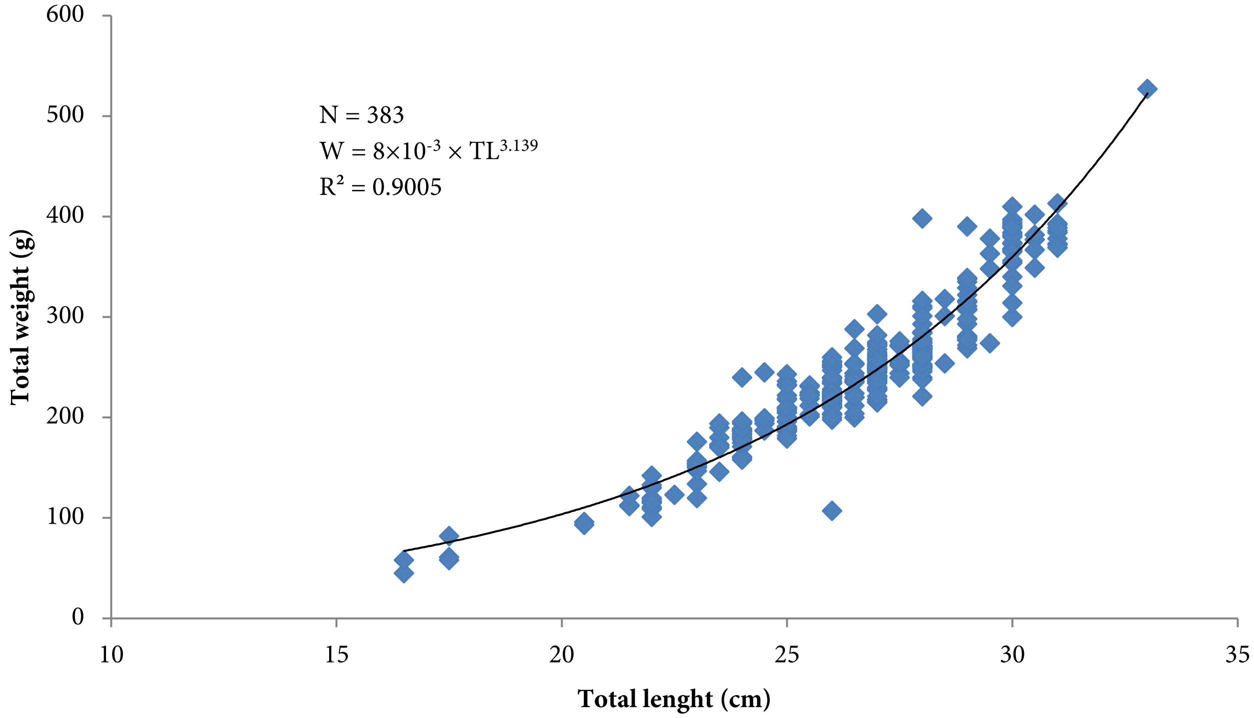

Figure 4. Length-weight relationship of frigate tuna (*A. thazard*)

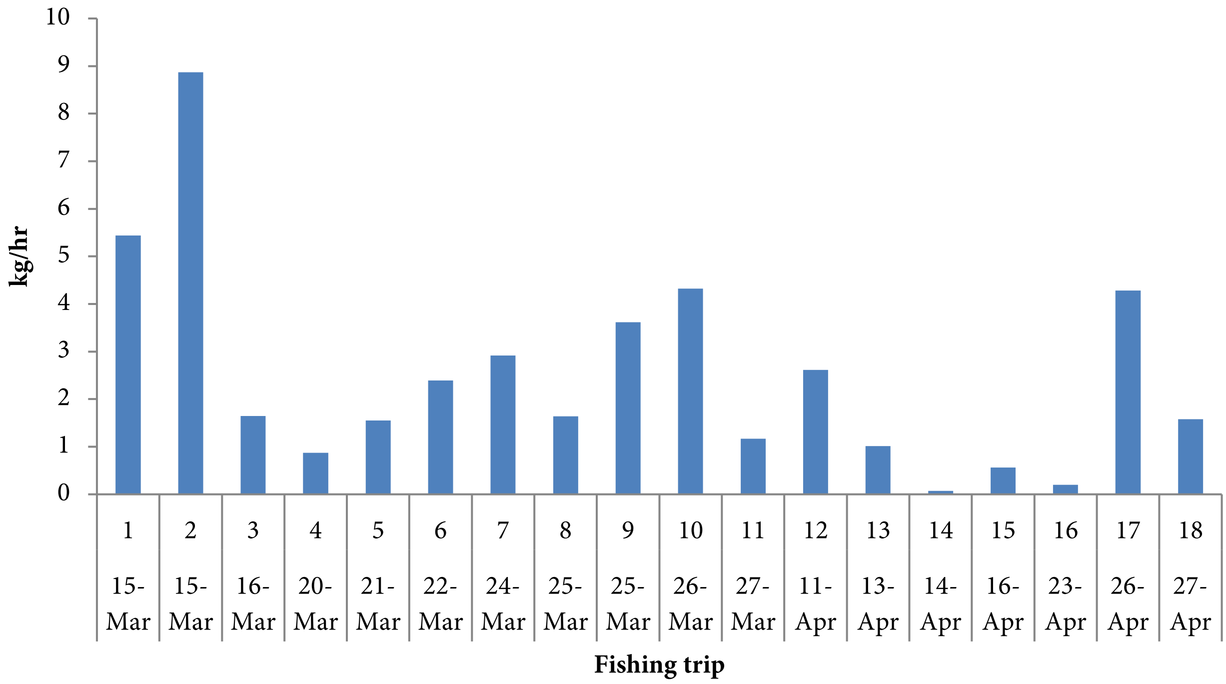

Figure 5. Catch per unit effort of frigate tuna using multiple handline

Conclusion

In conclusion, the size distribution of frigate tuna (*A. thazard*) was within the size range reported by the literature. Furthermore, the length-weight relationship of frigate tuna showed a positive allometric growth indicating that the fish live in the normal environment suitable for their growth. Based on the catch per unit effort result, we can still say that the multiple handline is relatively efficient to catch frigate tuna. Therefore, frigate tuna fishing in Tawi-Tawi waters, southern Philippines, is one of the important sources of food and livelihood for many local fishers.

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Compliance With Ethical Standards

Authors' Contributions

Both authors have contributed equally to this paper. In addition, both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

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RESEARCH ARTICLE

A study on the training and education of inspectors to prevent, deter and eliminate illegal, unreported and unregulated (IUU) fishing in Turkey

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ABSTRACT

The aim of this study was to examine present training conditions of inspectors and the current situation in Turkey to prevent, deter and eliminate IUU fishing within the scope of the training of qualified inspectors which was one of port states' duties according to the PSMA to Prevent, Deter and Eliminate IUU Fishing. In Turkey, the inspectors of the Ministry of Agriculture and Forestry and the Command of Coast Guard had sufficient training according to the Annex-E of Article 17 of the PSMA to Prevent, Deter and Eliminate IUU Fishing. The trainings related to the provisions stated in the Annex-E of Article 17 of the PSMA to Prevent, Deter and Eliminate IUU Fishing should be planned or necessary studies should be carried out by the Ministry of Agriculture and Forestry for authorized institutions and organizations other than the Ministry of Agriculture and Forestry and the Coast Guard Command. Legal arrangements should be made on the training of inspectors in order to prevent IUU fishing in the Fisheries Law.

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Introduction

Illegal, unreported and unregulated fishing (IUU fishing) is a global problem affecting both exclusive economic zones and the high seas (Boto et al., 2009). In the context of the Code of Conduct for Responsible Fisheries and its overall objective of sustainable fisheries, the issue of IUU fishing in world fisheries is of serious and increasing concern. IUU fishing undermines efforts to conserve and manage fish stocks in all capture fisheries (FAO, 2001). To prevent IUU Fishing on the national and international level, in 2001, the Food and Agriculture Organization of the United Nations (FAO, 2021) signed an International Plan of Action to Prevent, Deter and Eliminate IUU Fishing within the framework of the code of conduct for responsible fisheries (FAO, 2001).

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The Agreement on Port State Measures (PSMA) to Prevent, Deter and Eliminate IUU Fishing was approved by the FAO Conference at its Thirty-sixth Session on 22 November 2009 (FAO, 2009). Turkey has been part/has deposited to "the PSMA to Prevent, Deter and Eliminate IUU Fishing" on 9 November 2010 (FAO, 2021). Turkey signed this Agreement as the 18th country together with 23 countries which are United States of America, European Union (EU)–Member Organization, France, Russian Federation, Canada, Australia, New Zealand, Kenya, Angola, Brazil, Chile, Iceland, Indonesia, Norway, Samoa, Uruguay, Sierra Leone, Gabon, Ghana, Benin, Peru and Mozambique (FAO, 2009). Besides, the EU candidate membership period of Turkey is still in progress (EC, 2021). The PSMA to Prevent, Deter and Eliminate IUU Fishing came into force with the Official Gazette of the EU numbered L 191/3 and dated 22.07.2011 (EU, 2011).

Port states duties are determined in the PSMA to Prevent, Deter and Eliminate IUU Fishing. The training and education of qualified inspectors are among port state duties. Each Party shall ensure that its inspectors are properly trained taking into account the guidelines for the training of inspectors in Annex E of the 17th Article of the agreement (EU, 2011; FAO, 2021).

Elements of a training programme for port State inspectors according to Annex E should include at least the following areas: Ethics; Health, safety and security issues; Applicable national laws and regulations, areas of competence and conservation and management measures of relevant Regional Fisheries Management Organizations, and applicable international law; Collection, evaluation and preservation of evidence; General inspection procedures such as report writing and interview techniques; Analysis of information, such as logbooks, electronic documentation and vessel history (name, ownership and flag State), required for the validation of information given by the master of the vessel; Vessel boarding and inspection, including hold inspections and calculation of vessel hold volumes; Verification and validation of information related to landings, transhipments, processing and fish remaining onboard, including utilizing conversion factors for the various species and products; Identification of fish species, and the measurement of length and other biological parameters; Identification of vessels and gear, and techniques for the inspection and measurement of gear; Equipment and operation of Vessel Monitoring System (VMS) and other electronic tracking systems; and Actions to be taken following an inspection (FAO, 2021; EU, 2011).

There are very few studies about the IUU fishing in Turkey (Öztürk, 2013; Öztürk, 2015; Göktürk & Deniz, 2017). The aim of this study is to examine present training conditions of inspectors in Turkey to prevent, deter and eliminate illegal, unreported and unregulated fishing within the scope of the training of qualified inspectors which is one of port states' duties according to "the PSMA to Prevent, Deter and Eliminate IUU Fishing".

Materials and Methods

National and international legislations were examined. The activities of inspector institutions in Turkey in the field of fisheries inspection were investigated. Written and oral interviews were conducted with the auditors or inspector institutions.

Results

Organizations and Institutions Authorized for the Prevention of Illegal, Unregistered, and Irregular Fishing in Turkey

In accordance with the Presidential Decree No: 1 on the Organization of the Presidential the Protection Services of Fishery Products is executed by the Directorate General of Fisheries and Aquaculture under the Ministry of Agriculture and Forestry (Anonymous, 2018), by the Fisheries and Aquaculture Branch Directorate in provinces (MAF, 2021a). The control of the fisheries in seas and inland waters in Turkey is performed by the organizations and institutions which are also stated in the Fishery Law.

According to the 33rd Article of the Fishery Law, the personnel appointed for the protection and control of fishery products, seas and inland waters at the Ministry of Agriculture and Forestry and institutions regarding fishery as well as the associates of Police, Gendarme, Coast Guard, Customs and Forestry Enforcement Institution, Municipal police forces, Guardians, Wardens Attached to Public Legal Entities, Headmen or Board of Elders of the village in places where no police and gendarme stations exist are all authorized for the inspection of fishery at seas and inland waters. Due to the Fishery Law and the prohibitions based on this Law, these organizations and institutions are authorized and charged with the tasks of taking the minutes of crimes which are in the scope of this Law, seizing the caught fishery products and the fishing gears used during the crime, imposing administrative fines within the scope of their authority (Anonymous, 1971).

According to the 34th Article of the Fishery Regulation, the superior local administrative chief has been authorized for imposing administrative fines for illegal fishing. Local Authorities can assign their fine rights to organizations or institutions having inspection competence on condition that they declare beforehand (Anonymous, 1995).

As per the 35th Article of the Fishery Regulation, the commanders of the Coast Guard boats are authorized to give administrative fines in seas within the Turkish Territorial Waters and Exclusive Economic Zone, the Istanbul and Dardanelles Straits and in ports and gulfs (Anonymous, 1995).

Ministry of Agriculture and Forestry

Within the 412th article of the Presidential Decree No: 1 on the Organization of the Presidential the Directorate General of Fisheries and Aquaculture is also stated among the service units of the Ministry (Anonymous, 2018). Within the 416th Article of the Presidential Decree No: 1 Law, the tasks of the Directorate General of Fisheries and Aquaculture are specified and in the 1-d clause of this article the task is specified as “Fostering fisheries and the production sources of fishery and raising productivity, carrying out inspections (Anonymous, 2018).

Within the first clause of the Article 33 of the Fishery Regulation, it is stated that “the Ministry inspects and controls fishery product producers, merchants, industrialists, tradesmen, importers and exporters who deal with these products and their workplaces, fish markets, auction places, fishery processing and assessment facilities, the products obtained from these places, regions of fishery products, ports, shelters, landing places and the capture tools of fishers and carries out the necessary procedures according to the legislation (Anonymous, 1995).

The authorized personnel of the Ministry of Food, Agriculture and Livestock is assigned to inspect fisherman shelters and their superstructures and to examine and control the management and control matters, any kind of document and record and carries out the necessary procedures as per Article 20 of the Regulation on the Fisherman Shelter (Anonymous, 1996).

Personnel trained in the field of fisheries work in the Directorate General of Fisheries and Aquaculture and Branch Directorates of the Ministry of Agriculture and Forestry. In-service Training Programs are prepared in line with the decisions taken by the Ministry of Agriculture and Forestry by the Education Board and the proposals of the Central Service Units of the Ministry (MAF, 2021b). In-Service Training activities are carried out through the Personnel Training System with a transparent, traceable and evaluable method in cooperation with the Central and Provincial Organizations, Universities and other public institutions and organizations (MAF, 2021b).

Distribution of illegal fishing activities determined by the Ministry of Agriculture and Forestry by ratios between 2012 and 2015 (9-month period) are given in Figure 1 (Atalay, 2015).

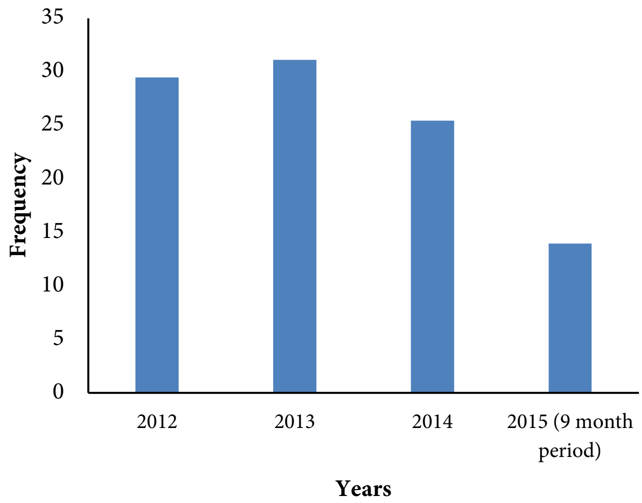

Figure 1. The variation of IUU fishing activities between 2012 and 2015 (9-month period) (Atalay, 2015).

General Directorate of Security

As per the 1st Article of the Security Affairs Law, the Minister of Interior is responsible for the countries’ general security and public order (Anonymous, 1937). The Minister of Interior performs these tasks by means of the General Security Directorate and Gendarmerie General Command acting within the circle of their own laws and when necessary, it utilizes from the army with the decision of the Cabinet (Anonymous, 1937).

As per the 3rd Article of the Security Affairs Law, the police organization; is divided into two sections as general (these are the police and gendarmerie which are armed forces) and special the general law enforcement (Anonymous, 1937). Special law enforcement; are the law enforcement forces except for the general law enforcement which carry out determined duties and which are founded as per intentional laws (Anonymous, 1937). Special law enforcement, Forest Enforcement Officers, Customs Enforcement Officers and Municipal Police Officers, etc. can be expressed (Akman & Bayram, 2018; Arslan, 2018). The gendarmerie and the special law enforcement agencies are liable to their own laws and the law enforcement is liable to the provisions of this law (Anonymous, 1937). The liaison, communication and working methods of the gendarmerie and special police and other law enforcement forces are determined by regulations (Anonymous, 1937).

According to the 4th Article of the Security Affairs Law, the security organization is an armed force which is divided into two as uniformed forces and civilian forces (Anonymous, 1937). As per the 5th Article of this Law uniformed police forces consist of units with or without vehicles (Anonymous, 1937). Police units with vehicle are equipped with mounted means or motor vehicles or bicycles and other living or lifeless vehicles (Anonymous, 1937).

According to the 8th Article of the Security Affairs Law, the police is divided into administrative political and judicial parts and as per the 9th Article, the administrative police is responsible for the procurement of public order (Anonymous, 1937).

As per the 3rd Article of the Regulation on the Tasks of the Gendarmerie and Police in Provinces Districts and Sub-Districts and their Authority Form and Interactions, the Area within the Municipality Borders of the Provinces and Districts is under the responsibility of the police, the area outside the municipality borders of the provinces and districts is under the responsibility of the Gendarmerie (Anonymous, 1961). Considering the personnel, tools and service requirements of the law enforcement certain areas outside the municipality borders can be determined within the area of responsibility of the police; some areas which are within the municipality borders but are far from residential areas can be determined within the area of responsibility of the Gendarmerie (Anonymous, 1961).

As per the 2nd Article of the Law Regulating the Tasks and Authorities of the Police, one of the tasks of the police related to security is to prevent illegal activities which are not complying with Laws, By-laws, government orders and the public order within the framework of the provisions of this law hereby (Anonymous, 1934).

The in-service training of the Turkish Police is given by the General Directorate of Security Training Department, and the entire pre-service training is provided by the training units called Police Chiefs Training Centre, Police Vocational School, Police Vocational Training Centre (GDS, 2021).

The duty types of the General Directorate of Security consist of Traffic Police, Marine Police, Motorcycle Police, Air Police, Juvenile Police, Anti-Terrorism, Fight Against Smuggling and Organized Crime, Riot Force and Special Operations (GDS, 2021).

The Marine Police is the police force responsible for ports, gulfs and inland waters. It is the unit responsible for the administrative and judicial procedures at sea on behalf of the security agency (GDS, 2021).

The maritime police carried out controls and inspections to prevent poaching, especially in the coastal waters of Istanbul, Izmir and Tekirdağ provinces. It was seen that the controls and inspections were carried out in coordination with the Ministry of Agriculture and Forestry (ISPAFD, 2020; AA, 2021; MM, 2021).

The police inspected showrooms selling fishing equipment to hinder the production and sale of tırıvırı (local name in Turkish) and parachute (a piece of fish line net which is attached to a fishing line to catch fish) with the personnel of the Ministry of Agriculture and Forestry (HC, 2010; HC, 2014; IASFA, 2019a).

According to need, Fishery Law, fishery gears and methods, fisheries management, landing points, shelters, wholesale and retail outlets, etc. trainings were given to the Marine Police, tasked with IUU Fishing preventions, by the Istanbul University Faculty of Fisheries, Istanbul and Izmir Directorates of Agriculture and Forestry (HC, 2015; IPAFD, 2015; IASFA, 2019b).

The Gendarmerie General Command

Except for the administrative, judicial and military tasks of the Gendarmerie stipulated in the 7th Article of the Law on the Organization, Tasks and Authorities of the Gendarmerie other Duties of the Gendarmerie is the execution of other laws and statute provisions and resolutions and orders of this laws (Anonymous, 1983a).

The Gendarmerie Command is attaching great importance to protect the environmental and ecological balance and if possible, to rehabilitate the damaged environment. Therefore, it has founded 41 Environmental Protection Teams to work within the structure of the Directorates of Public Order of the Gendarmerie Command of 38 Provinces in order to make research on maritime pollution, air pollution and solid waste pollution and their resources under their responsibility and to send the results of the researches as reports to the concerned institutions to get the necessary procedures done (APGC, 2021; GGC, 2021).

The environment protection teams; consist of the Team Commander, Team Deputy Commander and Environmental and Wildlife Conservation Specialist. One of the duties of the Environment Protection Teams is to control activities violating the Fishery Law and to determine offences by using technical tools such as video and photo cameras in situations which are against the legislation (APGC, 2021; GGC, 2021).

To prevent IUU fishing, the personnel who will be assigned in the Environment Protection Teams should primarily get theoretical and operative training in the Gendarmerie General Command on fishery legislation.

Command of Coast Guard

It is specified in the 4th Article of the Command of Coast Guard Law that it is under the responsibility of the Command of Coast Guard to seize people violating the Fishery Law to monitor and prevent them, and to seize the offenders and follow necessary proceedings (Anonymous, 1982).

The Command of Coast Guard personnel undergoes a training provided by the Coast Guard School Command in order to fulfil and carry out fishing controls and inspections successfully within the authority entrusted by the Fishery Law and Command of Coast Guard Law.

The Fishery Course contains the following topics; the General Assessment on the Fishery in Turkey, Commercially Important Species that are caught in Turkish waters (identification of fish species, the measurement of length and other biological parameters etc.), Fishery Gears and Fishing Methods (with the inclusion of techniques for the inspection and measurement of gear), IUU fishing, Provisions of Practice and Fining Procedures, Fishery Licenses, Prohibitions Related to Fishing And Law Enforcement Provisions, Fishery Production and Judicial Legislation, VMS, Fishery Information System, The Problems during the Practice of Fishery Legislation.

In the fishery lesson which is provided in the scope of the trainings and courses within the structure of the Coast Guard School Command, practical training is very important. Within this scope, “A Protocol on the Practical Training Principals” has been signed between the Coast Guard Education and Training Command and the Akdeniz University.

In the lectures video demonstrations are provided on capturing and fishing tools. Existing fishery and fishing gears in the Fisheries Port and Fisher’s Auction are examined in the fishery Laboratory. For some issues, external conference experts are invited from the relevant institutions/universities.

Useful publications for the fishery lessons are provided periodically for the Coast Guard Education and Training Command personnel. The Coast Guard Magazine also publishes studies related to fishery.

In the application process of the fishery legislation the administrative acquired and cancelled sentences are examined in the lessons, the legislation amendments are followed, the photos, Compact Disc’s and proceedings change of ownership by the Coast Guard Boat Commanders and which will be used as evidence for violating Fishery Law are examined in the lessons, the concerned proceedings related to case studies are filled by the trained staff and how to behave in similar situations is taught to the cadets.

“The Fishery Booklet” and “Maritime Fishing Gears and Methods” will be useful on the fishery for the Coast Guard personnel are prepared by the Command of Coast Guard

Distribution of illegal fishing activities determined by the Command of Coast Guard by ratios between 2016 and 2021 (6-month period) are given in Figure 2 (CGC, 2021).

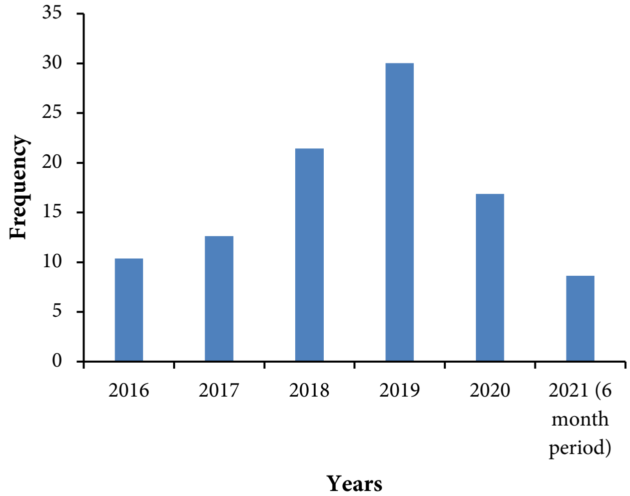

Figure 2. The variation of IUU fishing activities between 2016 and 2021 (6-month period) (CGC, 2021).

Directorate General of Customs Enforcement

According to Article 72/L of the Customs Regulation, the Ministry of Commerce of the Republic of Turkey determines the procedures and principles regarding customs surveillance and inspection in loading, transportation and unloading by all sea vehicles belonging to real or legal persons between ports and piers in the Customs Territory of Turkey (Anonymous, 2009).

Customs Enforcement Officers is a law enforcement agency working under the Ministry of Trade of the Republic of Turkey, and its primary duty is to combat smuggling (RTMT, 2021). In order to become a Customs Enforcement Officer, it is necessary to have a bachelor's degree and to be successful in the written proficiency exam and interview exams to be held after the basic education and internship phase. Customs Enforcement Officers receive training on fisheries law (Soydan & Ünal, 2019; HT, 2021; KN, 2021)

Directorate General of Forestry

According to the 20th and 30th articles of the Regulation on the Duties and Working Principles of Forest Enforcement Officers, Forest Enforcement Officers are authorized to apply the fisheries law for those caught from lakes and streams in the forest without obtaining permission from the forest administration (MAFF-FGD, 1996).

As per the 4th Article of the Forest Law, forests are categorised with regard to possession and administration as A) State forests; B) Forests of public establishments run by private corporations; C) Private forests. In terms of quality and character forests are divided as: A) Protection forests; B) National parks; C) Production forests (Anonymous, 1956).

As per the 6th Article of the Forest Law, any kind of work related to state forests and forests regarded as state forests are carried out by the General Directorate of Forests (Turkey, 1956). All forests, belonging to others but the state, are subject to the control of the General Directorate of Forests (Anonymous, 1956).

As per the 10th Article of the Regulation Forests Regarding Special Forests and Authorized Public Institutions, Special Forests and Forest regarding Authorized Public Institutions are operated and administered under the control and supervision of the forest administration (Anonymous, 2016).

In accordance with the 16th Article of National Parks Law, within the areas which are in the scope of this law, protection services and pursuing offences is provided by the Forest Enforcement Officers based on the provisions related to the pursuit of offences in the fifth section and fourth part of the Forest Law (Anonymous, 1983b).

According to Article 12 of the Regulation on Appointment and Relocation of Forest Enforcement Officers, Forest Enforcement Officers are required to graduate from the departments of Forestry and Forest Products, Forest Management, Forestry, Forest Products, Non-Wood Forest Products, Sapling Cultivation, Sapling Growing, Sapling and Seedling, Pruning and Grafting, Hunting and Wildlife, Hunting and Wildlife one of the associate degree programs of higher education institutions (Anonymous, 2011).

It was determined that the Forest Enforcement Officers did not get any in service training on inspection of fishery.

Municipal Police Forces

As per the 7th Article of the Metropolitan Municipality Law, one of the duties of the Metropolitan Municipality is to perform constabulary duties within their areas of responsibility (Anonymous, 2004a).

As per the 14th Article of the Municipality Law, performing constabulary services are under the responsibility of the Municipality (Anonymous, 2005).

In accordance with the 51st Article of the Municipality Law, the Municipal Police is responsible for the peace, health and order of the region and therefore enforces orders and bans taken the Municipality Assembly and executes the envisioned penalties and other sanctions for those who violate these laws (Anonymous, 2005).

As per the 10th Article of the Regulation on the Municipal Police Force, it is under the responsibility of the Municipal Police to use its authority and perform its tasks specified in by-laws and regulations in order to ensure the order within the Municipality borders and to preserve the peace and health of the people living there (Anonymous, 2007).

As per the 11th Article of the Regulation on the Municipal Police Force, the Municipal Police can enter public places, perform necessary controls, can demand documents from the owner or operator (of a business enterprise) and can write a record of these people within the borders of the municipality in order to fulfil its tasks granted by laws, regulations by-laws and authorized municipal organs (Anonymous, 2007).

According to Article 13 of the Regulation on the Municipal Police Force, it is necessary to be at least a high school graduate or equivalent to become a Municipal Police Officer (Anonymous, 2007). Depending on the need, it is required to have an associate degree and a bachelor's degree in subjects such as law, environment and food in order to be a Municipal Police Personnel (AMM, 2021).

According to article 28 of the Regulation on the Municipal Police Force, the basic and technical training of the personnel of the municipal police is carried out in cooperation with public institutions and organizations with training centres, relevant departments of universities or relevant non-governmental organizations when necessary (Anonymous, 2007). Based on the demand Municipal Police Forces were given Fisheries Inspection Training on retail outlets, fishing and length bans by Istanbul Agriculture and Forestry Directorates (BM, 2015; ISPAFD, 2015).

Guardians, Gatekeepers and Wardens Ground to Public Private Corporations and Members of the Council of Elderly

In accordance with the 2nd Article of the Law for the Formation of Headmanship and Council of Elderly of Neighbourhoods in Cities and Towns, Headmanship and the Council of Elderly consist of one headmanship and four members. The Council has four auxiliary members (Anonymous, 1944). As per the 32nd Article of the Statute on Headmanship and Council of Elderly in Cities and Towns, the Headmanship and Council of Elderly is authorized to notify in writing one of the official veterinary institutions in the town or the nearest civilian administration as soon as possible in case of epidemic and contagious diseases which are specified in the Veterinary Services, Plant Health, Food and Feed Law; as per the Law on the Interdiction and Pursuit of Smuggling, the duties of the Headmanship and Council of Elderly are as follows: A) to inform in writing the head of the civilian administration responsible for preventing and following illegal activities, Customs and Monopoly Officers, head of the police, commissary or his deputies, the Commander of the Gendarmerie or his staff or Coast Guard Officers and members in coasts on activities stipulated as illegal by laws B) in places where there aren't any civilian administration members responsible for preventing and following illegal activities to prevent smuggling itself and inform Customs and Monopoly Officers and the head of the civilian administration, C) to attend searches in private houses suspected with concealing smuggled goods and in shops, stores and commercial houses, warehouses, storehouses, warehouse, inns, hotels, pensions, cinemas, theatres, casinos, coffeehouses, pubs, when they are not open to the public (Anonymous, 1945).

It was determined that the Headmanship and Council of Elderly did not get any in service training on inspection of fishery.

Discussion

In Turkey, the Ministry of Agriculture and Forestry is the responsible institution for the protection and control of fishery products. It had been determined that people who have been trained in aquaculture and fisheries work in the control and inspection of fishery products. It was determined that in-service training activities were carried out in a transparent, traceable and evaluable method in cooperation with Central and Provincial Organizations, Universities and other public institutions and organizations through the Personnel Training System.

The General Directorate of Security has the authority to control catch yields of fishery within its area of responsibility in Turkey. One of the duty types of the General Directorate of Security is the Marine Police. The Marine Police is the police force in ports, gulfs and inland waters. It was determined that the maritime police conducted controls and inspections to prevent illegal fishing. It was determined that the maritime police received training on fishery control according to the need.

In Turkey, it was detected that Environmental Protection Teams were established within the Gendarmerie General Command in order to conduct research on marine pollution, air pollution and solid waste pollution and their sources under their responsibility, and to report the results of the research to the relevant institutions and take necessary actions. One of the duties of Environmental Protection Teams was to control activities against the Fisheries Law. It was determined that the person who would work in the Environmental Protection Teams should receive theoretical and operational training on environmental and fisheries legislation.

Within the scope of training and courses organized by the Coast Guard School Command, it had been determined that training is given on subjects such as Fish Species (identification of fish species, the measurement of length and other biological parameters etc.), Fishing Gears and Fishing Methods (with the inclusion of techniques for the inspection and measurement of gear), Fishery Legislation, Illegal Fishing Methods and European Union Fishery Policy, VMS, Fishery Information System in Turkey.

Customs Enforcement Officers is a law enforcement agency working under the Ministry of Trade of the Republic of Turkey. It was determined that Customs Enforcement Officers received training on fisheries law.

It was detected that the Forest Enforcement Officers, who had trained in the field of forestry and hunting and wildlife, working in the Forestry Organization, had not received any training on the control of fisheries in Turkey.

In Turkey, it was found that the basic and technical training of Municipal Police Personnel was carried out in cooperation with training centres and public institutions and organizations, relevant units of universities or, when necessary, relevant non-governmental organizations. Fisheries Inspection Training was given to the municipal police teams by the Istanbul Agriculture and Forestry Directorates upon request.

It was determined that neighbourhood and village headmen had duties such as preventing and following issues regarding the health of animals and smuggling, informing the relevant institutions, and being present during searches in Turkey. It was found that Bolu Directorate of Agriculture and Forestry provided in-service training to Village and Neighbourhood Headmen on Pasture Law, inheritance and sales of agricultural lands, animal health and support. In addition, it was detected that trainings were organized on the subjects Animal Husbandry and Fishing, Source of Life, Village Law No. 442, etc. for the Neighbourhoods and Headmen by the Çorum Governorship.

In the forum on IUU Fishing held in South and East Africa (Mozambique, Angola, Comoros, France, Kenya, Madagascar, Maldives, Somali Tanzania, Food and Agriculture Organization etc.) in 2007, it was determined that the inspectors and observers have insufficiency in training and need to update their education (Limitada, 2007). In November 2010, the Regional Schedules of Action of Western Africa (Senegal), Caribbean (Belize), Central Africa (Libreville, Gabon), Eastern Africa (Kampala, Uganda), South Africa (Maputo, Mozambique) and Pacific (Honiara, Solomon Islands) the training need of inspectors, observers and legislation personnel was specified (Tarabusi, 2010; Mobiha, 2010; Purvis & Mindjimba, 2010; Njifonjou, 2010; Grant, 2010; Sall, 2010). One of the results of the Report of the Workshop on Capacity Building of Developing States for Port State Measures and Catch Documentation Schemes of the Korea Republic held on 19-21 April 2011 was that the port state inspectors require adequate training (Tuna-org, 2011). Training and human capacity building was one of the topics agreed on at the FAO Regional Workshop on PSM to Combat IUU Fishing held in South Africa in 2008 for further action and cooperation in strengthening and harmonization of port state measures (FAO, 2008b). The subjects that were aimed to be developed within the scope of this subject were species identification, port state measures, measures against IUU fishing, VMS interpretation and Capacity development for legal expertise and skills (FAO, 2008b).

The first PSMA Inspectors Training Course was held in Vigo, Spain with the participation of twelve senior fisheries inspectors from Costa Rica, Ecuador, Panama and Peru in 2018 by FAO (FAO, 2018). FAO continued the capacity building trainings to implement PSMA in Guinea in 2019. Fifteen fisheries inspectors and seven additional government representatives from the Ministry of Fisheries, Coast Guard, Customs and Navy attended the training (FAO, 2019). Fish Force Academy was established to combat fishing crimes in South Africa. Similarly, the Australian government ran a capacity-building program among Fisheries Monitoring and Surveillance Officers across the Southeast Asian region. It was reported that Non-Governmental Organizations (NGOs) worked closely with coastal states in global geographies such as Italy, Gabon, Ghana, Namibia, Benin and Cape Verde to help combat IUU fishing (Wirajuda et al., 2019).

Conclusion

Turkey became a party to the "International Agreement on the Measures of the Port States for the Prevention of IUU Fishing" on the 9th of November 2010. According to the mentioned above agreement one of the responsibilities of port states is the training of high calibre inspectors. As per the agreement each party is authorized to provide the training of the inspectors in the country.

As per Article 5 with the title Integration and Coordination at the national level of the Agreement on the Measures of the Port States for the Prevention of IUU Fishing, each party integrated Port State measures with other measures to prevent, deter and eliminate IUU fishing and fishing related activities in support of such fishing, taking into account as appropriate the IPOA-IUU; and take measures to exchange information among relevant national agencies and to coordinate the activities of such agencies in the implementation of this Agreement (FAO,2009; EU, 2011).

Article 16 of the Fishery Law states that the Ministry of Agriculture and Forestry can plan training and education actions to improve the professional knowledge and experience of fishermen (Turkey, 1971). But the Fishery Law doesn't consist of anything regarding the training of inspectors. Besides, the integration of the training of inspectors should be provided within the scope of the 416th article of the Presidential Decree No: 1.

In Turkey, the inspectors of the Ministry of Agriculture and Forestry and the Command of Coast Guard had sufficient training according to the Annex-E of Article 17 of the PSMA to Prevent, Deter and Eliminate IUU Fishing. The trainings related to the provisions stated in the Annex-E of Article 17 of the PSMA to Prevent, Deter and Eliminate IUU Fishing should be planned or necessary studies should be carried out by the Ministry of Agriculture and Forestry for authorized institutions and organizations other than the Ministry of Agriculture and Forestry and the Command of Coast Guard.

International Forums on IUU Fishing (CH, 2009; FCWC, 2011; NFDS, 2016; IUUWatch, 2017; CH, 2018; CH, 2020) and Global Fisheries Enforcement Training Workshops (FAO, 2007; FAO, 2008a; FAO, 2017; GFETW, 2021) were held. To prevent IUU Fishing on a national and international level it was evaluated that it will be beneficial to participate in workshops on the prevention of IUU Fishing and Global Applied Training Workshop and to coordinate planned and continuous workshops, seminars and symposiums between the inspecting organizations in Turkey on the prevention of IUU Fishing.

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

Length-frequency distribution and relative condition factor of *Brycinus nurse* in the New Calabar River, Niger Delta, Nigeria

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ABSTRACT

This study assessed the length-frequency distribution and condition factor of *Brycinus nurse* from two sampling stations in the New Calabar River. A total of 401 individuals of *B. nurse* were collected from artisanal fishers and assessed between February 2020 and January 2021.The collected samples were analyzed for growth pattern using FISAT II. The mean values were 11.80±1.59 cm and 9.52±1.28 cm for total length and standard length The mean weight was 20.63±8.38 g. The length- weight relationship revealed an exponent b value of 2.539, showing negative allometric growth. The condition factor ranged from 1.09 to 1.42 indicating that the species is in good growth condition. The size structure of *B. nurse* recorded indicated that most individuals were relatively medium to small sizes and were not allowed to grow to adults as a result of overfishing. Therefore, proper management and intervention is needed to manage overexploitation of *B. nurse* in the New Calabar River.

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Introduction

Brycinus nurse (commonly referred to as nurse tetra) is a pelagic, potamodromous ray-finned fish species (Riede, 2004) belonging to the family Alestidae. It inhabits rivers, lakes, irrigation canals, and fringing vegetation. *B. nurse* (Rüppell, 1832) is widely distributed in west Africa (Paugy, 1990; Paugy, 2003), in lower Guinea, where it is present in the Cross and Mèmé rivers. Also, in the Chad basin and the Nile River (Getahun, 2007) up to Lake Albert. It feeds on zooplankton, caridina, insects, snails, and vegetation (Bailey, 1994), and less frequently, small fishes, mainly *Haplochromis* spp. It spawns at the beginning of the rainy season. Dwarf populations are described in lake basins.

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B. nurse is a native to freshwater systems in Africa, thriving well in both lacustrine and riverine conditions (Boulenger, 2002), particularly in the Niger Delta, Nigeria. *B. nurse* is easily identified by its bright red tail fin. Other fins are tinged with red, and there is a black patch on the tail peduncle (Adesulu & Sydenham, 2007). This species is said to be of commercial importance due to the fact that they are widely consumed locally and has food value (Reed et al., 1967; Saliu & Fagade, 2004). Like other commercially important fish species in Nigerian inland water, this fish family is currently subjected to intense fishing pressure and pollution due to the lack of proper management policies. As a result of this unregulated exploitation, there is an urgent need to monitor and assess the state of the fish stock.

Growth studies are particularly important for describing the status of a fish population and for predicting the potential yield of a fishery. Unfortunately, age and growth determination in fishes of tropical waters are not easily calculated. This is because water temperatures vary only slightly throughout the year, making the formation of growth marks on the scales and other hard parts uncertain (Arowomo, 1982). Mohanraj (2000) opined that a better understanding of growth and size/age relationships is a must for one applying equilibrium yield models to the management of a fishery. This study was therefore designed to investigate biological information such as length-frequency distribution and relative condition factors of *B. nurse* in the New Calabar River, Niger Delta, Nigeria.

Materials and Methods

Description of the Study Area

The study area is the stretch of the New Calabar River. The New Calabar River lies between longitude 06°53 53086'E and latitude 04°53 19.020'N in Choba, Rivers State, Nigeria. The entire river course is situated between longitude 7°60'E and latitude 5°45'N in the coastal area of the Niger Delta and empties into the Atlantic Ocean. The New Calabar River region has an annual rainfall between 2000 and 3000 (mm) (Abowei, 2000). The New Calabar River is a black water type (RPI, 1985) located in Rivers State, Nigeria. It lies on the eastern arm of the Niger Delta and empties into some creeks and coastal lagoons bordering the Atlantic Ocean. At the source at Elele-Alimini, the water is fresh and acidic but brackish and tidal at the mouth. Aluu is the upstream part of the river where the river is fresh and tidal (Erondu, 1983), whereas it is brackish at a little distance downstream (Choba and Ogbogoro) The New Calabar River is among the essential water resources in the Niger Delta region of Southern Nigeria; it is in the vicinity of the rapidly expanding oil city of Port Harcourt in Rivers State, Southern Nigeria. The river is subjected to effluent discharge from industries sited along its banks. Also, surface run-off resulting from soil erosion, lumbering activities, forestry operations, dredging activities, and domestic sewage inputs may lead to wide scale contamination of the river (Dienye & Woke, 2015). Fish samples were collected from two sampling stations in the New Calabar River; Choba and Ogbogoro station.

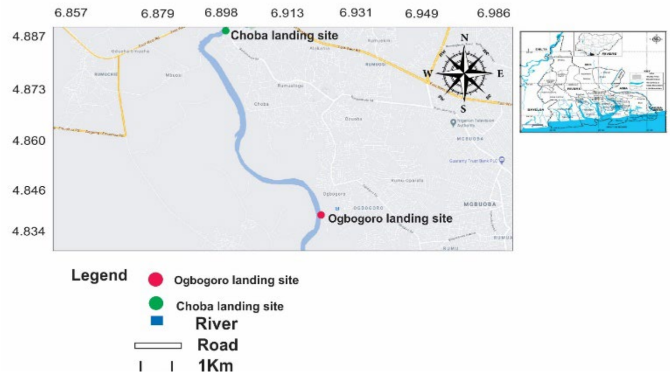

Figure 1. Map showing the sampling stations

Data Collection

Sampling was done twice a month from February 2020 to January 2021 from the two sampling stations with the assistance of local artisanal fishers. The fish species was identified using fish identification keys (Paugy, 2003; Adesulu & Sydenham, 2007). Measurements were taken at the landing sites of the fishers. The measurements collected were: the total length (TL), fork length (FL), standard length (SL), and girth length (GL) which is a contouring measurement to find the maximum circumference around the body of individuals of the species in the sampling stations in centimeters (cm) using a measuring board with a 30 cm ruler measured to the nearest ±0.1 cm. The body weight (W) was measured in grams (g) to the nearest ±0.01 g with an electronic sensitive scale.

Data Analysis

Analysis was carried out on the data obtained from the samples using FISAT II (FAO-ICLARM Stock Assessment Tools) as explained in details by (Gayanilo et al., 2005). The following stages of analytical methods were used;

The length-frequency distribution for *B. nurse* was estimated by using 1 cm intervals of TL.

Length-weight relationship was estimated using the equation (1):

$$W = aL^b \qquad (1)$$

Where, W=weight of fish in grams (g), L=length of fish in centimeters (cm), a=regression constant which describes the rate of change of weight with length (intercept), b=regression coefficient which is the weight at unit length (slope).

The equation would be transformed to log to estimate the parameters a and b (Nehemiah et al., 2012) as provided in the equation (2):

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

The relative condition factor is the ratio of observed weight (w) of a fish at a given length to the expected weight (w) of a fish of the same length as calculated from the length weight regression (Le Cren, 1951).

Ponderal index (k) was calculated using the equation (3) of Fulton (1904):

$$K = 100 \times \left(\frac{W}{L^3}\right) \quad (3)$$

where W is the weight of the fish in gram (g), L is the total length in cubic centimeters (cm^3) for the length (L). The scaling factor of 100 was used to bring the relative condition factor (K_n) close to the unit for each individual was calculated using the equation (4) of Le Cren (1951):

$$K_n = \frac{W}{aL^b} \quad (4)$$

where W is the body weight in (g), L is the total length in (cm), a and b are the LWRs parameter. The variation in the length and weight between the two sampling stations; Choba and Ogbogoro was done using T-test.

Results

Length Frequency Distribution

In Figure 2, mean was 11.8 cm the highest frequency distribution, the lowest length was 8.90 cm and the highest length recorded throughout the study period was 16.80 cm, and the total length of 10 to 12 cm was the dominant class.

Length-Weight Relationship

Descriptive statistics on the length (cm) and weight (g) measurements are presented in Table 1 with the mean total length estimated as 11.80 cm, and the mean weight as 20.63 g. The number of individuals (N) was 401 sampled across 12 months. The highest number of samples was recorded in June (70) while the lowest was in September (15). The b value was found to be 2.539. The value of the coefficient of correlation (r^2) estimated for the species was 0.8193 showing that the relationship between length and weight of the fish was highly significant (Figure 3).

Table 2 below compared the variation in the length and weight between the two sampling stations; Choba and Ogbogoro stations recorded higher mean values in all the parameters measured (TL, SL, FL, GL and W). indicating a significant difference between the two stations at $p<0.05$.

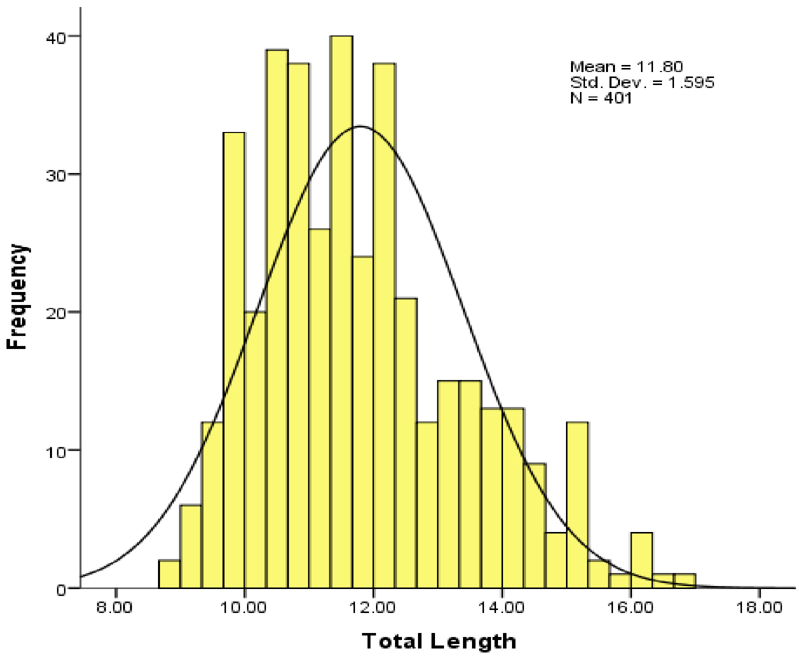

Figure 2. Length-frequency distribution of *B. nurse* during the study period

Table 1. Descriptive statistics on the length (cm) and weight (g) measurements

Parameters	N	Minimum	Maximum	Mean	SD
Total length	401	8.90	16.80	11.80	1.59
Standard length	401	7.00	13.30	9.52	1.28
Fork length	401	7.70	14.50	10.43	1.45
Girth length	401	5.70	11.70	7.64	1.07
Weight	401	10.00	55.20	20.63	8.38

Note: *N* is the sample size; *SD* is the standard deviation

Table 2. Comparisons of length (cm) and weight (g) between stations

Parameters	CHOBA	OGBOGORO	t-value	p-value
Total length	11.62±1.58	12.08±1.59*	-2.85	0.01
Standard length	9.34±1.22	9.81±1.32*	-3.62	0.00
Fork length	10.24±1.38	10.74±1.51*	-3.47	0.00
Girth length	7.31±1.00	8.17±0.97*	-8.43	0.00
Weight	19.62±8.40	22.24±8.12*	-3.08	0.00

Note: * Significant at p<0.05

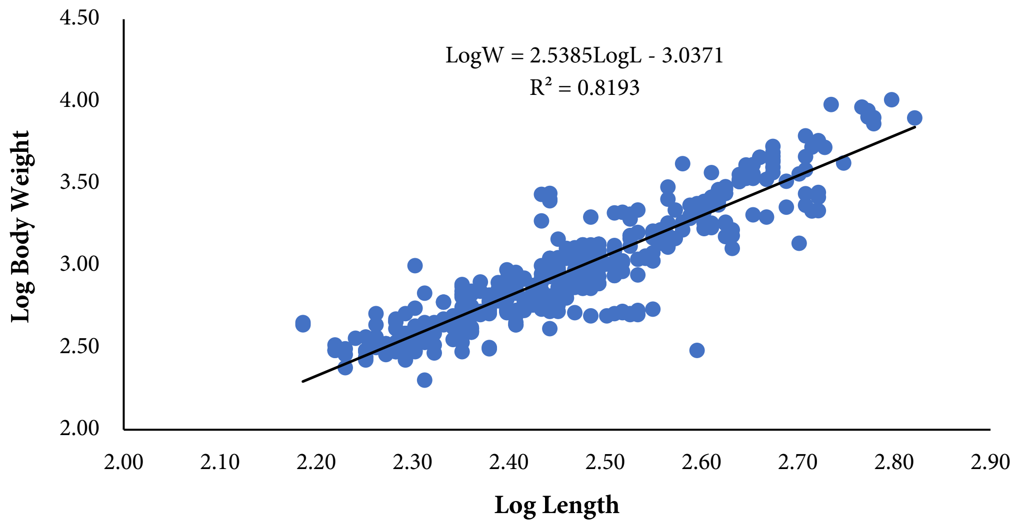

Figure 3: Length-weight relationship of *B. nurse* during the study period

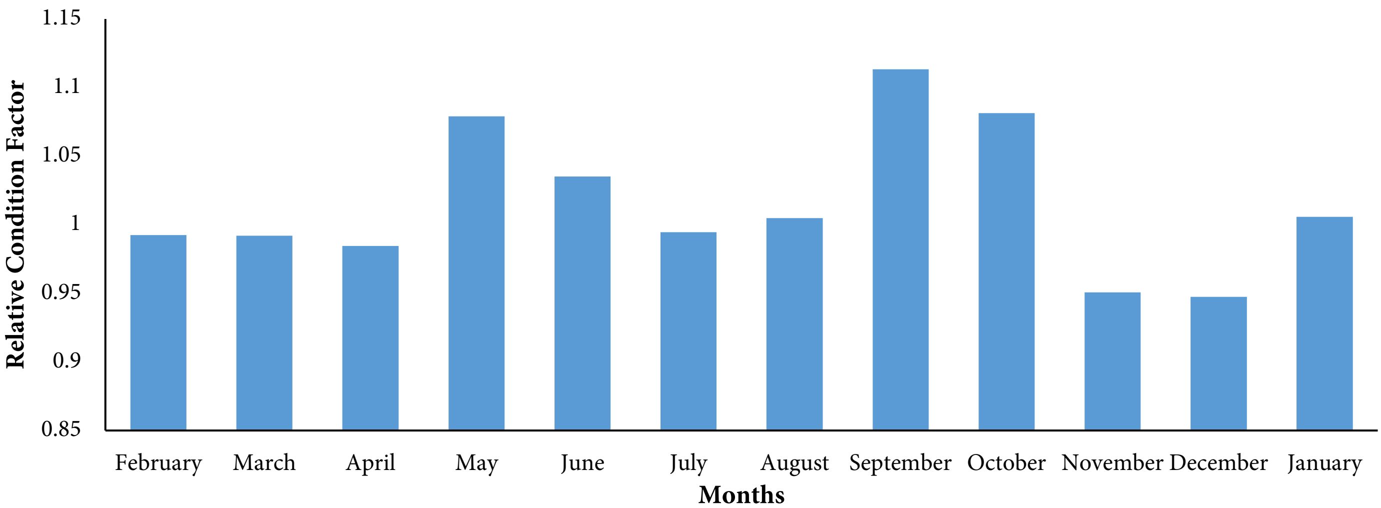

Figure 4. Month wise relative condition factor (K_n) of *B. nurse* during the study period

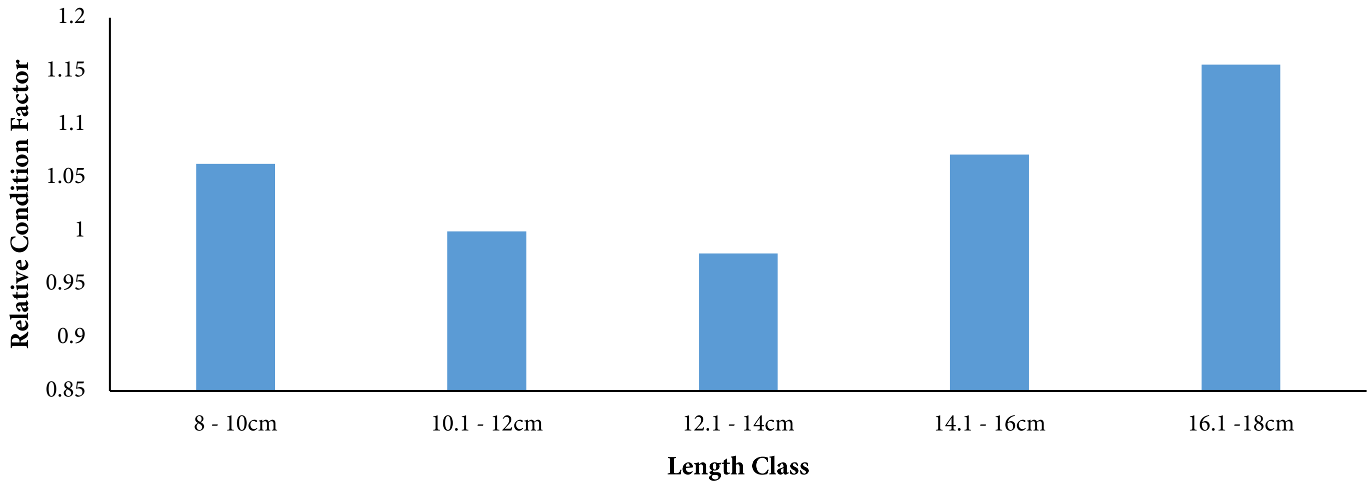

Figure 5. Length wise relative condition factor (K_n) of *B. nurse* during the study period

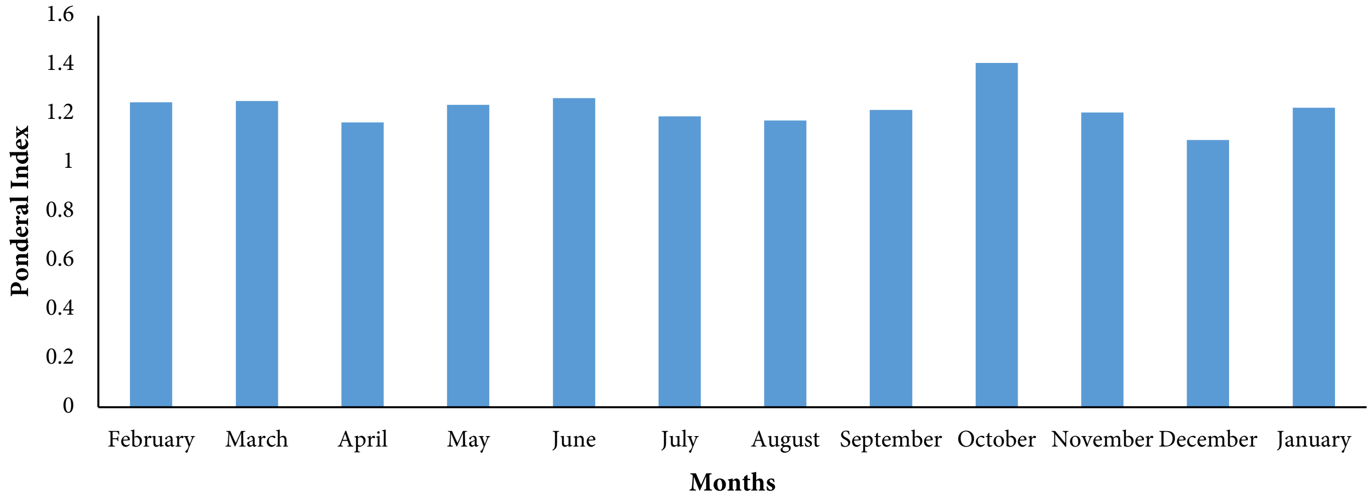

Figure 6. Month wise ponderal index (*K*) of *B. nurse* during the study period

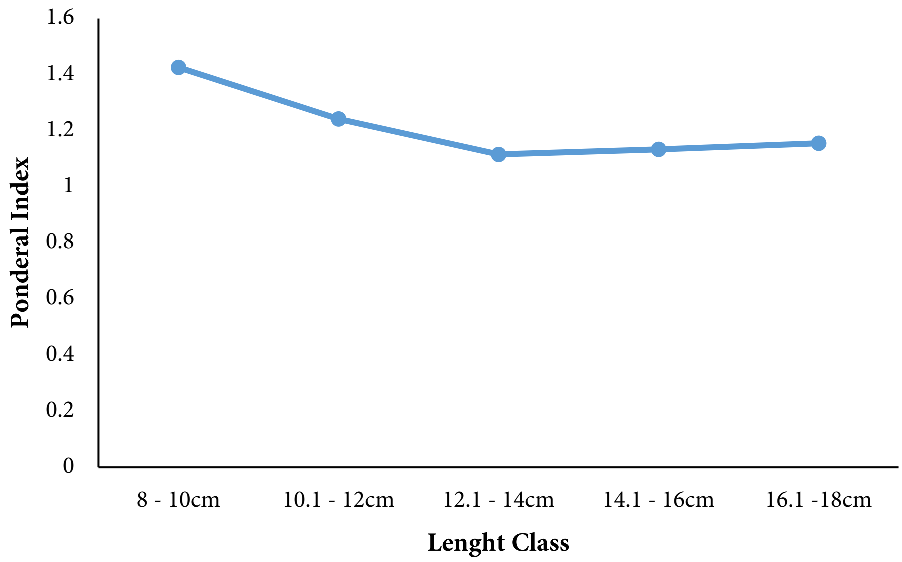

Figure 7. Length wise ponderal index (*K*) of *B. nurse* during the study period

Relative Condition Factor (K_n)

The Figure 4 below shows the monthly relative condition factor (K_n) for *B. nurse*; the highest value was 1.11 recorded in September, followed by 1.08 in October, while the lowest was 0.94 in December. Also, Figure 5 shows the length wise relative condition factor; the highest value of 1.15 was recorded in the length class of 16.1-18 cm and the lowest value of 0.97 was recorded in the length class of 12.1-14 cm.

Ponderal Index (K)

The figure 6 shows the month wise ponderal index (*K*) for *B. nurse* with the highest value observed in October (1.40), and lowest in December (1.09). *K* was determined for *B. nurse* on a length wise basis in Figure 7 highest value range of 1.42 was recorded in the length class of 8-10 cm, and 1.11 with the lowest in the class 12.1-14 cm.

Discussion

The weight of *B. nurse* throughout the study ranged from 10.0 to 55.20 g, with an estimated mean weight of 20.63±8.30 g, while its total length was observed to be in the range of 8.9 to 16.8 cm with an estimated mean total length (TL) of 11.80±1.59 cm. Olopade et al. (2019) recorded the weight of *B. nurse* that ranged from 5.0 to 210 g (mean=39.44±2.73 g) and a total length which ranged from 7.30 to 23.0 cm (mean=14.58±0.25 cm) in the New Calabar River. These results are in contrast with those from this study, and this variation in length and weight could possibly be as a result of some factors such as: growth stages, level of exploitation of the fish species in the water body as well as predation by other fish species, nature of the aquatic environment (pollution), and abundance of food for the fish species.

The length weight relationship of this study revealed that *B. nurse* had an exponent *b* value of 2.439, which shows a negative allometric growth. The value, however, falls within the acceptable range of 2.5 and 3.5, which is typical for tropical fish stocks (Carlander, 1969; Froese, 2006). A similar observation was also reported in *B. nurse* from the mid Cross River basin by Iyabo (2014), with a '*b*' value of 2.425 and 2.958 recorded by Lalèyè (2006) in the Ouémé River, Bénin. Also, Dankwa (2003) recorded 2.959 in Volta Lake Ghana. These are in contrast with the findings of this study as they exhibit closely isometric growth. While Getahun (2007) recorded a value of 3.086 for the same exponent in the River Bia; Abobi & Ekau (2013) reported 3.0737 for *B. nurse* from the White Volta River in Ghana; and Olopade et al. (2019) recorded 3.5405 in the New Calabar River, all showing positive allometric growth. The differences in these findings may be due to several factors that affect the growth of fish, including habitat, seasonal variation, sexual dimorphism, gonadal maturation, availability of food, and general health of the fish (Ozcan & Balik, 2009).

Adesulu & Sydenham (2007) reported that the condition factor in fish is mainly affected by the amount of food in the stomach and the stage of egg development that will affect the weight of the fish, and the weight of the fish is a function of the condition factor in fish. The condition factor and relative condition factor of *B. nurse* in this study were in the range of 1.09-1.42, indicating that the population is in good growth condition as the value is greater than 1. *K* values greater than 1 imply that the fishes thrive well in their current habitat (Komolafe & Arawomo, 2011).

According to Ikomi & Sikoki (2001), the '*K*' value for B. nurse in the River Jamieson, Nigeria ranged from 2.33 to 2.65 in the wet season and 1.99-2.21 in the dry season. The peak value was obtained in July and the least value was in September where the value dropped dramatically. Concerning size and sex variations, mean *K* values decreased with an increase in the fish size within the range of 7-11 cm. After that, the *K* values increased with additional size. The condition factor varied with the size of the fish. It was inversely related to increasing length in the medium size group. Moreover, with the large size group, *K* values increased with increasing fish size. A similar observation was made by Paugy (1979). However, Brown (1985) reported an inverse relationship with all size groups. The high rainy season peak in *K* values may be related to the food regime of the fish. During this season, the fish were able to utilize the rich food resources and accumulate a lot of fat, which probably resulted in their improved well-being (Ikomi & Sikoki, 2001). However, these are in contrast with the results of *B. nurse* in this study, where the condition factor values were unrelated to particular periods, sex, or length size, although all values were above unity, indicating that the fish were in good condition.

Conclusion

This study provides important baseline knowledge on the size structure and general well-being of *B. nurse* in the New Calabar River. The length-weight relationship revealed negative allometric growth (less than 3) for *B. nurse*. The species is also in good growth condition as revealed in the condition factor (*K*) which ranged from 1.09 to 1.42. The maximum size of *B. nurse* recorded in this study indicated that most individuals were relatively medium to small sizes, and were not allowed to grow to adults as a result of overfishing. This information will help in the development of efficient management strategies of the species and also, proper management and intervention are needed to prevent and manage the over exploitation of *B. nurse* in the New Calabar River.

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Compliance With Ethical Standards

Authors' Contributions

Author HE and OA designed the study, HE wrote the first draft of the manuscript, SA performed the 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

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

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RESEARCH ARTICLE

Some biological parameters of *Patella caerulea* from the Black Sea

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ABSTRACT

Limpets are common inhabitants of the midlittoral and upper infralittoral zones and play an important ecological role in the coastal ecosystem. The study aimed to assess growth, meat yield, morphological aspects, condition and reproduction features of *Patella caerulea*. About 58% of collected Mediterranean limpets were concentrated in the 25.0-34.9 mm shell length group. The average meat yield ratio was calculated as 39.34%. Mean growth increments for shell length (SL) and total weight (TW) were 23.99% and 97.99%, respectively. Results exhibited relatively high correlation coefficients among variables. Mean condition factor value was calculated as 14.2. Spawning occurs over a short period with ovigerous females observed on two month periods. Mean fecundity was calculated as 90,983±28,675 eggs/g whereas mean egg diameter was estimated as 160.6 µm. This study presents first baseline information about biological and morphological of Mediterranean Limpets population in Black Sea.

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Introduction

Marine gastropods are situated at several levels in the food chain, which increases their determining role in the functioning of marine ecosystems (Hakenkamp & Morin, 2000). Limpets of the genus *Patella* are grazing gastropods, common inhabitants of the hard substrate communities in the mid-littoral and upper infralittoral zones of the East Atlantic and Mediterranean coasts in temperate latitudes (Vafidis et al., 2020). Limpets are considered the "keystone" species of the mid-littoral zone and are widely collected for human consumption and as fishing bait (Menge, 2000). They play an essential role in controlling algal coverage and consequently, the ecological succession and biological communities established in coastal zones (Prusina et al., 2015; Vafidis et al., 2020).

The Mediterranean limpet *Patella caerulea* Linnaeus, 1758 is among the most common species of rocky shores in the infralittoral and midlittoral, especially Mediterranean (Küçükdermenci et al., 2017). *P. caerulea* is considered endemic to the Mediterranean Sea (Christiaens, 1973). It is very

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prevalent in Turkish seawater (Öztürk & Ergen, 1996). This species primarily feed by scraping algae off rocks and plants such as Cyanophyceae, *Ulva lactuca*, *Corallina elongate* (Silva et al., 2008; Ayas, 2010). *P. caerulea* is a hermaphrodite protandric species and may reach a length of 70 mm (Kastanevakis et al., 2008). Mediterranean limpet fulfills the criterion an ideal bioindicator for heavy metal contamination is extensively used in marine monitoring programs (Storelli & Marcotrigiano, 2005).

Various studies have been conducted on the morphology, biology, ecology, and distribution of *Patella* species, especially in the Mediterranean Sea (Navarro et al., 2005; Espinosa et al., 2006; Prusina et al., 2014; Prusina et al., 2015; Bouzaza & Mezali, 2018). In Turkey, generally, morphometric, taxonomic, distribution, and heavy metals content studies on *P. caerulea* have been conducted (Bat et al., 1999; Akşit & Falakalı Mutaf, 2007; Ayas, 2010; Çulha & Bat, 2010). Although *P. caerulea* is the most dominant intertidal gastropod on the rocky shore of the Black Sea in Turkey, there is no information about these species in the region yet. The present study aims to make an extensive assessment of population structure and characteristics, growth, meat yield and reproductive status of *P. caerulea* in the Black Sea. To our knowledge, this is the first study in Turkey of the species, on the Black Sea.

Material and Methods

Study Area and Sampling

This study was conducted along the Turkish coast of the Black Sea (between 41.149538° N, 37.273525° E–40.971953° N, 38.059734° E). In this study, *P. caerulea* were collected from the intertidal zone of the coastline monthly from April 2018 to March 2019. Limpets were collected by hand with a penknife, and injured limpets (broken, shattered, severed) were not included in the sampling. Collected specimens were put into drums filled with seawater and then transferred to the laboratory. Collected samples were identified on the basis of form and external sculpture of the shell, the color of the pallial tentacles and the color of the foot (Christiaens, 1973).

Growth and Morphological Analyses

Shell length (SL – the longest distance between the anterior and posterior of limpet), shell width (SWi – the longest distance perpendicular to the anterior-posterior axis), and shell height (SH – the longest vertical distance from the apex to the shell base) of samples were measured using a digital caliper to the nearest 0.01 mm. Total wet weight (TW), meat weight (MW), and shell weight (SWe) was measured with an electronic balance of 0.001 g accuracy. The growth performance of samples according to *SL* and *TW* was determined with the formula below:

$$\text{SL increment (\%)} = [(SL_n - SL_{n-1})/SL_{n-1}] \times 100 \quad (1)$$

$$\text{TW increment (\%)} = [(TW_n - TW_{n-1})/TW_{n-1}] \times 100 \quad (2)$$

where n is the number of size classes (Ricker, 1975). The *SL*, *SWi* and *SH* relationships with each one were determined using the equation (3).

$$W = aL^b \quad (3)$$

Linear relationships between *SL* and *TW*, *SWe*, and *MW* were also analyzed (Le Cren, 1951; Pauly, 1980; Erkoyuncu, 1995). The condition factor (*K*) was calculated using the formula (Equation 4):

$$K = 100 \times \frac{TW}{SL^3} \quad (4)$$

where *TW* was total weight (g), and SL was shell length (mm) of collected samples (Bagenal, 1978).

Reproduction, Fecundity, and Egg Diameter

Sex was determined using macroscopic methods. The spawning period was determined by analyzing the monthly variation in the gonad maturity as well as the gonadosomatic index (GSI). In this study, 191 females were used to calculate the gonadosomatic index as described by (Bagenal, 1978) (Equation 5):

$$GSI = \left[\left(\frac{Gonad\ weight}{Meat\ weight}\right) \times 100\right] \quad (5)$$

The limpet eggs were removed from 26 ovigerous females in the spawning period (in October and November), and the total weight of eggs was measured using a balance with a sensitivity of 0.0005 g. Fifty eggs were taken from different regions of an ovary, and egg diameters were measured with an calibrated ocular micrometer. Eggs were counted with an ocular micrometer as (Equation 6):

$$F = n\left(\frac{W_0}{X}\right) \quad (6)$$

where W_0 denotes the weight of gonad and n represents the number of eggs in the subsample, X stands for subsample weight (g), F represents the number of eggs (Bagenal, 1978; Kwei, 1978; Jones et al., 1990).

Data Analysis

Statistics and data analyses were performed by statistical software SPSS v26.0. The normality of the data was checked using the Kolmogorov-Smirnov test, depending on the sample size. In the former analysis, homogeneity of variance was tested using Levene's test. Since the data in this study were not normally distributed, monthly morphological values were compared using the Mann-Whitney U-test. Monthly and combined variables were analyzed with the Pearson correlation and regression analysis to investigate the relationships among morphological characters (Sokal & Rohlf, 1969; Düzgüneş et al., 1983). Hierarchical Ward cluster analysis after Z score correction was used to determine closely related variables (Lopez et al., 2004). The sex ratio of samples, the expected 1:1 male to female ratio was assessed using the chi-square (χ^2) test (Düzgüneş et al., 1983).

Results and Discussion

Size Distribution and Meat Yield

In the present study, 1830 *P. caerulea* individuals were sampled during the study period. Shell length distribution of collected samples showed that 57.98% of Mediterranean limpets were in a range of 25 to 34.9 mm. It was observed that 77.40% of the samples collected were smaller than 5 g, according to the TW (Figure 1). Ayas et al. (2008) reported the SL, SWi, and SH in a range of 19–39 mm, 15–33 mm and 5–11 mm from samples caught in the Mersin Bay (Mediterranean). Küçükdermenci et al. (2017) reported the minimum and maximum SL as 24.4 and 30.7 mm in Izmir Bay (Aegean Sea), respectively. Vafidis et al. (2020), who conducted a study in the Pagasitikos Bay (Aegean Sea), determined the average SL, SWi, and SH as 23.36, 18.96, and 6.35 mm, respectively. In our study, biometric parameters were more variable compared to similar studies, possibly attributed to variation in the number of samples or sampling regions. The environmental and ecological factors (food availability, water parameters, and meteorological factors) may lead to morphological variations in limpets of the same species that live in different ecosystems (Crothers, 1983). Monthly biometric measurements and statistical differences of *P. caerulea* are presented in Table 1. A closer look at the monthly SL reveals that the maximum value was 31.70 mm in December and the minimum value of 25.80 mm in May. Minimum SL, SWi, SH, TW, and MW were determined in May and July. High SL, SWe, TW, and SW values were obtained in April and December.

Average MW of *P. caerulea* was calculated as 1.49±0.02 g with a rate of 39.34% of TW. In other words, 1 kg of edible meat can be obtained from approximate the 2.5 kg live weight of *P. caerulea*. It was determined that the minimum and maximum meat yield ratio was 35.32% in November and 43.68% in August, respectively. A weak relationship was found between the SL and MW. However, the maximum ratio of meat weight (41.56%) was observed in the 25.0-29.9 mm shell length classes (Figure 2). The mean meat weight of samples was significantly different by month (χ^2= 99.851; df= 11; .001). Similarly, Küçükdermenci et al. (2017) determined the percentage of meat yield as 37% in Izmir (Turkey) for *P. caerulea*. Hamad et al. (2019) who conducted a study in Libya, determined the average meat yield as 1.09 g. The present study's findings concerning meat yield are in agreement with the findings reported by Küçükdermenci et al. (2017) and Hamad et al. (2019). Water conditions, especially temperature, may also affect the meat yield of *Patella* species (Fretter & Graham, 1976). Thus, it is usual to observe differences in meat yield.

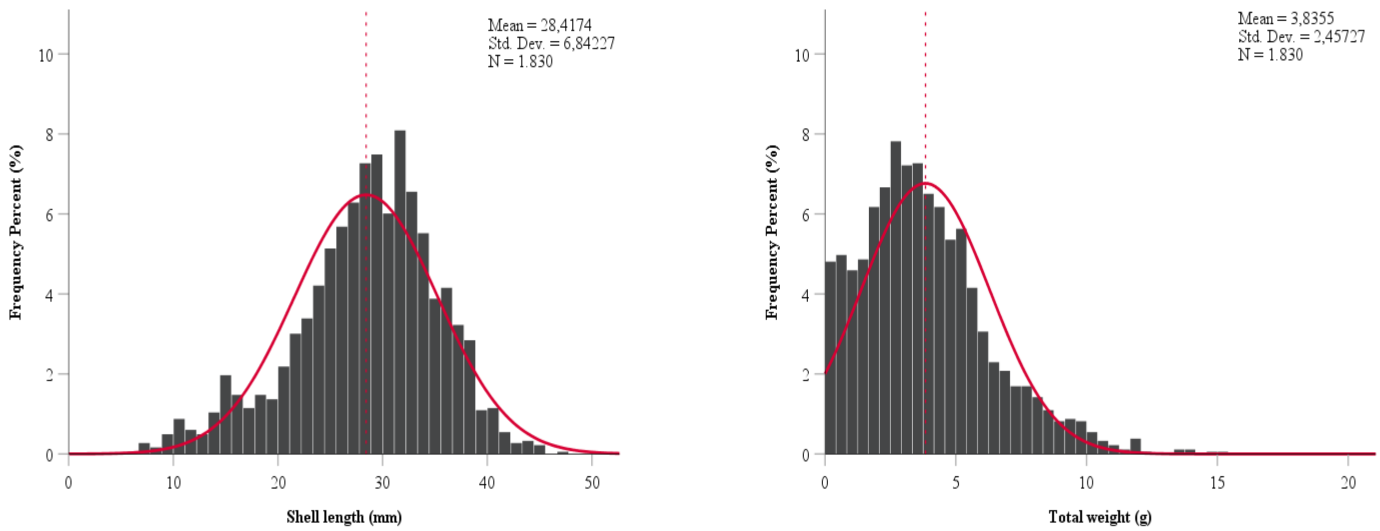

Figure 1. Density histogram of shell length (SL) and total weight (TW) of population and dashed red lines indicating the means and red lines representing the normal curve of the population for the *P. caerulea*

Table 1. Monthly and totally morphometric measurement values of *Patella caerulea* (N = 1830)

		April A	May B	June C	July D	Aug E	Sep F	Oct G	Nov H	Dec I	Jan J	Feb K	Mar L	Mean
SL (mm)	Mean	31.04	25.80	28.46	26.20	28.84	28.65	28.51	28.53	31.70	28.54	26.74	27.66	28.42
	±SE	0.54	0.71	0.51	0.41	0.38	0.44	0.68	0.56	0.49	0.57	0.54	0.61	0.16
	Min	7.26	6.31	9.84	10.13	15.99	9.27	6.72	7.75	10.09	10.33	9.32	12.10	6.31
	Max	44.29	43.42	40.43	43.35	38.72	47.05	44.75	45.00	43.02	45.26	38.86	40.40	47.05
	Dif.	a,f,g	b,c	b,d,e	c	a,d,h,i	a,e,h,i	a,b,c	a,b,c	f	b,c,d,e,g	b,c,h	b,c,i	**
SWi (mm)	Mean	25.14	20.62	22.92	20.91	23.17	23.22	23.12	23.15	26.03	23.22	21.51	22.51	22.99
	±SE	0.46	0.61	0.46	0.36	0.33	0.39	0.61	0.50	0.44	0.50	0.47	0.55	0.14
	Min	4.82	4.09	6.61	7.23	12.13	6.53	5.03	5.38	7.47	7.15	6.30	8.55	4.09
	Max	35.98	35.78	36.26	36.85	31.18	38.12	39.02	36.49	36.49	38.04	32.94	35.53	39.02
	Dif.	a,h,i	b,d,g,j	b,c,e	d	e	a,e,f	a,b,d,e	a,c,e,g	h	c,e,i,j	b,d,e	b,d,e	**
SH (mm)	Mean	10.46	9.10	10.05	9.09	10.10	10.31	10.84	10.49	10.85	9.75	9.49	9.72	10.04
	±SE	0.23	0.31	0.25	0.19	0.17	0.21	0.34	0.25	0.20	0.23	0.22	0.27	0.07
	Min	2.29	1.54	2.94	2.64	4.83	2.35	1.14	1.71	3.22	2.61	2.67	3.15	1.14
	Max	15.64	17.10	18.85	15.57	14.88	19.15	22.81	17.69	15.60	16.91	15.67	18.23	22.81
	Dif.	a,e,f,g	b,c,d	a,b	b	a,c,e,f,g	a,d,e,f,g	a,e,f,g	a,e,f,g	a	b,e	b,f	b,g	**
TW (g)	Mean	4.93	3.30	4.08	3.00	3.76	4.08	3.93	3.91	4.51	3.75	3.16	3.55	3.84
	±SE	0.21	0.23	0.21	0.13	0.14	0.17	0.26	0.21	0.17	0.20	0.17	0.21	0.06
	Min	0.06	0.02	0.10	0.10	0.51	0.12	0.04	0.04	0.14	0.15	0.08	0.24	0.02
	Max	14.62	11.87	10.75	10.41	7.88	12.47	15.05	13.97	10.39	12.02	8.90	13.44	15.05
	Dif.	a	b,c,d	a,b,d	c	d,g,i,j	a,d,e,j	a,b,c,d	a,d,f,i,j	a,g,h	b,c,d,h	c,i	b,c,j	**
SWe (g)	Mean	2.23	1.56	1.78	1.31	1.66	1.78	1.79	1.85	2.22	1.75	1.48	1.68	1.76
	±SE	0.10	0.12	0.09	0.06	0.06	0.07	0.12	0.10	0.09	0.10	0.08	0.10	0.03
	Min	0.02	0.01	0.04	0.04	0.25	0.05	0.02	0.02	0.08	0.06	0.05	0.11	0.01
	Max	6.71	6.67	5.71	5.10	4.24	5.20	6.49	7.08	5.55	5.57	4.87	7.58	7.58
	Dif.	a,i,j	b,d,e	a,b,c,e	d	e	e,f	a,e,g,i	a,e,h,i	i	c,e,j	b,d,e	b,d,e	**
MW (g)	Mean	1.85	1.24	1.68	1.20	1.64	1.69	1.61	1.37	1.60	1.40	1.20	1.39	1.49
	±SE	0.08	0.09	0.09	0.06	0.06	0.07	0.10	0.07	0.06	0.07	0.06	0.08	0.02
	Min	0.02	0.01	0.05	0.04	0.20	0.04	0.01	0.01	0.05	0.05	0.03	0.10	0.01
	Max	5.19	4.52	4.38	4.38	3.44	5.90	5.37	4.05	3.46	4.23	3.87	4.57	5.90
	Dif.	a	b,d,h	a,e,g,i	b,c	a,e,g,i	a,e,g,i	a,d,e,g,i	b,e,f,h	a,f,g,i	b,g,h	c,h	b,h,i	**

Note: Superscript and capital letters denote comparisons between two months (a capital and a superscript). There is no statistical difference between a capital letter and each superscript letter (P < 0.05). A – November, B – December, C – January, D – February, E – March, F – April, G – May, H – June, I – July, J – August, K – September, L – October.

Table 2. Proximity matrix of six morphometric variables for *P. caerulea*

Proximity Matrix						
	SL	SWi	SH	TW	SWe	MW
SL	0.00					
SWi	435.36	0.00				
SH	4582.08	2252.68	0.00			
TW	8306.20	5012.97	578.12	0.00		
SWe	9614.60	6047.51	946.03	62.59*	0.00	
MW	9788.57	6188.20	1001.81	79.88*	3.39*	0.00

Note: Values with * indicates that the morphometric characters are very close.

Table 3. Mean (±SE) and size increment rates (%) of SL (mm) and TW (g) for sampled *P. caerulea* during the study

Size classes	N	%	Mean SL	Mean TW	SL increment (%)	TW increment (%)
5.00-9.99	18	0.98	8.52±0.26	0.08±0.01	-	-
10.00-14.99	72	3.93	12.89±0.19	0.30±0.01	51.18	264.85
15.00-19.99	119	6.50	17.36±0.14	0.70±0.02	34.73	136.81
20.00-24.99	275	15.03	22.86±0.08	1.69±0.03	31.69	141.99
25.00-29.99	539	29.45	27.65±0.06	3.09±0.03	20.95	82.43
30.00-34.99	522	28.52	32.34±0.06	4.88±0.05	16.95	58.10
35.00-39.99	238	13.01	37.01±0.08	7.39±0.11	14.43	51.39
40.00-44.99	43	2.35	41.62±0.20	9.93±0.30	12.46	34.35
45.00-49.99	4	0.22	45.59±0.49	11.32±1.05	9.54	13.92
Combined	1830	100	28.42±0.16	3.84±0.06	23.99	97.98

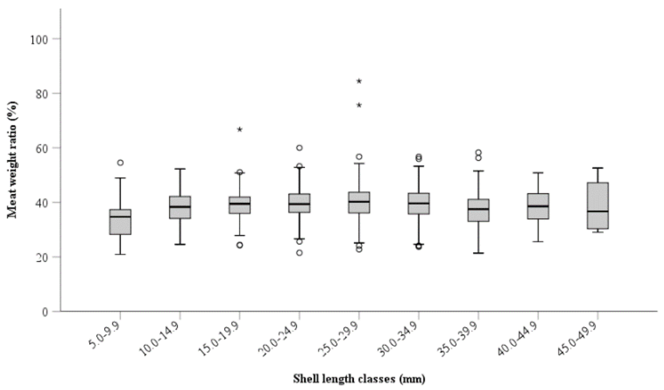

Figure 2. Boxplot of the percentage of meat weight (%) for the Mediterranean limpets according to shell length classes. The whiskers represent the ranges for the bottom 25% and the top 25% of the data values while bold horizontal lines represent the mean values. Circles and stars outside the whisker represent outliers (95%).

Biometric Relationships and Growth

According to the proximity matrix, close relationships were exhibited between SWe and MW (3.39), TW and SWe (62.59), and TW and MW (79.88). For instance, the relationships of SL with SWe and MW were highly distant. However, there appeared to be weak relationships of SL and SWi with other variables, a tendency similar to that presented by the correlation analysis (Table 2, Figure 3).

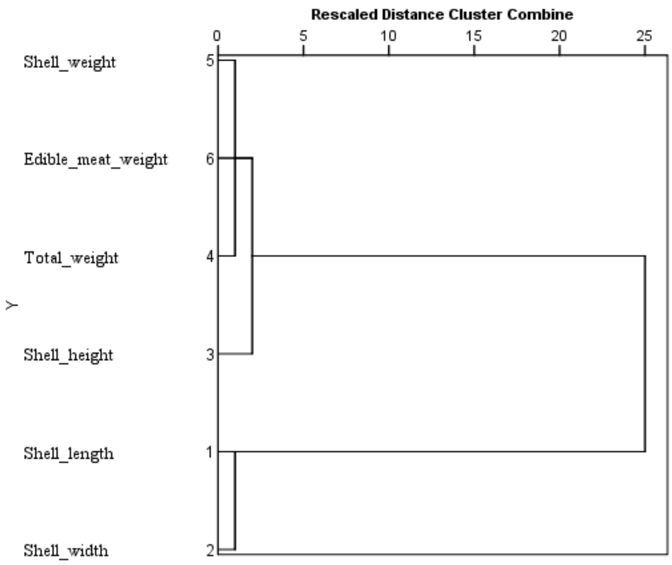

Figure 3. Dendrogram topology of the morphometric variables of *P. caerulea*.

The mean growth increment value was calculated as 23.99% and 97.99% according to the SL and TW, respectively.

According to SL and TW growth increments, the highest growth (51.18% and 264.85%, respectively) occurred in 10.0–14.9 mm size class individuals (Table 3). Both growth types inversely correlated with the size classes but seemed to be close relations. Furthermore, the growth of individuals decreased with the increase of SL size classes and almost plateaued after 50 mm. The most remarkable difference between SL and TW increments concerning the models is that the TW growth increment increases after 40 g weight classes (Figure 4).

In this study, the finding showed that the correlation coefficients among variables were quite high (Table 4). According to Pearson correlation values, the strongest and weakest correlations were determined between SL and SWi (R^2= 0.982) and between ST and MW (R^2= 0.868), respectively. A linear relationship and strong interaction (R^2= 0.95) were reported between SL and SWi of *P. caerulea* in the Mediterranean was found by Bouzaza and Mezali (2018). These findings are consistent with our results. Unlike these findings, Belkhodja and Romdhane (2012) reported that curvilinear regression equations between morphometric measures for Mediterranean limpets in Tunisia. Compared with our findings, weaker correlation coefficients (R^2= 0.962 and R^2= 0.908 for sheltered and exposed, respectively) were obtained of morphometric measurements of *P. caerulea* by Vafidis et al. (2020). *Patella* species may show significant morphological variability depending on environmental variation (Mauro et al., 2003). Correlations among the morphometric parameters are of particular significance in terms of understanding of smoothness of organism shell structure. It should be underlined, however, that big sample sizes could make a slight difference among the correlations that are significant (Aydın et al., 2014). The relationship of SL with TW and the linear relationship of SL with SWi and SH are presented in Figure 5. However, the b value of SL and TW of individuals was found as 3.09 (positive allometry). Considering the growth value (b > 3), it can be said that the sampled limpets have well enough environmental conditions, and the total growth of the individuals is provided as required. The b value was reported as 2.89 between SL and TW in the exposed site in Pagasitikos Gulf. Isometric growth type for SL and TW was reported by Vafidis et al. (2020). This value may vary depending on many conditions such as the number of samples, catch season, characteristics of the aquatic ecosystem, gonadosomatic index, and nutrition (Bagenal, 1978).

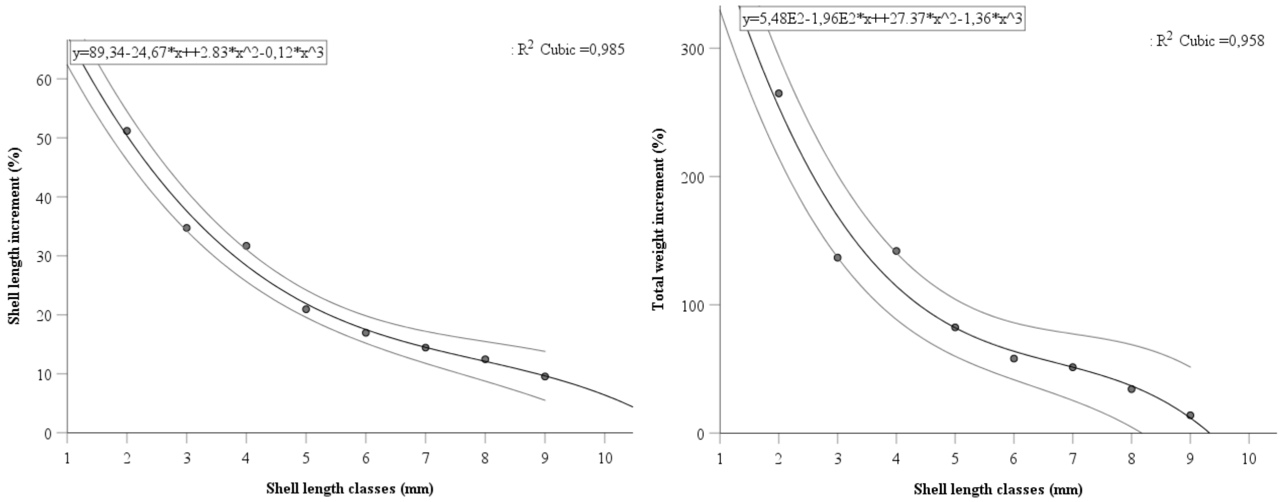

Figure 4. Growth increments (%) indicating the size classes for the Mediterranean limpets, where shell length from 5.0 to 49.9 mm are represented as 1 to 10. Grey lines in the graph represent the mean confidence intervals.

Table 4. Pearson correlation coefficients among morphometric variables for *P. caerulea*

Variables	SL	SW_i	SH	TW	SW_e	MW
SL	1					
SW_i	0.982	1				
SH	0.910	0.913	1			
TW	0.899	0.904	0.896	1		
SW_e	0.882	0.891	0.878	0.967	1	
MW	0.880	0.880	0.868	0.955	0.890	1

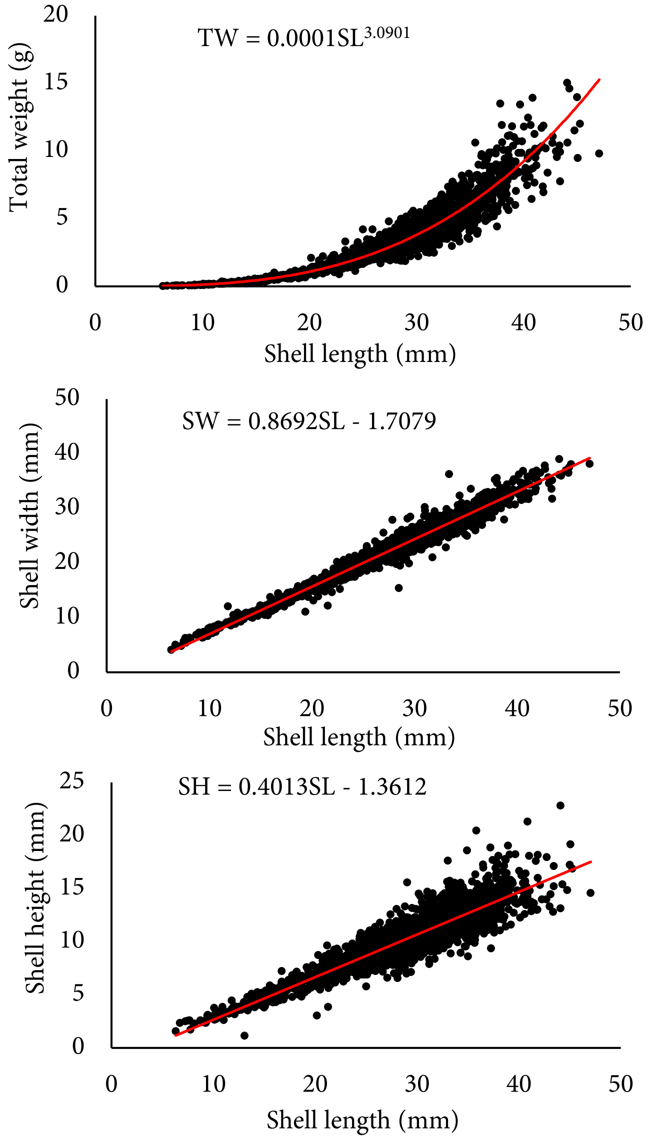

Figure 5. Nonlinear regressions SL-TW, linear regression SL-SWi and SL-SH for *P. caerulea* from the study area (N = 1380). The red lines represent the regression curve.

Condition Factor (K)

The mean condition factor (K) value was calculated as 14.2 for sampled individuals from the study area. The maximum K value occurred in June with 15.47, whereas the minimum K value occurred in December with 12.79. A significant increase in the condition of the individuals was observed in the summer with 15.03 (Figure 6). The mean K value of individuals was significantly different by monthly (χ^2= 164.21; df= 11; .001). The highest condition factor can signify the period before spawning in gastropods; water temperature may also affect both the index and reproduction of *Patella* species (Fretter and Graham, 1976; Vafidis et al., 2020). Based on this statement, it was seen that the increase in the K value before the ovulation period was significant. Thus, it was determined that the spawning season started in October and ended in November. The maximum and minimum K value of *P. caerulea* was reported as 41.20±1.18 in winter and as 30.46±1.54 in autumn, respectively, by Küçükdermenci et al. (2017). Vafidis et al. (2020) conducted that the maximum K value occurred in April (32.762±7.38), whereas the minimum K value occurred in February (26.58±4.47). The findings of our study were lower than the findings in other studies. Factors, such as different areas, spawning periods, and water parameters, may cause monthly changes in the condition of the limpets (Fretter & Graham, 1976; Belkhodja et al., 2011).

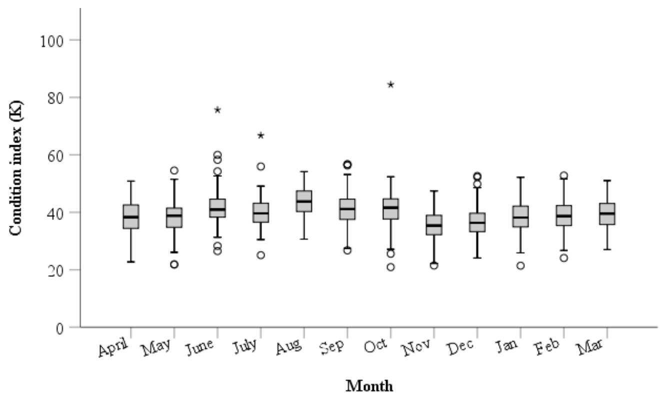

Figure 6. Boxplot of the monthly condition factor (K) for the *P. caerulea*. Horizontal lines in the boxes represent the mean values, and whiskers represent the standard deviations. Error bars represent 95% confidence intervals.

Reproductive Characteristics

Ovigerous females were observed for only two months (October and November), implying that spawning takes place for quite a short period for *P. caerulea*. The overall sex ratio of all individuals was calculated as 1:1.93 (M/F), with a significant deviation from the expected 1:1 ratio (χ^2= 29.19; df= 1; .05) in the spawning period. The GSI values of *P. caerulea* ranged from 1.41 to 12.32%, with a mean value of 5.69%. It has been reported that the Mediterranean limpets spawn period is in winter in south Eastern Australia (Parry, 1982), whereas in Eastern Tunisian coasts was between February and May (Gharred et al., 2019). These values are different from those obtained in the present study, suggesting that the regional differences may play a significant role in the spawning period. The fecundity of 26 ovigerous females ranged from 39,773 to 127,273 with an average value of 90,983±28,675 eggs/g. The egg diameter of samples ranged between 115.0 μm and 242.5 μm (mean 160.6 μm). Gharred et al. (2019) reported the mean egg diameters for three sites on the eastern Tunisian coast as 56.93, 41.22 and 40.71 μm, respectively. The egg diameters of Mediterranean limpets are unequivocally higher than those obtained in the present study. The present monitoring study suggests that reproduction parameters of Mediterranean limpets may exhibit variations depending on the regions.

Conclusion

In conclusion, due to its ever-changing spatiotemporal structure, the Black Sea should be closely monitored in terms of biodiversity, food chains and ecosystems. Thus, it is essential and valuable to know the current status, growth, and reproductive performance of available populations. This study presents baseline information on the Mediterranean Limpets population in this area, where no studies have been carried out. This study assessed growth, morphometric parameters and population aspects of the Mediterranean limpet, contributing valuable new data on *P. caerulea* along the Black Sea coastal zones.

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Compliance with Ethical Standards

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MA, AES and UK. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

Ethics committee approval is not required. All authors declare that this study does not include any experiments with human or animal subjects. All applicable international, national, and institutional guidelines for the care and use of animals were followed by the author.

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RESEARCH ARTICLE

Rosemary (*Rosmarinus officinalis*) as a preservative agent in canned bonito (*Sarda sarda*)

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ABSTRACT

This study was conducted to determine the shelf life and quality of traditional canned fish made from bonito (*Sarda sarda*) fish. The effect of the rosemary plant on some quality parameters and storage time of bonito fish with tomato sauce stored for 510 days at room temperature was determined by sensory, physical, chemical and microbiological freshness control methods. Nutrient composition analyses were performed in bonito fish before the canning process. Crude protein, crude oil, moisture and crude ash values were found to be, 23.125±1.062%, 2.783±0.339%, 72.717±0.652% and 1.150±0.212%, respectively. The amount of carbohydrates was determined as 0.275 g/100 g, the amount of energy was determined as 157.43 kcal/100 g. During storage, the total amount of volatile basic nitrogen (TVB-N), the number of thiobarbituric acid (TBA), pH, water activity (aw), the total number of aerobic mesophilic bacteria (TAMB), the total number of yeast and mold (TYM) were calculated. When TBA, pH, aw and microbiological analyzes were evaluated, both groups did not exceed the consumption limit values during the storage period. But in the research, the antioxidant effect of rosemary was clearly seen.

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Introduction

Nutrition, which is one of the basic requirements of life, is an important factor affecting all age groups and their quality of life, from the health of the unborn baby to the health of the elderly (Talak et al., 2010). Fish is healthy food and is the main player in human nutrition, ensuing about 20% of protein consumption to a third of the world's population which is more obvious in developing countries (Béné et al., 2007). Currently, chilling, freezing, salting, canning, drying and smoking, are the

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most frequently used techniques used for efficient safekeeping (Kumolu-Johnson et al., 2010). Bonito is the most abundant fish species in the Black Sea of Turkey and this species is consumed fresh domestically or exported as frozen (Caglak et al., 2012). Changing eating habits over time and increasing the number of working people necessitates the development of ready-to-eat foods. For this reason, antimicrobial or antibacterial agents are added to foods for protection purposes (Altuğ, 2001). Canning is a well-established and traditional way of providing food products with a long shelf life at constant ambient temperatures and is suitable for worldwide distribution (Bratt, 2010). Rosemary extract, whose antioxidant activity is well known (*Rosmarinus officinalis*) (Erkan et al., 2008, Bousbia et al., 2009), is the most tested active natural agent in both food simulation systems and real foods (Akhtar et al., 1998, Serdaroglu & Felekoğlu, 2005). Among the plants reported to have antioxidative activity, rosemary in its ground form or as an extract is commonly used in many food applications. The high antioxidant capacity of rosemary is due, substantially, to the phenolic diterpenes, carnosic acid, carnosol and rosmarinic acid (Sáenz-López et al., 2002). It has been reported that rosemary extract has antioxidant properties, it is a natural source of antioxidants compared to BHA and BHT in this regard (Tewari & Virmani, 1987).

The effects of rosemary extract on the quality of fish have been reported by several authors (Kenar, 2009; Ozogul et al., 2011; Kvangarsnes et al., 2021; Fouad et al., 2021). However, the effects of dry rosemary on the quality of canned bonito with tomatoes have not been reported so far.

This study was conducted in order to determine the quality of fresh bonito fish, which are loved and consumed in Turkey, canned rosemary with tomatoes and tomatoes were made by traditional methods.

Materials and Methods

Material

In this study, dry rosemary (*Rosmarinus officinalis*), purchased ready-made from the spicer, was used. To boil in tomato sauce, 200 g of dry rosemary were wrapped in a clean cloth. 150 bonito (*Sarda sarda*) fish with an average length of 28.91±2.85 cm and an average weight of 273.44±14.51 g, caught in Sinop in October, were used as fish material. 60 kg of tomatoes were obtained from the local market of Sinop, after being peeled, they were cut into 2 equal parts with a food processor. One group was boiled with the addition of rosemary and salt, the other group was boiled with the addition of salt only. An average of 5 liters of sunflower oil was used to add to each jar.

Methods

The fish purchased from the fishing stalls were cleaned after removing the head and internal organs and cooked in the oven at 180°C for 25 min without adding any salt and spices. The fish, the skin and the bones of which are extracted are placed in 370 ml jars with 150 g of fish meat. Fish meat is placed in jars in such a way that there is ahead, abdomen and tail. On average, 200 g of dried rosemary was added to 15 l of tomato juice. Jars with 200 ml of tomato sauce and 20 ml of sunflower oil added to them were autoclaved at 121°C for 30 minutes after closing the lids. The jars removed from the autoclave were turned over on a clean cloth for sealing control and allowed to cool. Sensory, chemical, physical and microbiological quality parameters were evaluated 15 days decently. The research continued for 510 days.

The methods of total crude protein Kjeldahl (AOAC, 1990), crude oil analysis (Bling & Dyer, 1959), crude ash analysis (AOAC, 1984), moisture analysis (Ludorf & Meyer, 1973) were used in nutrient composition analysis. Carbohydrate and energy amounts were calculated according to Merril & Watt (1973). The analysis of the nutritional composition of fresh bonito fish was carried out in two repetitions, three in parallel. During the storage period; the total volatile basic nitrogen (TVBN) (Antonacopoulos & Vyncke, 1989), the thiobarbituric acid level (TBA) [Erkan & Özden (2008) and Tarladgis et al. (1960) the methods were used together], the pH (Curran et al., 1980). An automatic water activity machine, the Novasina LabSwift-Aw meter, was used to determine the water activity (aw), the total aerobic mesophilic bacteria count (TAMB) and the total yeast and mold (TYM) count (Baumgart, 1986; Kılınç & Çaklı, 2005) and sensory analysis (Anonymous, 1988), of the canned bonitos in both groups were determined. In sensory analysis, it was taken according to 6 panelists. According to General Principles of Canned Fish Canned Fish (Anonymous, 1988), quality ranking of canned fish, first quality products get 15 points, second quality products get 14.9-13.0, third quality products get 12.9-11.0 and fourth quality products gets 10.9-6.

Results

The quality of the raw fish is the basic factor that governs the final product quality. This applies as much to canned fish as to any other food product. Estimation of the quality parameters is possible using both organoleptic and analytical methods in the laboratory and chemical and microbiological testing are have been done in this research. Crude protein, crude oil, crude ash and moisture values were determined as 23.125±1.062%, 2.783±0.339%, 1.150±0.212% and 72.717±0.652%, respectively (Figure 1). Carbohydrate (Figure 1, column right) and energy (Figure 1, column left) amounts were determined as 0.275 g/100 g and 157.43 kcal/100 g, respectively.

The pH value, which was determined as 5.47±0.01 in the group R at the beginning of the storage and as 5.39±0.008 in the control group, was determined as 5.73±0.06 and 6.03±0.01 on the 510th day, the last day of storage, respectively (Figure 2). As a result of analysis of variance and Tukey test, the difference between groups was found to be significant ($p<0.05$).

When the TVB-N results were examined, the average values of groups R and C on the first day were determined as 14.3±0.56 mg/100 g, 15.33±1.12 mg/100 g, respectively (Figure 3). The amount of TVB-N increased in both groups during the storage period. The amount of TVB-N was determined as 35.49±2.75 mg/100 g, exceeding the consumable limit value in the C group on the 450th day, and 28.02±2.75 mg/100 g in the R group on the 2nd day. And it has been determined that it does not exceed the consumable limit value. As a result of the variance analysis and Tukey test, the effect of storage time and groups on the amount of TVB-N was found to be significant ($p<0.05$).

In the study, the TBA value of groups R and C was determined as 0.88±0.02 mg malonaldehyde/kg and 0.67±0.101 mg malonaldehyde/kg, respectively, on the first day. An increase due to storage was observed in both groups. TBA values of groups R and C were determined as 4.95±0.12 mg malondialdehyde/kg and 7.18±0.22 mg malondialdehyde/kg, respectively, on day 510 (last day of storage) (Figure 4). As a result of the variance analysis and Tukey test, the effect of storage time and groups on the amount of TBA was found to be significant ($p<0.05$).

Water activity (aw) values were found to be 0.982±0.001, 0.98±0.001 in the R and C groups, respectively, on the first day of storage. On the 510th day, which is the last day of storage, it was determined as 0.96±0.002 and 0.959±0.097 in groups R and C, respectively (Figure 5). The variance analysis and Tukey test performed showed that the server storage time and the effect of the groups were significant ($p<0.05$).

Preparation of raw materials for canning, processing issues from the fresh product to the finished product, from the intestinal microflora of the fish to cross-contamination should be considered. Comprehensive planning of canned products in the production environment should be done, it is important to avoid contact between the beginning and the end of production. Microorganisms that can cause fish spoilage include yeasts, molds, bacterial cells and spores (Bratt, 2010). The results of the total number of mesophilic aerobic bacteria (TMAB) were determined as 2.13±0.04 log cfu/g in group R and 2.10±0.06 log cfu/g in group C on the first day (Figure 6). Considering the changes in the number of TMAB in the study, there was an increase due to storage in both groups, and on the 510th day, the last day of the last storage, the number of TMAB in R and C groups was 0.20 log cfu/g and 5.98±0.09 log cfu/g, respectively. As a result of analysis of variance and Tukey test, the effect of storage time and groups on the amount of TMAB was found to be significant ($p<0.05$).

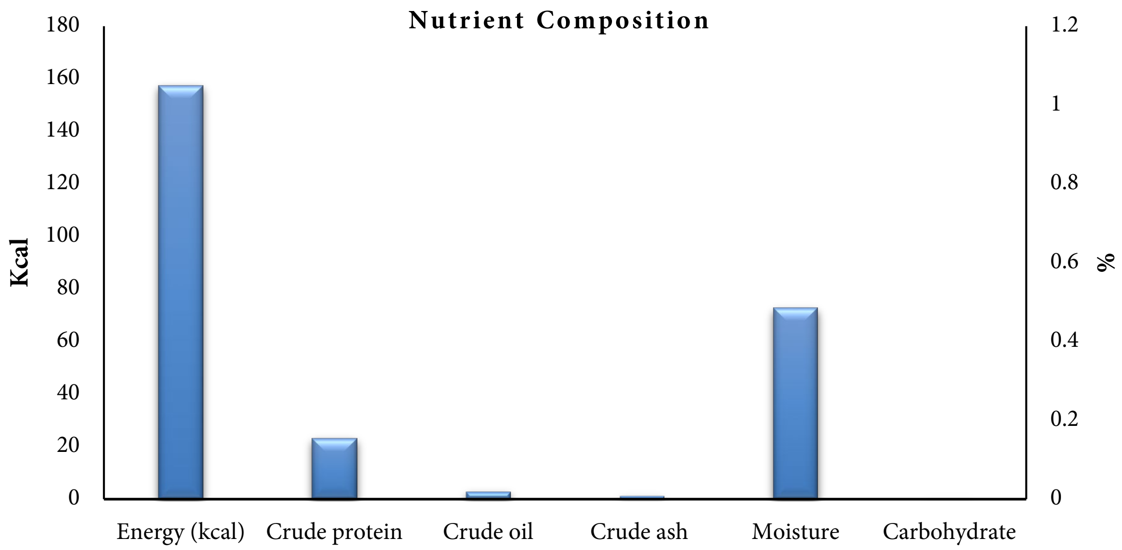

Figure 1. Nutrient composition of bonito

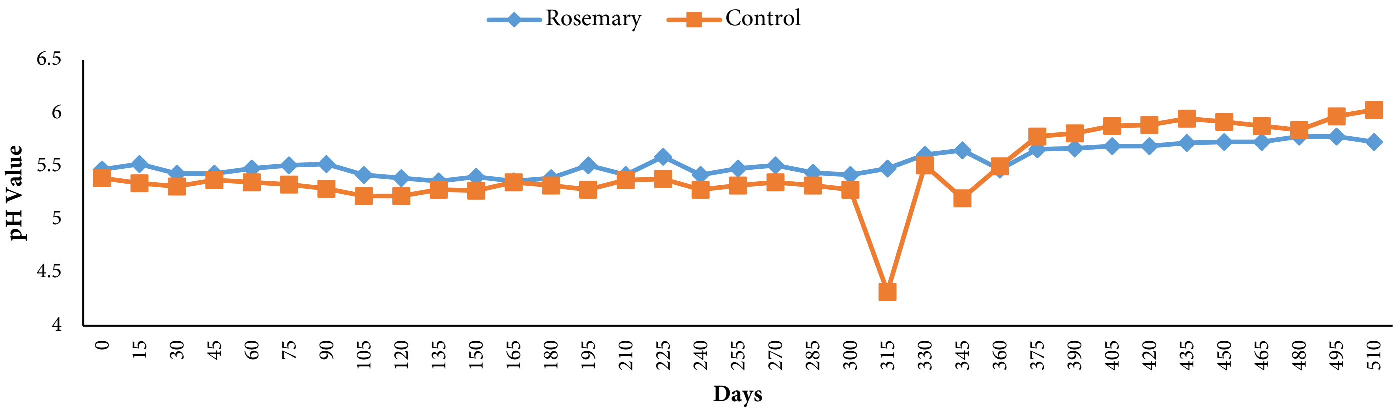

Figure 2. pH change during storage

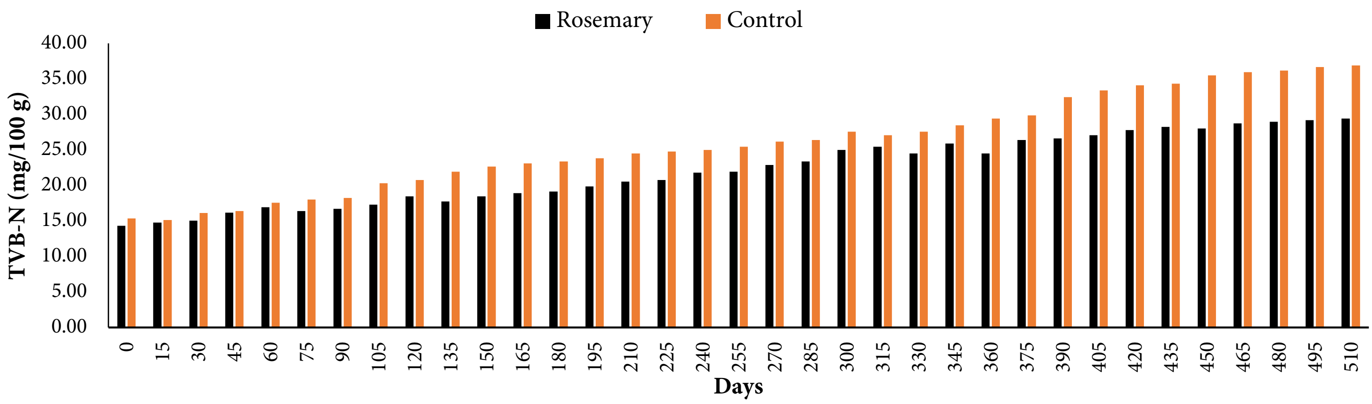

Figure 3. TVB-N change during storage

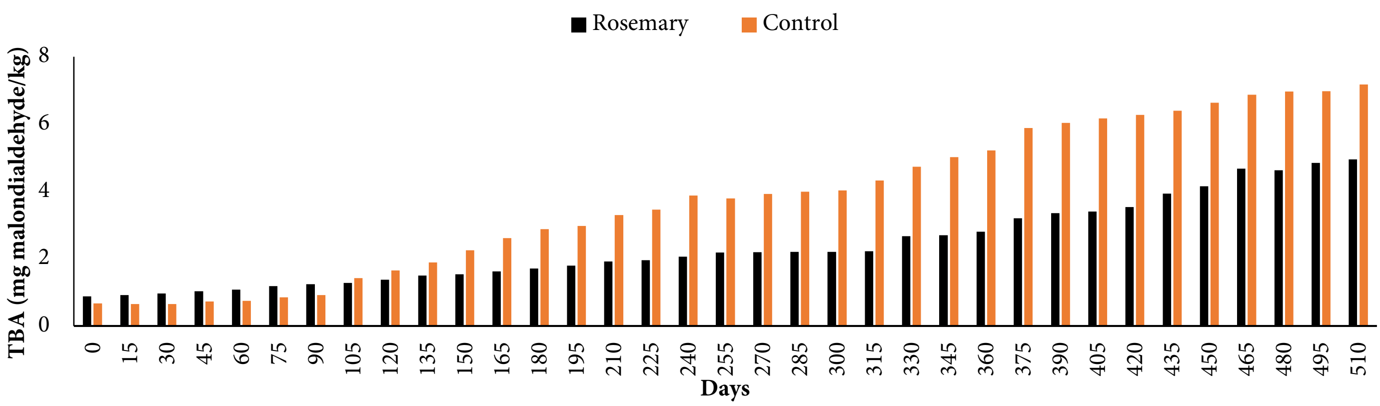

Figure 4. TBA change during storage

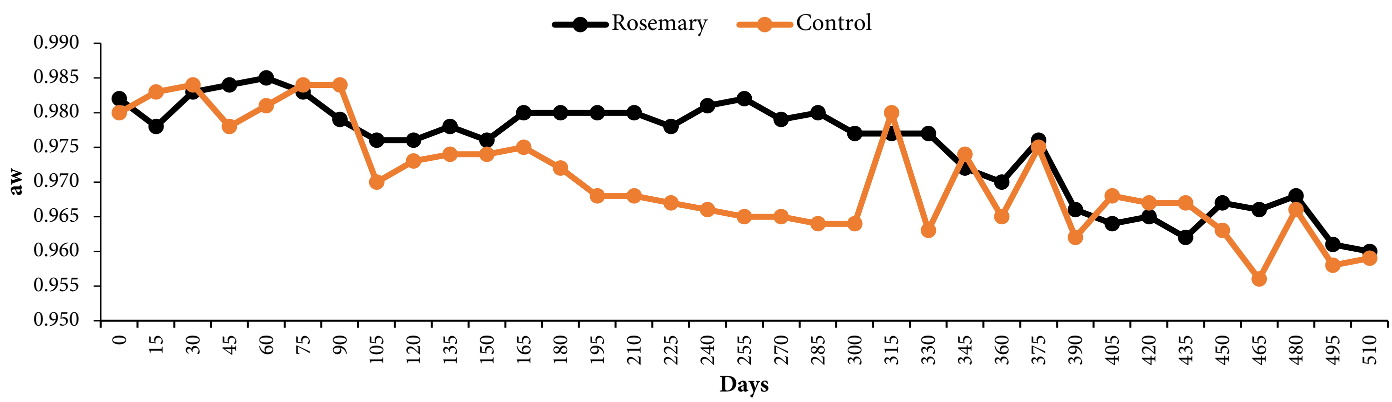

Figure 5. Water activity (aw) values

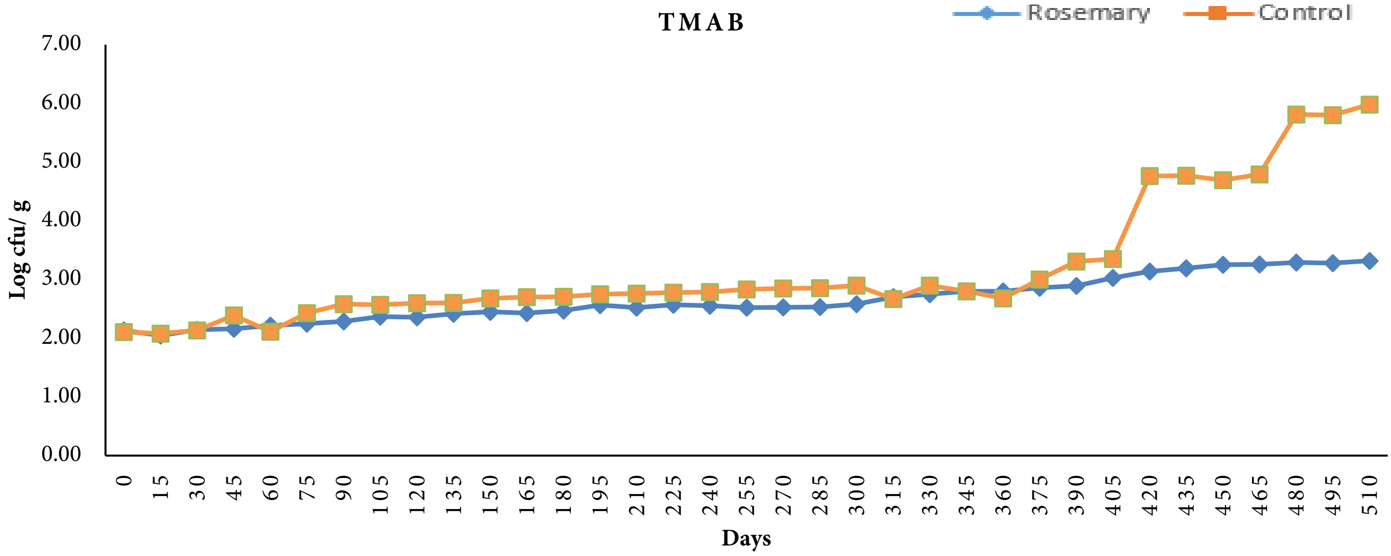

Figure 6. TMAB change during storage

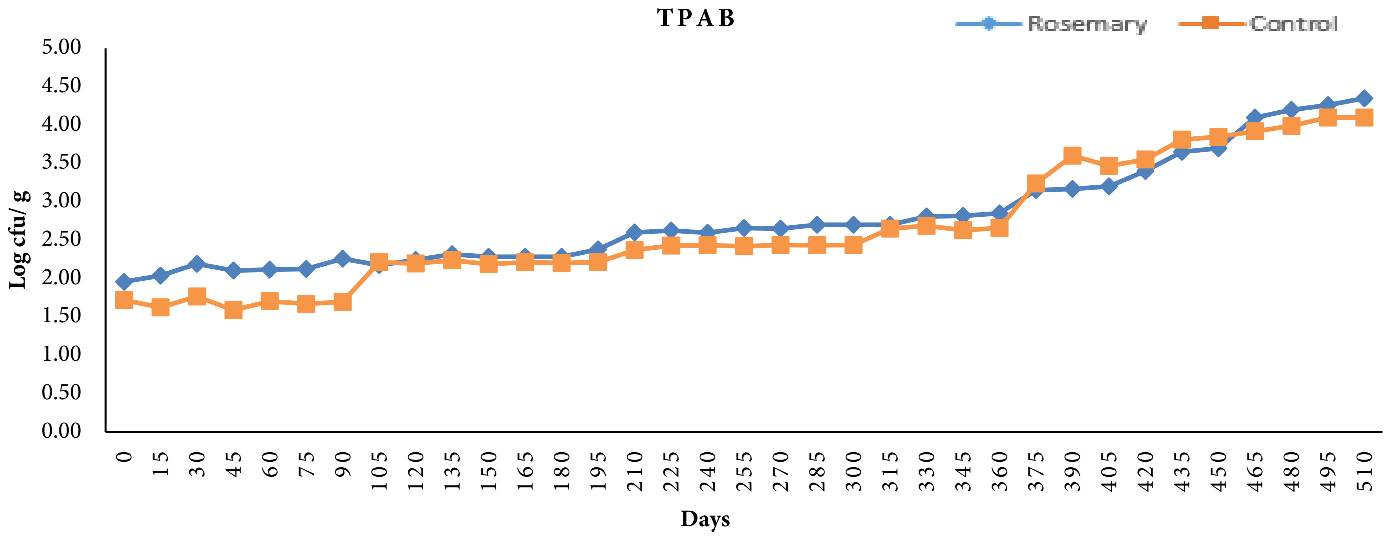

Figure 7. TPAB change during storage

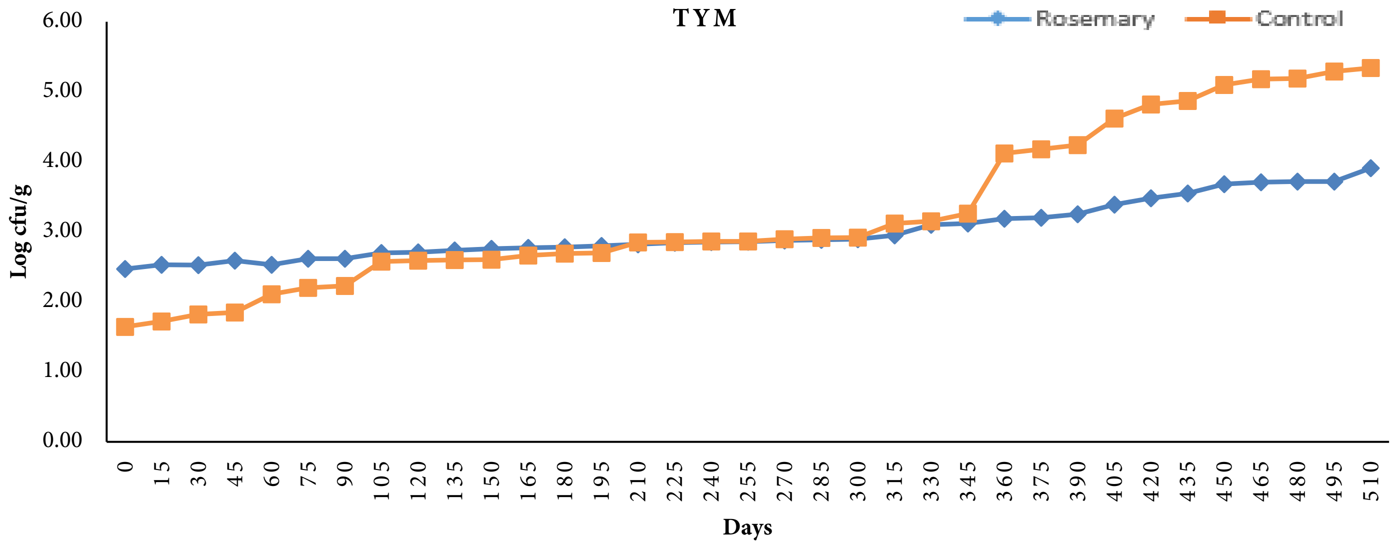

Figure 8. TYM change during storage

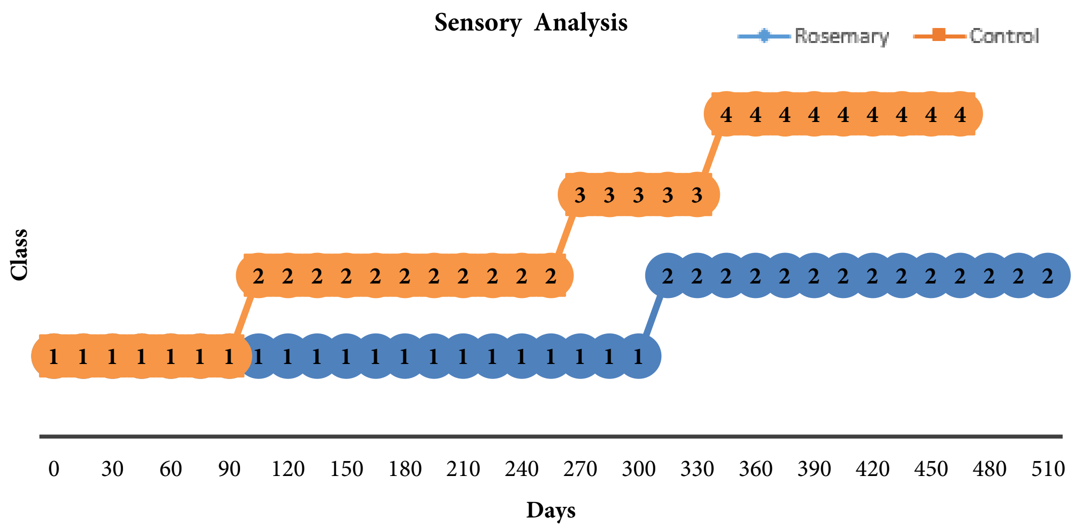

Figure 9. Sensory analysis during storage

The first-day results of the total psychrophilic aerobic bacteria (TPAB) count of groups R and C were determined as 1.96±0.03 log cfu/g and 1.72±0.12 log cfu/g, respectively (Figure 7). There was a steady increase in both groups during storage. On the 510th day, the last day of storage, TPAB results were 4.35±0.30 log cfu/g and 4.10±0.04 log cfu/g in groups R and C, respectively. As a result of analysis of variance and Tukey test, the effect of storage time and groups on the amount of TPAB was found to be significant ($p<0.05$).

Total yeast and mold (TYM) counts on the first day of storage in groups R and C were determined as 2.46±0.02 log cfu/g and 1.64±0.12 log cfu/g, respectively, and an increase was observed in both groups depending on the storage period. On the 510th day, which is the last day of storage, yeast and mold numbers of groups R and C were determined as 3.91±0.3 log cfu/g and 5.34±0.09 log cfu/g, respectively (Figure 8). As a result of analysis of variance and Tukey test, the effect of storage time and groups on TYM amount was found to be significant ($p<0.05$).

In order to determine their quality, sensory analysis was performed in both groups for 510 days and it was calculated which quality class of canned food products they were. The panelists generally accepted the rosemary-added group with appreciation according to the control groups in terms of appearance, taste, smell and consistency. It has been determined that from the first day of storage to the 300th day, it is a R group 1st class product, and from the 310th day to the 510th day, which is the last day of storage, it is a 2nd class product. In the C group, the canned goods that were 1st class until the 105th day were determined as 2nd class until the 270th day, 3rd class until the 330th day, 4th class until the 480th day, and then they were declared as "non-consumable" by the panelists (Figure 9). As a result of variance analysis and Tukey test, 105% of the storage was performed. the effect of storage time and groups from day to day was found to be significant ($p<0.05$).

Discussion

Recently, studies on obtaining natural antioxidants from different sources have increased. For this purpose, new natural antioxidants have been discovered as a result of studies on plant and spice species (sage, green tea leaves, pomegranate peel, grape seeds, nettle, basil, thyme, rosemary, etc.) (Vareltzis et al., 1997; Serdaroğlu & Felekoğlu, 2005; Da Silva Afonso & Sant'ana 2008; Al-Bandak et al., 2009; Turhan et al., 2009). Investigating the nutritional components of red and white muscles in bonito fish, Öksüz et al. (2008), Duyar et al. (2016) and Kınay et al. (2020) showed parallelism with the results of the study.

When nutritional composition examined, crude protein, crude oil, crude ash and moisture values were determined as 23.125±1.062%, 2.783±0.339%, 1.150±0.212% and 72.717±0.652%, respectively. Kınay et al. (2020) calculated crude protein, crude oil, crude ash and moisture values as 23.88±0.575%, 360±0.051%, 70.61±0.675%, 0.97±0.010%, respectively. Duyar et al. (2016) investigated the effect of coating and vacuum packaging the nutritional composition of bonito fish and reported that crude protein was 25.78±0.62%, crude oil was 3.66±0.38%, moisture was 69.57±0.61%, and raw ash was 0.98±0.10%. The results show parallelism with these studies.

The pH value, which was determined as 5.47±0.01 in the group R at the beginning of the storage and as 5.39±0.008 in the control group, was determined as 5.73±0.06 and 6.03±0.01 on the 510th day, the last day of storage, respectively. Many canned fish products contain ingredients with a pH above 4.6 and a water activity above 0.85. Thus, canned fish products are considered low acid canned foods. Regulatory requirements for the production of low acid canned fish products take into account target organisms that must be controlled to produce commercially sterile products (Bratt, 2010). The pH data are in parallel with the values set in the corresponding book.

In quality classification according to TVB-N values, samples containing 25 mg/100 g TVB-N are very good, samples containing 30 mg/100 g TVB-N are good, samples containing 30-35 mg/100 g TVB-N are marketable, 35 mg/100 g TVB-N Samples containing more than 100 g of TVB-N are considered degraded (Varlık et al., 1993). The amount of TVB-N increased in both groups during the storage period. On the 450th day, when C group exceeded the consumable limit value and was determined as 35.49±2.75 mg/100 g, R group was determined as 28.02±2.75 mg/100 g. It was determined that group R did not exceed the TVB-N value and the consumption limit value until the end of storage (510 days). In his study examining the sensory, chemical and microbiological effects of natural antioxidants obtained from aromatic plants on fish fillets, Kenar (2009) reported that the TVB-N value increased during the storage period, and the TVB-N values of rosemary extract with sardine fillets were lower than the control group. The results show parallelism with the study.

Oily fish contain high levels of unsaturated fatty acids. For this reason, it is attacked by atmospheric oxygen and causes bitterness. This is why some oily fish, such as sardines and mackerel, have a shorter shelf life than lean fish, even when frozen (Slabjy & True, 1978). TBA value, which is one of the most important criteria of spoilage in meat, emerges as a result of fat oxidation and is considered as one of the most important criteria of quality criteria (Günlü, 2007). TBA change amounts of rosemary and control groups were determined as 0.88±0.02 mg malonaldehyde/kg, 0.67±0.101 mg malonaldehyde/kg on the first day, respectively. A steady increase was observed in both groups during storage. Yerlikaya & Gokoglu (2010), Kenar (2009), Yasin & Abou-Taleb (2007), and Duman et al. (2012) reported that oxidation occurred more in the control groups in terms of TBA amount in their study where they used plants (rosemary, sage tea, marjoram, thyme) as natural antioxidants. The results show parallelism with the present study.

It has been reported that canned foods are included in the high moisture content foods group with aw values above 0.90. It has been stated that such foods carry the risk of bacterial spoilage, and at aw values below 0.90, deterioration related to fermentation and mold growth due to yeasts may begin (Özay et al., 1993). They determined aw values in various foods (canned foods, soft drinks, meat, milk and flour products, confectionery, honey and jams, shelled and dried fruits, cereals, legumes, spices, instant soups, etc.). In their studies in which some cans were examined in terms of aw, it was reported that aw values were in the range of 0.994-0.998 (Özay et al., 1993) and the results show parallelism.

Gökoğlu (1993) examined the sensory characteristics such as color, taste, texture and smell in canned sardine according to the 1-9 evaluation system and they found average values between 6 and 6.5 in fish. Erüstün (1984) reported that in the sensory evaluation of canned sardines produced by five different companies, after 9 months of storage, the products of four companies were of first quality and the products of the other company were of second quality. It has been determined that from the first day of storage to the 300th day, it is a R group 1st class product, and from the 310th day to the 510th day, which is the last day of storage, it is a 2nd class product. In the C group, the canned goods that were 1st class until the 105th day were determined as 2nd class until the 270th day, 3rd class until the 330th day, 4th class until the 480th day, and then they were declared as "non-consumable" by the panelists. The results show parallelism with the study.

In the production of canned fish products, as with most foods, the microbiological content of the raw material is essential for the effective preservation or stability of the end product. After harvesting, microbial growth on and in the raw fish must be minimized (Bratt, 2010). It has been reported that the number of microorganisms in nutrients is an important criterion in terms of both human health and quality (Karaçam, 1998), and the microbial flora in fresh fish is 6 log cfu/g (Huss, 1988). Gargacı (2010), Kenar (2009), and Aysel (2008) reported that the control groups had a higher antimicrobial load in their studies with aromatic plants. Kobya et al. (2021), noticed that, plant extracts have the potential to be used as an antioxidant agent in processing technology. In general, considering the microbial flora detected in our study, it is thought that there is a microbial load because rosemary is taken from herbalists and is not in compliance with the standards and is not treated with radiation. Therefore, in our study, the antimicrobial properties of rosemary as TMAB and TPAB were not found. Duman et al. (2012) found that rosemary and thyme essential oils, waters and extracts showed antifungal effects at different rates on molds in studies conducted in culture media. Elgayyar et al. (2001), examining the effects of essential oils obtained from plants against selected pathogens and saprophytic microorganisms, it was reported that rosemary and thyme essential oils, juices and extracts showed antifungal effects at different rates on molds. The results show parallelism with all studies with the antioxidant effect of rosemary.

Conclusion

In our study, in which the effect of rosemary plant used in canned bonito fish prepared with the traditional method was investigated, it was determined that rosemary provides both sensory and antioxidant properties. The most important positive changes in our study were TVB-N, TBA and sensory aspects. It has been determined that rosemary increases the sensory and chemical consumability and reliability of the product after canning bonito fish. The preservation of fishery products, which is one of the foods whose storage and preservation is problematic, with different methods and technologies should be investigated in more detail.

Compliance With Ethical Standards

Authors' Contributions

Author HAD designed the study and AGK performed and managed statistical and chemical analyses. Both authors read and approved the final manuscript.

Conflict of Interest

The author confirms that no conflicts of interest exist and the funders had no role in study design, data collection, analysis, and decisions.

Ethical Approval

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

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RESEARCH ARTICLE

Spatial and temporal distribution of *Liocarcinus depurator* (Crustacea: Decapod) caught by beam trawl in the southeastern Black Sea

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ABSTRACT

This study was carried out to determine the temporal and spatial distribution of *Liocarcinus depurator* (Crustacea: Decapod) caught by beam trawl in the southeast Black Sea between December 2012 and November 2013. Sampling was executed at depths from shoreline to deeper 30 m by using a 2 m wide beam trawl from İyidere, Merkez and Çayeli stations. *L. depurator* was determined most intensely (1000 m^2) at 0-5 m depth at Çayeli station and during the summer season. *L. depurator* started to migrate from shallow to deeper in autumn and left the coast completely in winter. It approached the shore again in the spring and spread at all depths in the summer season. In addition, while it was distributed in three different habitats according to CPUE values, the highest CPUE value was calculated at Çayeli station, which has a macroalgae structure.

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Introduction

Blue-leg swimcrab *Liocarcinus depurator* is distributed in the North Sea, Atlantic Ocean, Mediterranean Sea and Black Sea (Horton & Lilley, 2008). It is a wide species of bathymetry, temperature and habitat (Zariquiey-Álvarez, 1968, Minervini et al., 1982; Pérès & Picard, 1965; Christiansen, 1982). It is found in a variety of substrates, although it is most commonly found in mud, coastal-littoral mud and both mid-shore and cullet-filled sands (Schembri & Lanfranco, 1984). It was reported that *L. depurator* is the main food source for Crustacea, Mollusca, Polychaete, Ophiuroid and other fish (Freire, 1996). Decapod crustaceans are important ecological components of the marine ecosystem and play a vital role in the intermediate trophic level (Farina et al.,1997). Studies on crab species in the Black Sea are generally on taxonomy and records (Anosov, 2000; Bilgin &

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Çelik, 2004; Ateş et al., 2010; Micu et al., 2011; Bilgin, 2019; Demirbas et al, 2021). There are also studies on the distribution of the species (Holthuis, 1961; Zariquiey Alvarez, 1968; Kattoulas & Koukouras, 1975; García Raso, 1984; D'Udekem d'Acoz, 1993; Manjón-Cabeza & García Raso, 1998; Abelló et al., 2002; Rufino et al., 2005). However, apart from the studies on the biology of the species in the Black Sea (Aydın et al., 2013; Aydın, 2018), there is no study on the distribution abundance and ecological aspects of the species in the Black Sea. The aim of this study is to characterize the seasonal, bathymetric, and spatial distribution sympatry of *L. depurator*, the benthic crab community in the southern Black Sea. This study is the first to determine the temporal and spatial distribution of this species in the Black Sea.

Materials and Methods

This study was carried out monthly between December 2012 and November 2013 by using a 2 m width and 15 mm mesh opening beam trawl, from 3 stations (İyidere, Merkez and Çayeli) at 4 different depth groups, on the coast of Rize, Turkey (Figure 1).

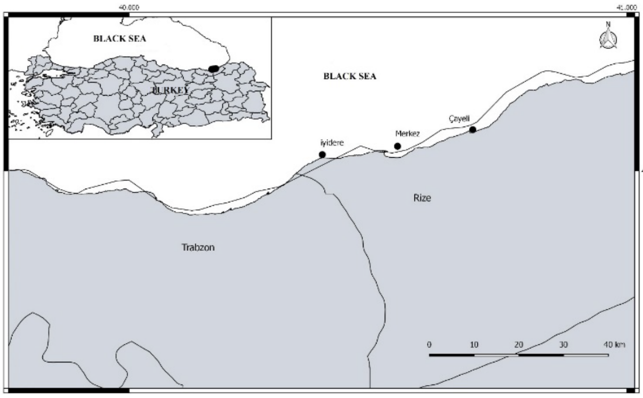

Figure 1. Study area

The amount of catch per unit effort (CPUE) was calculated according to the formula below for the *L. depurator* species.

$$CPUE = \frac{N_i}{t_i} \quad (1)$$

Here, *CPUE*=catch per unit effort, N_i=*i*. number of individuals in the tow, t_i=*i*. tow time (hour).

CPUE and abundance analyses according to location, depth and season groups were performed according to One-way analysis of similarity (ANOSIM) (Clarke & Warwick, 1994). It was made according to the similarity percentage analysis (similarity percentages: SIMPER) of the groups that make up the difference between the groups (depth, season, station) (Clark, 1993). Multiple statistical analyses (multivar) were performed using the PAST program v. 2.14 (Hammer et al., 2001).

Results

As a result of the beam trawling the bottom structure of the stations were determined as follow: *Iyidere station***,** the bottom structure was generally formed as sandy, crust and small rock debris. Sandy and rocky areas were dominant. This station generally consists of sand, gravel and shell structure in terms of bottom structure. *Merkez station***,** the bottom structure was generally consisted crust and small rock debris covered with macroalgae (*Zostera* sp., *Ulva* sp.). Sandy areas were dominant. *Çayeli station***,** the bottom structure of this station was partially covered with sandy and macroalgae (*Zostera* sp., *Ulva* sp. and *Cystoseira* sp.).

During the research, a total of 146 tow were taken from İyidere, Merkez and Çayeli stations using a 2 m wide beam trawler (Beam trawl). The differences in the amount of abundance between the depth groups were found to be statistically significant (Kruskal-wallis test, $p<0.05$). Number of individuals in 1000 m^2 (±standard error) area was calculated as 15697.4±2260.55 ind. in the 0-5 m depth group, 13050.3±5894.50 ind. in the 5-10 m depth group, 3900.7±1373.69 ind. in the 10-20 m depth group and 4362.5±1626.79 ind. in the 20-30+ m depth group, respectively (Figure 2).

The differences in intensity between the seasons were found to be insignificant between the following seasons: Spring-Winter (P=0.595) and Autumn-Summer (P=0.3195). *L. depurator* individuals were found in the highest abundance (ind./1000*m^2) in Summer, Autumn, Spring and Winter seasons, respectively. Number of individuals in 1000 m^2 area was calculated as 8123.7±4997.92 ind. in the spring, 4259.8±1586.10 ind. in the winter, 11514.3±2569.65 ind. in the autumn, and 12297.5±1981.91 ind. in the summer (Figure 3). The average number of *L. depurator* individuals in an area of 1000 m^2 in the research area was calculated as 9380.4±1637.47 ind. The highest density of *L. depurator* was recorded in Çayeli (Figure 4), although statistically significant differences (Kruskal-Wallis test, $P<0.05$) were observed among the stations. The differences between seasons in terms of abundance were found to be statistically significant and they were listed as: (Kruskal-wallis test) Spring-Autumn (P=0.002279), Spring-Summer (P=8.543E06), Winter-Autumn (P=0.01825) and Winter-Summer (P=0.0001551).

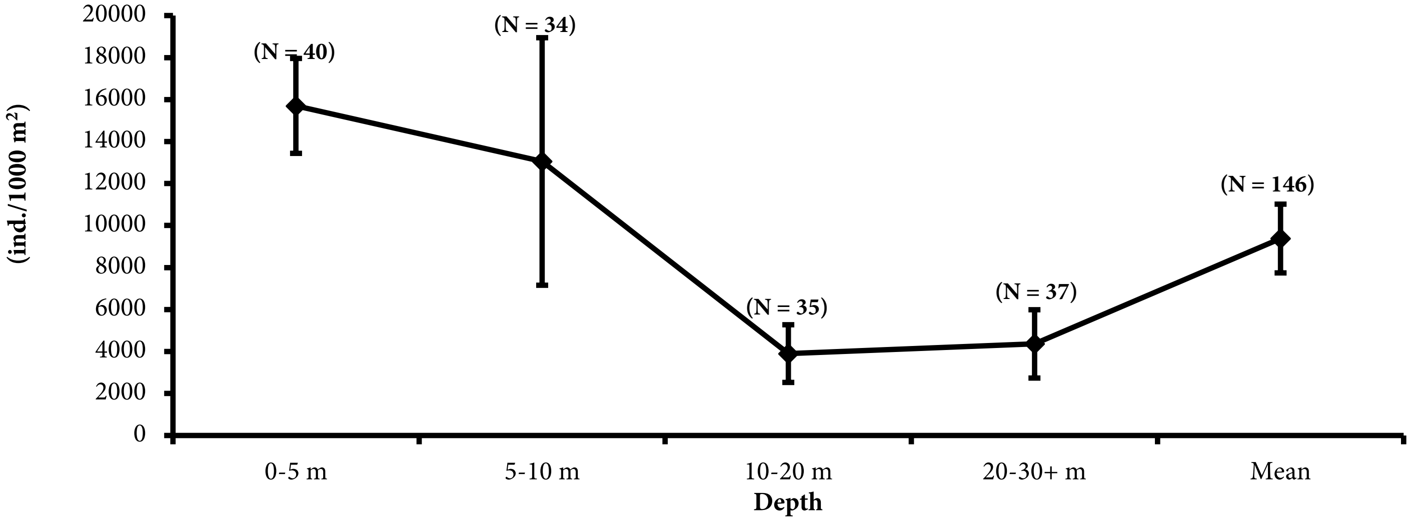

Figure 2. Average abundance of *L. depurator* by depth groups (ind./1000 m^2). The values in parentheses indicate the number of towing, middle point average and the bars indicate the standard error.

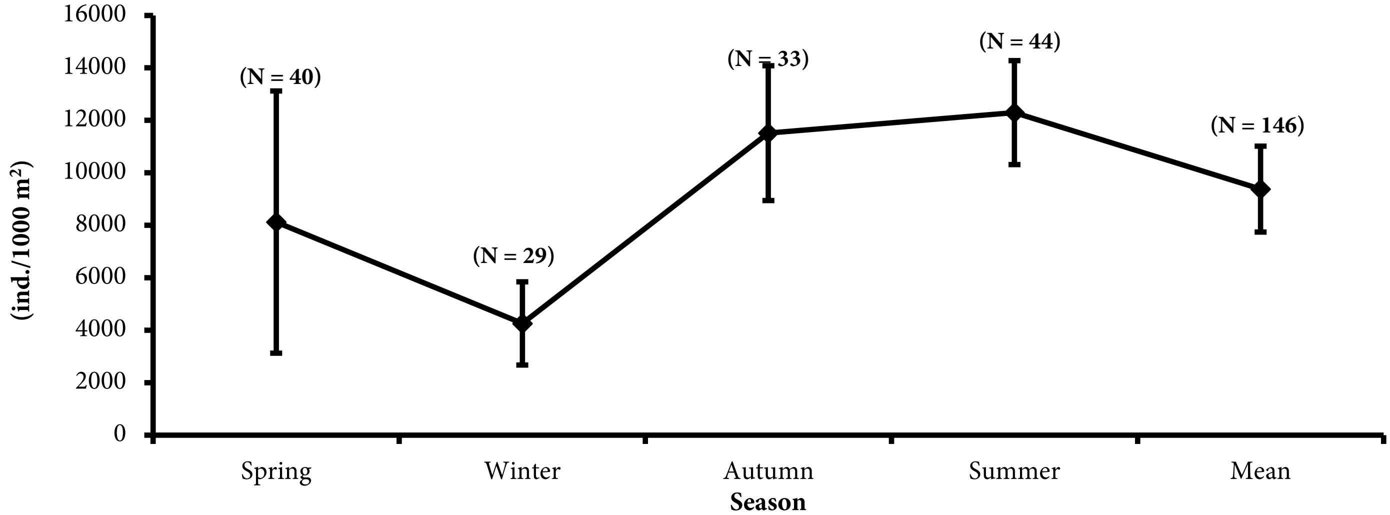

Figure 3. Average abundance of *L. depurator* according to seasons (ind./1000 m^2). The values in parentheses indicate the number of tows, middle point average and the bars indicate the standard error.

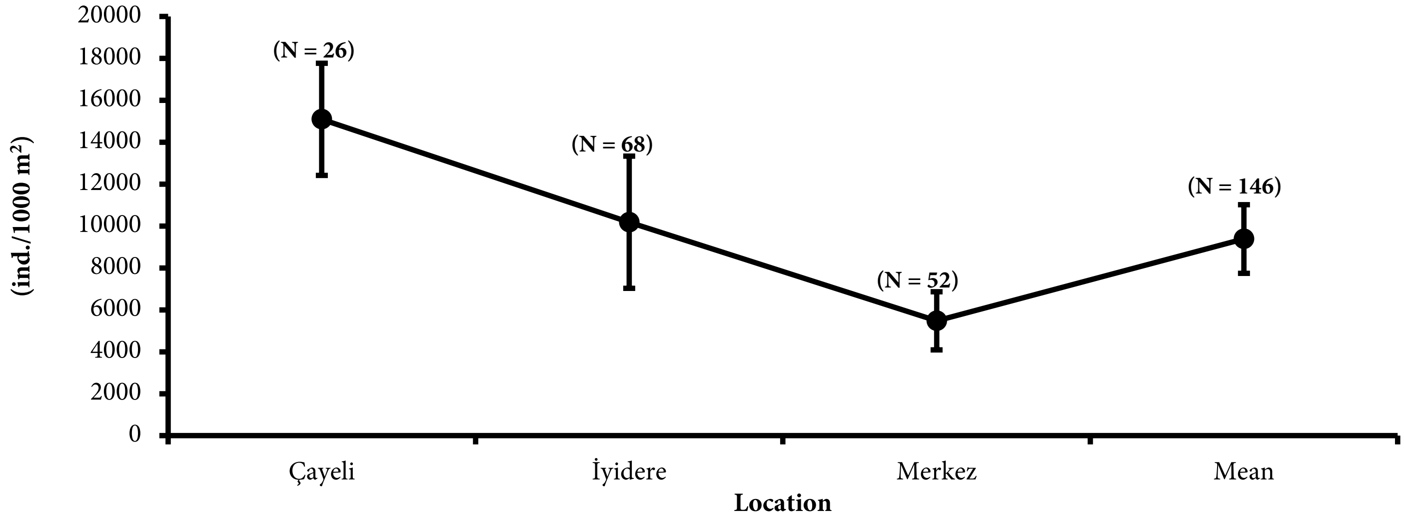

Figure 4. Average abundance of *L. depurator* species according to location (habitat) groups (ind./1000 m^2). The values in parentheses indicate the number of tows, middle point average and the bars indicate the standard error.

Spatial and Temporal Distribution of CPUE Values of *L. depurator*

The difference between the seasons in terms of catch amount in unit effort (CPUE: individuals/hour) was statistically significant and it is as follows: (Kruskal-wallis test) Spring-Autumn (P=0.004293), Spring-Summer (P=1.077E-05), Winter-Autumn (P=0.01152) and Winter-Summer (P=7.708E-05). The difference in the catch amount of CPUE between the seasons was insignificant between Spring and Winter (P=0.7084) and Autumn and Summer (P=0.2101) (Figure 5). *L. depurator* individuals were found in the highest abundance (individuals/hour) in Summer, Autumn, Spring and Winter seasons, respectively. The difference detected in terms of CPUE was similar to the difference between seasons in terms of intensity. *L. depurator* individuals were found in the highest abundance (individuals/hour) in Çayeli, Iyidere and Merkez stations, respectively (Figure 6). *L. depurator* individuals are at the highest abundance (individuals/hour) at the depth of 0-5 m, 5-10 m, 20-30 m, and 10-20 m, respectively (Figure 7).

One-way similarity analysis (one-way ANOSIM) was performed to determine whether the CPUE values of *L. depurator* species differ between seasons according to depth groups or not, and according to this analysis, the difference was found to be statistically significant (Global R=0.3225; P < 0.0001). According to the similarity percentage (SIMPER) test results, the first distinguishing depth group is 5-10 m, and the second distinguishing depth group is 0-5 m, between spring and winter. Among the other seasons (Spring-Autumn, Spring-Summer, Winter-Autumn, Winter-Summer and Autumn-Summer), the first depth group that distinguishes in terms of abundance of *L. depurator* is the 0-5 m depth group (Table 1).

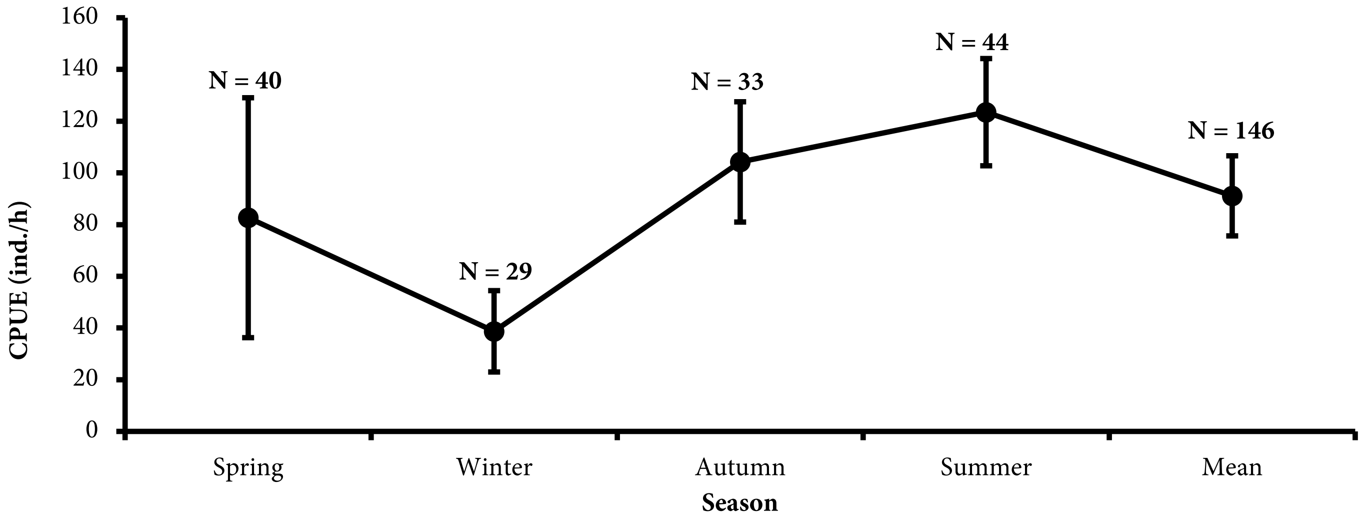

Figure 5. Average CPUE (ind./hour) values of *L. depurator* species by season. The bars represent the standard error, middle point average and the numbers represent the number of tows.

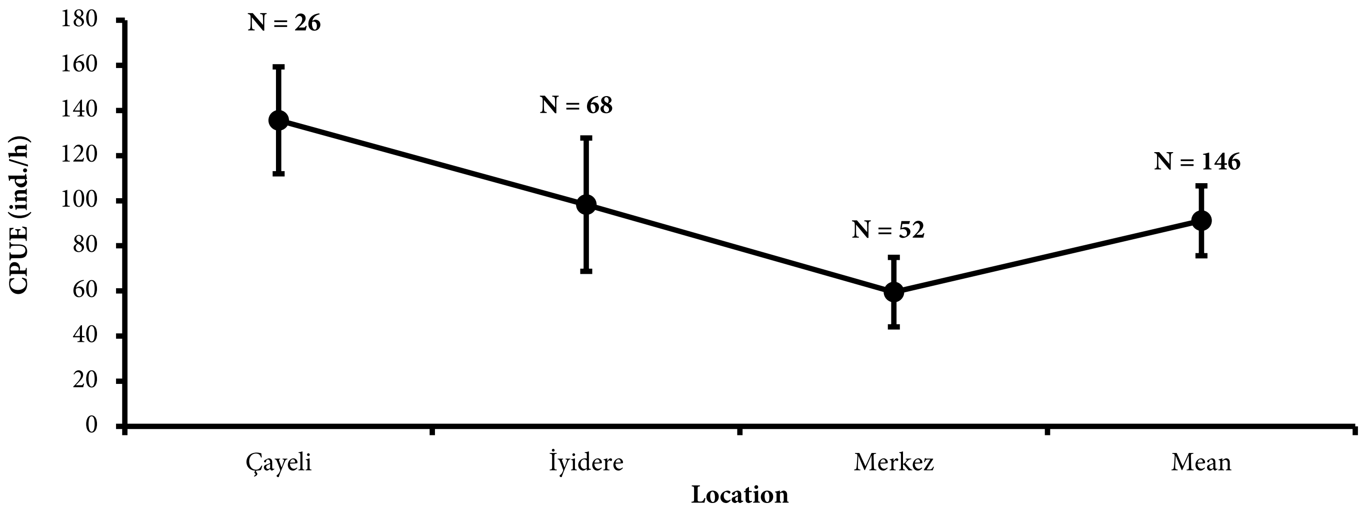

Figure 6. Average CPUE (ind./hour) values of *L. depurator* species by location. The bars represent the standard error, middle point average and the numbers represent the number of tows.

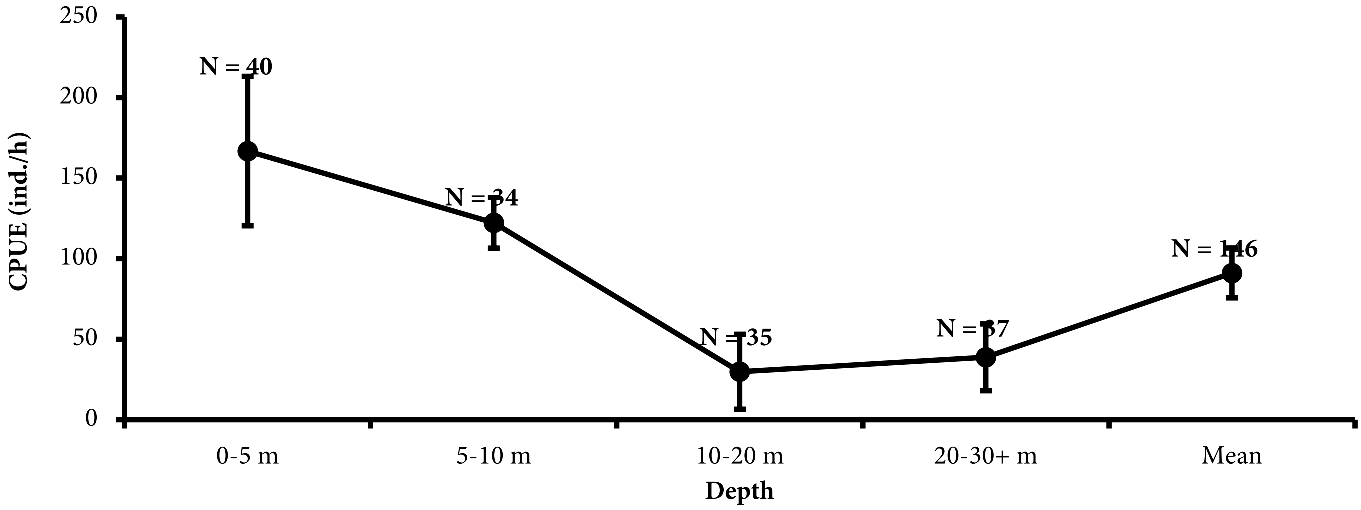

Figure 7. Average CPUE (ind./hour) values by depth groups of *L. depurator* species. The bars represent the standard error, middle point average and the numbers represent the number of tows.

Table 1. Similarity analysis of CPUE values between seasons according to depth groups (one-way ANOSIM) and similarity rates (SIMPER). Global R = 0.3225; P<0.0001.

Groups	ANOSIM		SIMPER				
	R	*P*	*Average dissimilarity*	*Distinctive depth group 1*	*Contrib%*	*Distinctive depth group 2*	*Contrib%*
Spring -Winter	0.1012	0.0576	84.45	5-10 m	37.03	0-5 m	29.22
Spring-Autumn	0.0275	0.2594	80.28	0-5 m	39.25	5-10 m	30.06
Spring-Summer	0.3389	0.0001	81.51	0-5 m	52.53	5-10 m	19.36
Winter-Autumn	0.2652	0.0061	89.57	0-5 m	40.74	5-10 m	27.76
Winter-Summer	0.7552	0.0001	96.69	0-5 m	60.75	20-30^{+} m	15.56
Autumn-Summer	0.0872	0.1511	67.89	0-5 m	34.24	5-10 m	19.69

Table 2. Similarity analysis of CPUE values between locations according to depth groups (one-way ANOSIM) and similarity rates (SIMPER). Global R=0.1142; P<0.0133.

Groups	ANOSIM		SIMPER				
	R	*P*	*Average dissimilarity*	*Distinctive depth group 1*	*Contrib%*	*Distinctive depth group 2*	*Contrib%*
İyidere-Merkez	0.1744	0.0033	78.93	0-5 m	37.26	5-10 m	22.93
İyidere-Çayeli	0.0945	0.0900	72.70	0-5 m	36.59	5-10 m	18.95
Merkez-Çayeli	0.0313	0.2478	63.76	0-5 m	37.40	5-10 m	14.05

Table 3. Similarity analysis of CPUE values between seasons according to locality groups (one-way ANOSIM) and similarity rates (SIMPER). Global R=0.1494; P<0.0002.

Groups	ANOSIM		SIMPER				
	R	*P*	*Average dissimilarity*	*Distinctive locality group 1*	*Contrib%*	*Distinctive locality group 2*	*Contrib%*
Spring-Winter	0.0037	0.3838	73.58	İyidere	55.53	Merkez	17.95
Spring-Autumn	0.0445	0.1336	85.11	İyidere	41.43	Çayeli	30.23
Spring-Summer	0.2388	0.0007	90.46	Çayeli	32.91	Merkez	30.63
Winter-Autumn	0.144	0.0136	82.06	İyidere	42.09	Çayeli	32.25
Winter-Summer	0.4292	0.0001	92.61	Çayeli	35.09	Merkez	32
Autumn-Summer	-0.0037	0.3838	73.58	İyidere	55.53	Merkez	17.95

One-way similarity analysis (one-way ANOSIM) was performed to determine whether the CPUE values of *L. depurator* species differ between locations according to depth groups or not, and according to this analysis, the difference was found to be statistically significant (Global R = 0.1142; P<0.0133). According to the percent of similarity (SIMPER) test results, the first distinguishing depth group among the locations is 0-5 m, and the second distinguishing depth group is 5-10 m (Table 2). One-way similarity analysis (one-way ANOSIM) was performed to determine whether the CPUE values of *L. depurator* differ between seasons according to location groups or not, and according to this analysis, the difference was found to be statistically significant (Global R=0.1494; P<0.0002). According to the percent of similarity (SIMPER) test results, the first location group that distinguishes between the seasons is Iyidere, and the second distinguishing location group is the Merkez, regarding the seasons are Spring-Winter and Autumn-Summer seasons. The first location group that distinguishes between the seasons is Iyidere and the second distinguishing location group is Çayeli, regarding the seasons are Spring-Autumn and Winter-Autumn. The first location group distinguishing between the seasons is Çayeli, and the second distinguishing location group is the Merkez, regarding the seasons are Spring-Summer and Winter-Summer (Table 3).

In the spring, *L. depurator* was found more abundant between 0 and 10 m depth, while it was found less abundant between 10 and 30+ m depth. In winter, *L. depurator* was determined in the least abundant at 0-5 m depth. In the summer, *L. depurator* was determined at all depths (Figure 8). CPUE values of *L. depurator* were determined at the lowest rate in Çayeli station in summer and winter seasons. In the other two stations, *L. depurator* was encountered in all seasons (Figure 9). *L. depurator* was found at all depths at three stations. The CPUE value decreased from 0-5 m depth to 20-30^{+}m depth at Iyidere and Çayeli stations. At the Merkez station, the lowest CPUE value was determined at a depth of 10-20 m (Figure 10). It was determined that the *L. depurator* started to migrate from shallow to deeper in autumn and left the coast completely in winter. It was observed that it approached the shore again in the spring and spread at all depths in the summer season. In addition, while it was distributed in three different habitats according to CPUE values, the highest CPUE value was calculated at Çayeli station, which has a macroalgae structure.

Discussion

L. depurator is a resistant species that can tolerate temperature changes (Christian, 1982; Peres & Picard, 1995) and can live in various habitats at different depths (Abello & Valladares, 1988; Minervi et al., 1982; Zariquiey-Alvarez, 1968). In this study, *L. depurator* individuals were determined as a result of shootings between 0 and 30^{+} m. The depth in which individuals are detected most abundance in an area of 1000 m^{2} is the depth group of 0-5 m, whereas the depth group with the lowest abundance is the depth group of 20-30^{+} m. Researchers working at similar depths (0-40 m) reported a high number of individuals in shallow waters which is consistent with this study, while they determined less number of individuals in deeper waters with increasing depth (Manjón-Cabeza & García Raso, 1998; García-Raso & Majón-Cabeza, 2002; González-Gordillo et al., 1990; Ramsay et al., 1998; González-Gurriarán et al., 1990; Freire et al., 1993; González-Gurriarán et al., 1991). Researchers working in deeper waters (between 50 and 800 m) reported *L. depurator* species more abundance compared to increasing depth at depths between 50 and 100 m, while they found a decrease in the number of individuals with increasing depth (Sarda et al., 1982; Ellis et al., 2000; Farina et al., 1997). The Black Sea, in which this study was conducted, is a semi-enclosed inland sea. In the southwest, it has connections with the world's seas as much as the Turkish Straits System allows. This restricted water exchange contains oxygen only from the surface to a depth of 150 m (15% of the total volume). In addition, at a deeper level it leads to the formation of an almost completely oxygen-free environment containing hydrogen sulphide (Ross et al., 1974). This feature of the Black Sea prevents the *L. depurator*, which was detected at much deeper depths (800 m) (Rufino et al., 2005), from living much deeper in the Black Sea. The differences in the number of individuals at different depths in the studies may be due to the fishing method (fishing equipment and features) used by the researchers, and the number and duration of shooting in the operations. Our results showed that *L. depurator* migrates from shallow waters to deeper waters in winter, while it migrates from deep waters to shallow waters during summer and spring. This is due to the water temperature, but may be related to the prolongation of daylight and reproductive behaviour. *L. depurator* has been detected in various habitats (sand, mud and shell) as well as having a wide distribution in depth. In this study, the highest amount of *L. depurator* individuals was detected in Çayeli, Iyidere and Merkez stations, respectively. In studies, *L. depurator* was commonly detected in muddy habitats (d'Udekem d'Acoz, 1992; Abelló, 1993; Relini et al., 1986; Pastore et al., 1998; Falciai, 1997). Secondly, their common

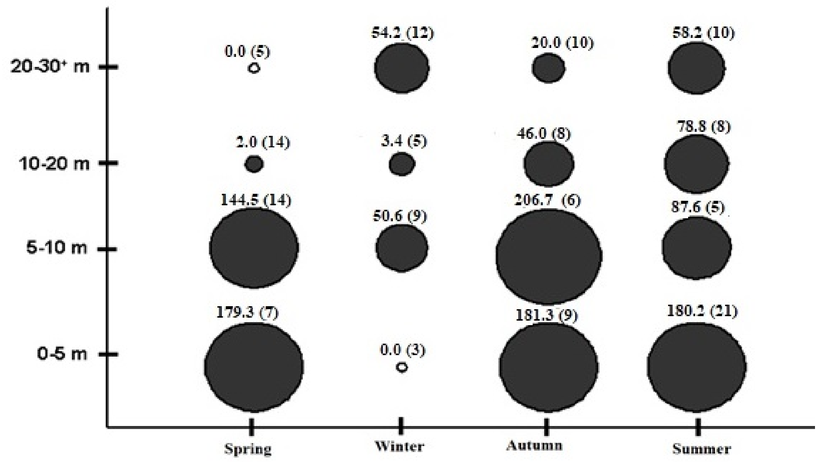

Figure 8. Average CPUE (ind./hour) values of *L. depurator* species by depth groups and seasons. The numbers in parentheses represent the number of tows.

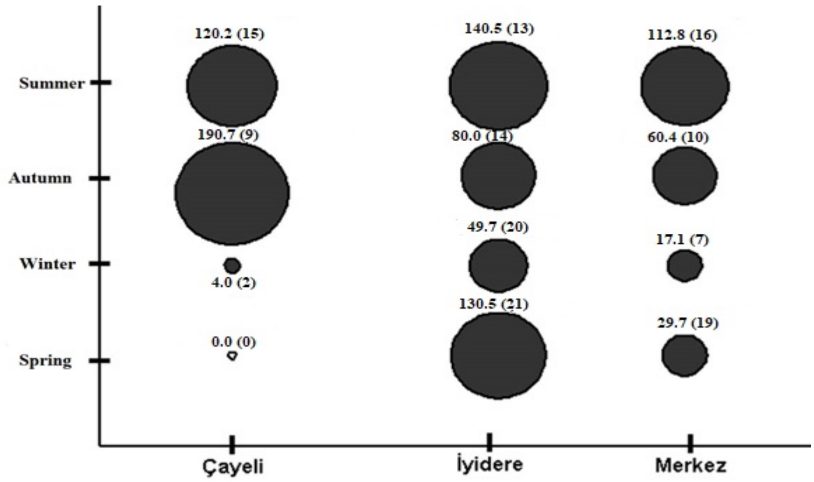

Figure 9. Average CPUE (ind./hour) values of *L. depurator* by location and seasons. The numbers in parentheses represent the number of tows.

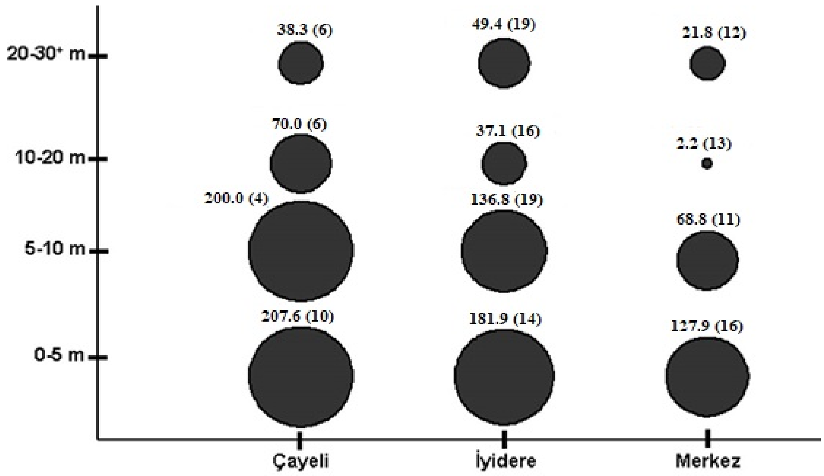

Figure 10. Average CPUE (ind./hour) values for *L. depurator* species by depth groups and locations. The numbers in parentheses represent the number of tows.

habitat is sand, gravel and shell (Stevic, 1979). In the algae habitat, Bilgin et al. (2007) determined the crab species living in the *Zostera marina* species in their studies in Sinop coasts, in the Black Sea. They found *Liocarcinus vernalis* in the first place in terms of abundance, while *L. depurator* was the second most intense and sampled along 12 months.

The detection of *L. depurator* in different habitats may be due to the fact that the researchers used different depths and fishing methods. As a matter of fact, *L. depurator* is more concentrated in shallow (mud-sand) waters and found less frequently in deeper waters (Minervini et al., 1982). This is an indication that the availability and abundance of *L. depurator* is under the influence of depth-habitat. Our findings showed that *L. depurator* density change with seasons, depth and habitat characteristics. However, considering the reproductive biology and fecundity characteristics of the species, it will be more useful to understand the role of *L. depurator* in the ecosystem.

Conclusion

L. depurator was distributed at all depths between 0-30^{+} m in autumn and summer seasons. However, with the decrease in temperature in the winter season, the *L. depurator* migrated from the coast to the deeper areas, while in the spring it migrated towards the coast with the increase in temperature. For an ecosystem-based fisheries management, revealing other bio-ecological characteristics of *L. depurator* will contribute to the literature and fisheries management. As a result, crabs have an important place in the benthic ecosystem as they form the food of other demersal creatures. In the light of these data, it shows that the place of crabs in the ecosystem is undeniably important.

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Compliance With Ethical Standards

Authors' Contributions

Author SB designed the study, HO wrote the first draft of the manuscript, performed and managed statistical analyses. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

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RESEARCH ARTICLE

Forecasting Shanghai containerized freight index by using time series models

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ABSTRACT

Recently, the container shipping industry has become unpredictable due to volatility and major events affecting the maritime sector. At the same time, approaches to estimating container freight rates using econometric and time series modelling have become very important. Therefore, in this paper, different time-series models have been explored that are related to the Shanghai Containerized Freight Index (SCFI). SMA, EWMA, and, SES, Holt Winter method are used to describe the data and model. Afterward, the Holt Winter method and SARIMA was applied to model and predict the SCFI index. MAPE, RMSE, AIC, BIC are used to measure the performances of the models and predictions. We observe that the SARIMA model provides comparatively better results than the existing freight rate forecasting models while performing short-term forecasts on a monthly rate. Results demonstrate that the increase will continue without losing momentum.

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Introduction

Nowadays, more and more companies are looking for driven decision-makers. Maritime industry intelligence and analytics departments and business managers have to regularly make forecasts of product sales, inventory, requirements, shipment rates, etc. Then, take strategic decisions based on these forecast values. For example, retail stores forecast sales. They use data of the consumers' past purchases and try to forecast sales for the coming days. Similarly, energy companies forecast production demand of reserves, and price forecast of reserves are used to determine long-term investment plans whereas demand forecasts are used for short-term production planning and competitive pricing. Banks and lending institutions forecast new home purchases and venture capital firms forecast market potential to evaluate business plans. The

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maritime industries operate in complex global markets and businesses are subject to external forces and constant environmental change. The ability to read, understand and respond effectively to the range of rapidly moving components that make up a market is essential to the modern company's survival. Forecasting is vital to increase profitability and save money in the maritime industry.

Freight prices are at their highest values in history. Container freight rates are breaking all-time records. World Container Index experienced a 480% increase by reaching $8,795.77 for each 40 ft container between January 2020 and August 2021 (Drewry, 2021). The reasons behind this increase can be listed as that the mobility which started with the pandemic period together with other factors such as maritime trade wars, previously established and strengthened joint service structures, alternative routes, empty container problems, oil price volatility, the ship that blocked the Suez Canal, the spread of vaccine and normalization, increasing demands, supply shortages, Christmas preparation in global trade, and expected rapid growth in world trade. This shows that a new era has begun in maritime trade in the light of recent developments.

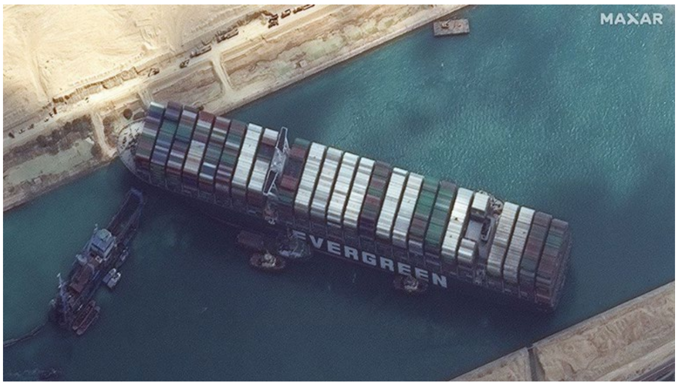

Figure 1. Ever Given: Ship that blocked Suez Canal (BBC, 2021)

A large container ship called Ever Given, owned by the Evergreen company, disturbed the sea traffic for 6 days in March 2021, when it went aground during its passage through the Suez Canal, which is the passage for 12% of world trade (Figure 1). This incident caused commercial delays and financial losses in supply chains around the world. It is estimated that there is a loss of an average of 50 billion dollars. Moreover, oil prices in international markets increased by 4% (BBC, 2021).

With such an extreme breaking point that is due to a ship blocked a canal for 6 days, the dimensions of the dangers facing the maritime industry can be seen quite clearly when compared to the global epidemic (pandemic) that lasted for months. Besides this, world trade is struggling with the empty container problem. Thus, many traders are facing challenges to deliver their export products to customers (Ship Technology, 2021).

Christmas preparations in global trade started early this year. Global buyers, who were unable to fill their shelves before Christmas due to the supply disruptions last year because of the pandemic, are in a rush to restock by moving their orders to an earlier time to avoid a similar situation. Experts warn about the acceleration of trade may cause a new crisis in the container market, and besides the equipment shortage, freight prices, which have increased by 300% in the last year and a half, may have an increasing trend (Dunya, 2021).

Forecasting is an important tool for maintaining the competitiveness of container lines and ports, formulating appropriate short-medium term strategies, and planning. In times of crisis or when all circumstances are normal, the importance of forecasting can be clearly noticed. Major fluctuations during the pandemic period affect index rates. Shanghai Containerized Freight Index (SCFI), one of the most important indices, is regarded as an important evaluation factor for container shipping in the maritime industry. SCFI is also a solid indicator in terms of supply and demand balance, container shipping, world trade movement, and the shipping industry.

SCFI focuses on Shanghai export container transport market spot rates, which are generally considered to be periodical and more sensitive. Shanghai export container transport market spot rates include a composite index, 15 shipping routes, and freight rates. The freight indices reflect the ocean freight and the associated seaborne surcharges of individual shipping routes on the spot market, where: Shipping routes: major container trade routes export from Shanghai to the following regions: Europe, Mediterranean Sea, US west coast, US east coast, Persian Gulf, Australia/New Zealand, West Africa, South Africa, South America, West Japan, East Japan, Southeast Asia, Korea. SCFI refers to volume weighted average prices of space book on spot market by common shippers, which are not influenced by peculiarity of shippers' enterprises or container volume. (Shanghai Shipping Exchange, 2020).

The composite index is the weighted average of all routes: the average spot freight rate of the specific route is divided by average price of its base period. The result multiplies its weighting and its base period index to obtain a value of each route. All the route values shall then add up to obtain the total value (Shanghai Shipping Exchange, 2020).

Forecasting is one of the most significant elements for all types of industries and the maritime industry is not an exception. Since it is impossible to predict the events that affect the shipping market and they happen suddenly, the changes in the market are also fluctuating and stochastic (Goulielmos et al., 2009).

However, such cycles and tendencies are not a new concept in the shipping industry. In fact, these are the integrated part of the industry for centuries (Stopford, 2009). Therewithal, being able to predict some of these swings can easily help carriers and shippers to capitalize on the fluctuations by allowing them to make the right decisions at the right times (Dixon, 2010).

In literature, several forecasting studies use maritime trade indices and volumes. The first study on time series is conducted by Klein & Verbeke (1987) and is the study carried out for the Antwerp port by using univariate time series with monthly data in Antwerp port. A multivariate time series model was used in another study for steel traffic flow in the Antwerp port (Klein & Verbeke, 1987). A long-term predictive value interval model was developed for forecasting the SCFI by fuzzy time series (Chou, 2017). Munim & Schramm (2017) proposed a state-of-the-art volatility forecasting method for container shipping freight rates. Another article on forecasting explored the use of SARIMA models in forecasting containers throughout several major international container ports while taking into consideration seasonal variations (Farhan & Ong, 2018). Shu et al. (2014) forecasted both freight and container outputs for Hong Kong and Kaoshsiung ports with ARIMA and Grey Model and modified with Fourier to increase the accuracy of these two models. Pang & Gebka (2017) used SARIMA, Additive Seasonal Holt-Winters (ASHW), Multiplicative Seasonal Holt-Winters (MSHW), and Vector Error Correction Models (VECM) to forecast the container output of Tanjunk Priok port, which is Indonesia's largest port.

In this paper, different time-series models have been explored that are related to the SCFI index. Different models and approaches such as SMA, EWMA, and, SES, Holt Winter method are used to describe the data and model. the Augmented Dickey-Fuller (ADF) was applied test on the trends and seasonality (time-invariant), which have been adjusted from the series so that the time series model can be applied. Later on, ACF and PACF have been calculated so that the time series can be determined for the model. For the model and forecasting of the SCFI index, the SARIMA and Holt Winter methods have been utilized. Moreover, in the evaluation of the models' and predictions' performances, the MAPE, RMSE, AIC, and BIC have been used.

Materials and Methods

In the study, Shanghai Containerized Freight Index data from February 2016 to July 2021 was used. These data are modeled with the Python programming language. The proposed SCFI index forecasting methodology includes the following stages: describing the data and model, model identification and estimation, evaluation and forecasting, results.

Data and Model Description

Time series are well-defined data sets collected at variable time intervals and at equal time intervals. Analysis of time series includes some stages. Firstly, the data to be modeled should have a normal distribution. If the data is not normally distributed, the data will need to be converted to ensure the normal distribution of data. The conversion of data (such as square root or logarithms) ensures the fixation of the variance in a series with varying variation (Dasyam et al., 2015).

Later on, whether the series is stationary or not. Box-Jenkins model assumes that the time series is stationary. A stationary time series has a stationary mean, stationary variance, and stationary autocorrelation. These values are determined by using autocorrelation (ACF) and partial autocorrelation functions (PACF) and Dickey-Fuller (ADF) test (Awal & Siddique, 2011). Correlograms (ACF and PACF graphs) can show a stationarity pattern or a unit root with significant lags. A more subjective way to evaluate is using (augmented) Dickey-Fuller (ADF) test statistics (Dickey & Fuller, 1979). The null hypothesis is that the series has a unit root. The alternative hypothesis is that the time series is stationary (or trend-stationary).

Model Identification and Estimation

In the study, smoothing methods and the Box-Jenkins method were used in time series analysis.

A simple moving average (SMA) is an arithmetic moving average calculated by adding recent prices and then dividing that figure by the number of time periods in the calculation average. SMA is given as follows:

$$SMA_{t+s} = \sum_{s=-h}^{h}(2h+1)^{-1}\, y_{t+s}, \qquad t = h+1, h+2, \dots, n-h \quad (1)$$

With $(t+s)$ observations of y_{t+s} , h denotes a positive integer, s is the seasonal indices, t is the time period (Khogali et al., 2002).

Exponentially Weighted Moving Average (EWMA) will allow us to reduce the lag effect from SMA and EWMA will put more weight on values that occurred more recently. EWMA formula is as follows:

$$EWMA_t = \alpha y_t + (1-\alpha)EWMA_{t-1}, \qquad t = h+1, h+2, \ldots, n-h \qquad (2)$$

where α is the weight and t is the time period of the series. EWMA is a recursive process that continues until reaches the $EWMA_0$ (Lucas & Saccucci, 1990).

The Holt-Winters method is a very common time series forecasting procedure capable of including both trend and seasonality. The method is also called "double exponential smoothing". Holt-Winter is used for exponential smoothing to make short-term forecasts by using "additive" or "multiplicative" models with increasing or decreasing trends and seasonality. Smoothing is measured by beta and gamma parameters in Holt's model. The Holt-Winters seasonal method consists of the forecast equation and three smoothing equations which are one for the level l_t, one for the trend b_t, and one for the seasonal component s_t, with smoothing parameters α, β, γ . m is used to define the seasonality of frequency. The component form for the additive model is as follows:

$$y_{t+h|t} = l_t + hb_t + s_{t+h-m(k+1)}, \qquad (3)$$

$$l_t = \alpha(y_t - s_{t-m}) + (1-\alpha)(l_{t-1} + b_{t-1}),$$

$$b_t = \beta^*(l_t - l_{t-1}) + (1-\beta^*)b_{t-1},$$

$$s_t = \gamma(y_t - l_{t-1} - b_{t-1}) + (1-\gamma)s_{t-m},$$

The component form for the multiplicative model is as follows:

$$y_{t+h|t} = (l_t + hb_t)s_{t+h-m(k+1)}, \qquad (4)$$

$$l_t = \alpha(y_t / s_{t-m}) + (1-\alpha)(l_{t-1} + b_{t-1}),$$

$$b_t = \beta^*(l_t - l_{t-1}) + (1-\beta^*)b_{t-1},$$

$$s_t = \gamma(y_t / l_{t-1} - b_{t-1}) + (1-\gamma)s_{t-m},$$

where k is the integer part of $(h-1)/m$, which ensures that the estimates of the seasonal indices used for forecasting come from the final year of the sample. The level of the equation shows a weighted average between the seasonally adjusted observation $(y_t - s_{t-m})$ and the non-seasonal forecast $(l_{t-1} + b_{t-1})$ for time t. The trend equation is identical to Holt's linear method. The seasonal equation shows a weighted average between the current seasonal index, $(y_t - l_{t-1} - b_{t-1})$, and the seasonal index of the same season last year. Triple exponential smoothing applies exponential smoothing three times, which combines double exponential smoothing (Holt's method) and simple exponential smoothing. Thus, the method is useful for determining the level, trend and seasonality, and rapidly makes us of them in a forecast.

Box-Jenkins method is based on discrete and linear stochastic processes. Major Box-Jenkins forecasting models are autoregressive (AR) integrated (I) moving average (MA) and seasonal (S) autoregressive integrated moving average. The general structure of this model is represented as ARIMA (p, d, q) × (P, D, Q). The main approach in the Box-Jenkins model is based on the combination of the value of the currently studied variable and the random shocks with the weighted sum of the previous values. Whether the series is stationary or not and it has a seasonal effect is decisive in model selection. Therefore, firstly, the properties of the time series are revealed, and a suitable model will be tried to be find.

Time series usually consists of cyclical properties that seasonal variation is used to eliminate the effects of seasonality. These models are named as SARIMA models. SARIMA models are often described as SARIMA$(p, d, q) \times (P, D, Q)$_$S$ where P is the order of the seasonal autoregressive (SAR) part. D is the order of the seasonal differencing. Q is the order of the seasonal moving-average (SMA) process. S is the length of the seasonal cycle. SARIMA model's formula is as follows:

$$\varphi(B)\emptyset(B^S)\nabla^d\nabla_S^D y(t) = \theta(B)\vartheta(B^S)\varepsilon(t) \qquad (5)$$

with

$$\emptyset(B^S) = 1 - \emptyset_1 B^S - \cdots - \emptyset_P B^{PS}$$

$$\vartheta(B^S) = 1 + \vartheta_1 B^S + \cdots + \vartheta_Q B^{QS}$$

$$\nabla_S^D y(t) = (1 - B^S)^D x(t)$$

In the formula, ∇ is the difference operator; $\varphi(B)$ is the autoregressive polynomial of p with autoregressive parameter of $\varphi_1, \varphi_2, \ldots, \varphi_p$; $\theta(B)$ denoted moving average polynomial of q, as the moving average parameter described as $\theta_1, \theta_2, \ldots, \theta_q$; stationary time series reflects as $\nabla^d x(t)$; $\varepsilon(t)$ is independent white noise and suits the Gaussian distribution.

$\emptyset(B^S)$ indicates seasonal autoregressive polynomial as $\emptyset_1, \emptyset, \ldots, \emptyset_P$ acts as the seasonal autoregressive parameter.

$\vartheta(B^S)$ stands for seasonal moving average polynomial as $\vartheta_1, \vartheta, \dots, \vartheta_Q$ acts as seasonal mobbing average parameter; ∇_S^D signifies the seasonal difference which goes through the D order (Yang et al., 2017).

Evaluation and Forecasting

In order to evaluate the forecasting model, the following evaluation criteria are introduced; where *y(t)* is the actual value of a point for the given time period *t*, *n* is the total number of fitted points, and where $y(t)$ is the actual value of a point for given time period t, n is the total number of fitted points, and $\widehat{y(t)}$ is the fitted forecast value for the time period t (Agrawal et al., 2018).

RMSE (Root mean square error):

$$RMSE = \sqrt{\frac{\sum_{t=1}^{n}(y(t)-\widehat{y(t)})^2}{n}} \quad (6)$$

MAPE (Mean absolute percentage error):

$$MAPE = \frac{100}{n}\sum_{t=1}^{n}\left|\frac{y(t)-\widehat{y(t)}}{y(t)}\right| \quad (7)$$

Root mean square error is an absolute measure of error that squares deviations to prevent positive and negative deviations from canceling each other.

Mean absolute percent error is a relative measure of error that uses absolute values to prevent positive and negative errors from canceling each other and uses relative errors to enable you to compare the predictive accuracy between time series models.

AIC and BIC measure the model's prediction error and are used for model selection. Given a set of constructed models for the series, the model with the minimum AIC, BIC values is preferred.

Akaike information criterion (AIC):

$$AIC = 2k - 2ln(L) \quad (8)$$

Bayesian information criterion (BIC):

$$BIC = klnn - 2lnL \quad (9)$$

where k is the number of estimated parameters, n is the time period of the series, and L is the maximum value of likelihood functions (Burnham & Anderson, 2004).

Results and Discussion

In the period from February 2016 to August 2021, SCFI is shown in Figure 2.

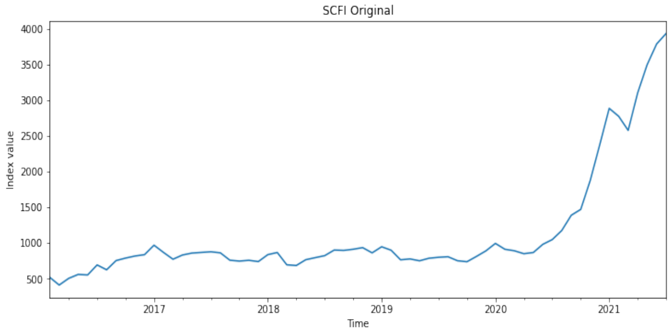

Figure 2. Time series plots of original SCFI

First, we focused on the comparison of Exponentially Weighted Moving Averages and Simple Moving Averages to determine the performance and complexity. The results are shown in Figure 3. The comparison includes the data between February 2016 and August 2021.

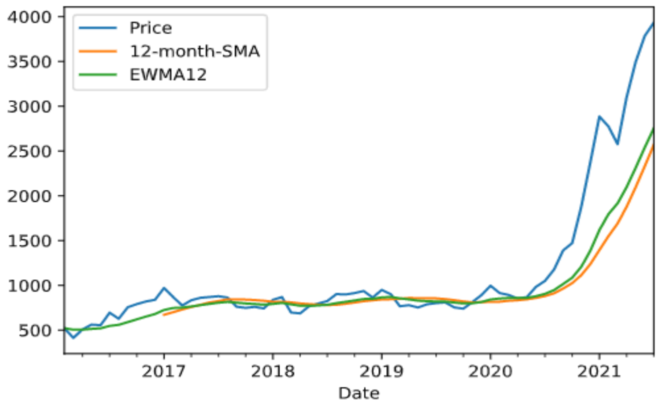

Figure 3. Simple moving averages to exponentially weighted moving averages

It is shown that this technique does a poor job of forecasting when there is a trend in the data as seen in Figure 3. As shown in the Figure 3, traders with a short-term perspective may not care about which average is used, since the difference between the two averages is usually a matter of mere cents. In the next section, we'll look at Simple Exponential Smoothing, Double and Triple Exponential Smoothing with the Holt-Winters Methods.

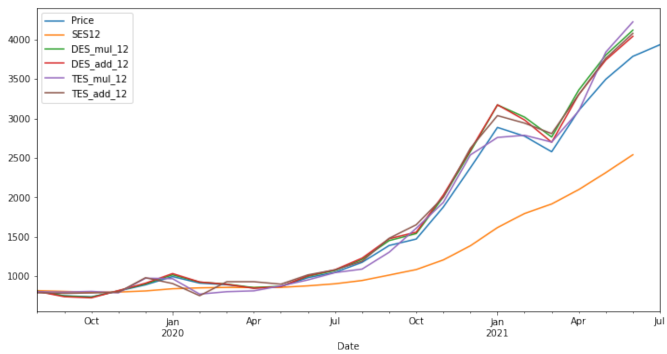

Figure 4. Comparison of model performances

In this section, we are going to focus on extending our models for future forecasts. To do this, we are going to divide our current data into testing and training sets and then work on the trained model to evaluate its performance. Because of the importance of forecast accuracy, a test should be performed to verify forecast accuracy by comparing forecast values with observational values. (Chen et al., 2018). According to Farhan & Ong (2018), while measuring forecast accuracy, it is better to use the combination of different measures to evaluate the forecast accuracy. MAPE and MAE are two of these combinations.

We can also address different types of change (growth/decay) in the trend. Our time series displays an exponential (curved) trend. We use a multiplicative adjustment. In Figure 4, we can see that Trible Exponential Smoothing is a much better representation of the time-series data. Although minor, it does appear that a multiplicative adjustment gives better results. Note that the green line almost completely overlaps the original data. RMSE and MAPE values were given in Table 1. In finding the performances of the resulting models, performance evaluation formulas provided in equations (1) and (2) were used. Also, forecast values for the August 2021- March 2022 periods are provided in Table 2.

Table 1. The Holt-Winters Methods prediction outcome and performance evaluation

SCFI	RMSE	MAPE
The Holt-Winters Methods	743.61	649.30

Table 2. The forecast values of SCFI by Using Holt-Winters Methods

Month	Forecast Values
2021-08-01	4673.516318
2021-09-01	5308.626677
2021-10-01	5517.084727
2021-11-01	6834.825919
2021-12-01	8140.522746
2022-01-01	9427.757152
2022-02-01	9072.097432

For time series modeling, time series data is said to be stationary if it does no exhibit trends or seasonality. That is, the mean, variance and covariance should be the same for any segment of the series and are not functions of time. There is a statistical hypothesis under this test.

H_0: Data has unit root and is non-stationary;

H_1: Data has no unit root and is stationary

The Augmented Dickey–Fuller (ADF) test is applied for testing the stationarity for the data series presented in Table 3.

Table 3. ADF test results for evaluating stationarity

SCFI	Original	1st order difference	2nd order difference
ADF (p-value)	1.000	0.161	<0.000188

From the Table 3, after second order difference, the *p* value is very less than the significance level of 5% and hence one cannot accept the null hypothesis H_0 and make a decision that data series are stationary.

Decomposition of time series is applied to describe the trend and seasonal factors in a time series observation. When we decompose a time series into components, we usually combine the trend and cycle into a single trend-cycle component. Thus, we think of a time series as comprising three components: a trend-cycle a seasonal, and a remainder component. In Figure 5, although small in scale compared to the overall values, there is a definite annual seasonality. The plot shows the decomposition of our time series data in its seasonal component, its trend component, and the remainder (Figure 5).

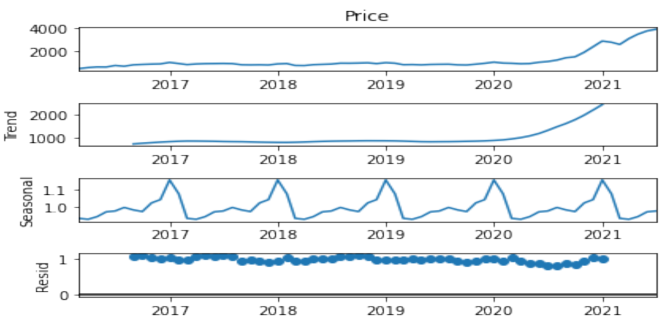

Figure 5. Decomposition of time series

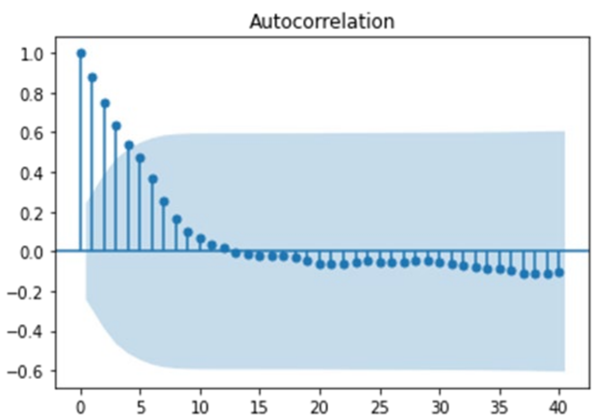

Figure 6. ACF plot of SCFI

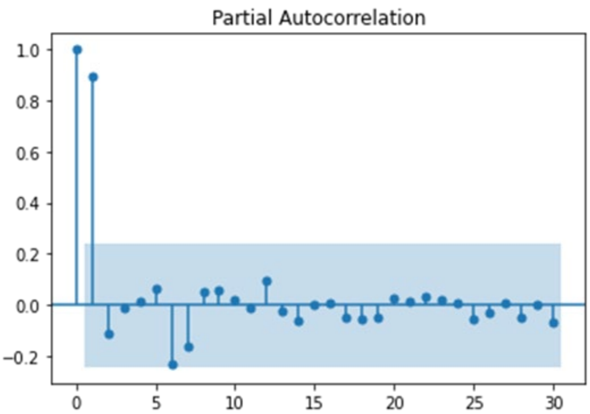

Figure 7. PACF plot of SCFI

The ACF and PACF plots helps us to confirm stationarity. The data are clearly non-stationary, with some seasonality, so we will first take a seasonal difference. The seasonally differenced data are shown in Table 3. The ACF and PACF plots of SCFI are examined and the degrees of seasonal and non-seasonal autoregression (AR) and moving average (MA) processes are determined. The linear decline of ACF is slow and that there is a significant meaningful block in PACF for SCFI. (Figure 6 and Figure 7).

Following is the sample PACF for this series. The first 2 lag value is statistically significant, whereas partial autocorrelations for all other lags are not statistically significant. According to the plot of the residuals in Figure 5. Residuals are normally distributed and follows a linear trend. Overall, the model shows good forecasting accuracy and used to predict future values. The model's forecast accuracies were calculated and compared by RMSE and MAPE. The RMSE and MAPE in SARIMA are also relatively low. The selected model shows good forecasting accuracy of the testing set. Considering that the second difference in the series is also stationary, the SARIMA (0,2,3) $(1,0,0)_{12}$ model is chosen as the appropriate model. The selected model is verified to be appropriate using the stepwise model selection method.

The comparison of the Holt-Winters methods and SARIMA with model prediction outcome and performance evaluation is shown in Table 4. SARIMA marginally performed with a tightly lower errors when compared to the Holt-Winters. The SARIMA models, where error values are lower, are found more suitable models for forecasting SCFI. According to the results in Table 5, SARIMA (0,2,3) $(1,0,0)_{12}$ is the found the most suitable model for forecasting SCFI.

Since model performance and diagnostics are controlled, the forecasting results can be examined 6-month forecasting is utilized with the SARIMA models. The forecasted values are also shown in Table 6.

Table 4. SARIMA and the Holt-Winters Methods prediction outcome and performance evaluation

SCFI	RMSE	MAPE
SARIMA (0,2,3) $(1,0,0)_{12}$	338.60	318.23
Holt-Winters Methods (Double Exponential Smoothing)	561.14	519.867
Holt-Winters Methods (Triple Exponential Smoothing)	3727.24	3037.74

Table 5. SARIMA models prediction outcome and performance evaluation (AIC and BIC)

SCFI	AIC	BIC
SARIMA(0,2,0)$(1,0,1)_{12}$	819.35	830.22
SARIMA(1,2,0)$(1,0,0)_{12}$	815.93	827.07
SARIMA (0,2,1)$(0,0,1)_{12}$	799.52	812.17
SARIMA (0,2,1)$(1,0,1)_{12}$	798.17	810.19
SARIMA (1,2,1)$(1,0,0)_{12}$	798.06	810.17
SARIMA (0,2,3)$(1,0,1)_{12}$	794.02	805.12
SARIMA (0,2,2)$(0,0,2))_{12}$	798.20	810.21
SARIMA(0,2,3)$(0,0,0)_{12}$	793.87	804.59

When the forecasting results are shown with graphs, SCFI forecasting results are showed that the increase will continue (Figure 8).

Table 6. The forecast values of SCFI by Using SARIMA

Month	Forecast Values
2021-08-01	4186.648130
2021-09-01	4555.021128
2021-10-01	4821.787789
2021-11-01	5228.400601
2021-12-01	5674.845754
2022-01-01	6127.352350
2022-02-01	6310.557688

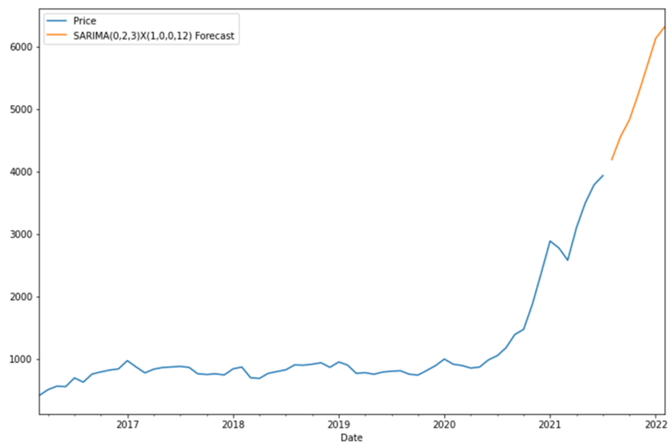

Figure 8. Forecasting of SCFI

Conclusion

In this study, we have tried to contribute to the improvement of the forecast accuracy of SCFI by using two different time series modelling approaches and appropriate criteria to provide the assumptions of the approaches. For this purpose, SCFI data was examined by Holt-Winters and SARIMA methods. The study results demonstrate SARIMA model seems to be more precise and accurate model. SARIMA model has the minimum MAPE and RMSE values when compared with Holt-Winters. As a result of time series analysis, the chosen SARIMA model can be used to predict future values because the forecasting accuracy is acceptable. It was found that the most suitable model is SARIMA (0,2,3) $(1,0,0)_{12}$. In line with the determined SARIMA model, forecast values for the period August 2021-February 2022 have been calculated. According to the results of the analysis, it is predicted that the increase will continue without losing momentum. Since the beginning of 2021, container shipping spot freight rates have continued to rise due to the major imbalance between demand and effective maritime container transport capacity. The Shanghai Containerized Freight Index (SCFI) rose above 4,000 points for the first time in history. This increase can be associated with goods that are unable to be transported due to pandemic (lockdown), the intensive transportation of the goods that are kept in stock, and the increasing demands before Christmas, blocking off the Suez Channel by Evergreen, which happened suddenly during the transportation process that started after the pandemic, short-term breaks in world supply chains, empty container problem due to above-mentioned facts and the pandemic, and rises in inflation in the world. As a result of these developments, it is seen that the limited ship capacity leads to an increase in the freights in this current situation where transportation costs are increasing. Shipping market analysts can benefit from the performance of the proposed satisfactory forecasting models and integrate them into their administrative toolkits.

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Compliance With Ethical Standards

Authors' Contributions

KK: Validation, Investigation, Data curation, Writing- original draft, Methodology, Visualization.

LT: Conceptualization, Writing- review & editing, Supervision.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

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

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