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RESEARCH ARTICLE

Additional Record of Rare Pilot Fish, *Naucrates ductor* (Linnaeus, 1758) with Some Biological Notes from the Northern Aegean Sea, Türkiye

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Key words:

Naucrates ductor
Rare species
Purse seine fishing
Population
Edremit bay

Abstract: In this study, four individuals of pilot fish *Naucrates ductor* which were accidentally caught by a commercial purse seiner in Edremit Bay (Aegean Sea) were investigated. The morphometric and meristic characteristics, sex, maturity stages, otoliths, age and stomach contents of the specimens were evaluated. This study contributes to the existing literature by providing additional records from specimens reported from the northernmost location reported so far as well as other biological information for the rarely observed pilotfish.

Anahtar kelimeler:

Naucrates doctor
Nadir tür
Gırgır balıkçılığı
Popülasyon
Edremit körfezi

Ege Denizi'nin Kuzeyinde Nadir Bulunan Malta Palamudunun *Naucrates ductor* (Linnaeus, 1758) Bazı Biyolojik Özellikleri ile Birlikte İlave Kaydı

Öz: Bu çalışmada Ege Denizi'nin Edremit Körfezi'nden gırgır balıkçılığı operasyonu ile tesadüfi olarak yakalanan 4 adet Malta palamudu *Naucrates ductor* incelenmiştir. Elde edilen bireylerin morfometrik ve meristik özellikleri, cinsiyet, olgunluk safhaları, otolit ölçümü, yaş ve mide içerikleri incelenmiştir. Nadir gözlemlenen türün en kuzeyden bireylerini değerlendirme imkânı sağlayan bu çalışma, ilave kayıtlar ve biyolojik notlar ile literatüre katkı sağlamaktadır.

Introduction

The pilotfish (*Naucrates ductor* (Linnaeus,1758)) (Carangidae) is an epi-pelagic fish species which is found in tropical and subtropical seas (Whitehead et al., 1986; Quigley, 2019). It is distributed in the Mediterranean; Corsini-Foka et al. (2015) and Ali-Basha et al. (2021) reported this species from Rhodes (Southwest Aegean Sea) and Syrian waters, respectively. Although Bilecenoğlu et al. (2014) stated that the pilot fish's distribution comprise all Türkiye Seas (Bilecenoğlu et al., 2014), none of these records accompanied any specimens. The pilot fish was considered as a rare fish species for Türkiye waters (Smith-Vaniz, 1986). Özgül (2015) identified 21 specimens from Kuşadası Bay when working on the species composition of fish aggregation devices and Akyol (2019) reported a single individual of pilot fish caught by a gillnet in Izmir Bay, Aegean Sea. Ichthyoplanktonic results suggested that pilot fish spawns around the Bodrum-Yalıkavak region, in the Southeastern Aegean Sea (Mater and Coker, 2002; Hoşsucu and Taylan, 2015).

Information on the pilot fish biology in the literature is also scarce. In terms of behavior, commensal relationship between pilot fish and large-size marine animals such as sharks, mobulid rays, bony fish and turtles (Massutí and Reñones, 1994; Vassilopoulou et al., 2004) as well as floating objects were reported (Massutí and Reñones, 1994; Reñones, et al., 1998). The pilot fish was caught as a by-catch in the Mediterranean using purse seine nets around floating objects (Reñones et al., 1999; Pipitone et al., 2000). With respect to foraging, the pilot fish feeds on jellyfish and drifting seaweed (Golani et al., 2006; Froese and Pauly, 2018). Members of Arthropoda, Annelida, Mollusca, Vertebrata and Tunicata species were reported in the stomach contents of the pilot fish (Pipitone et al., 2000; Vassilopoulou et al., 2004).

This study reports a new additional northernmost record of the pilot fish with some data on morphometric and biological characteristics. This information will help increase knowledge of pilot fish biology.

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Material and Methods

Four individuals of pilot fish were caught by a commercial purse seiner at a depth of nearly 100 meters, on 15th December, 2022 in Edremit Bay, Northern Aegean Sea, Türkiye (Coordinates: 39°25’584’’ N - 26°14’351’’ E) (Figure 1).

The specimens were transferred to the laboratory in a cooler. Individuals were identified based on Whitehead et al., (1986) and photographed. Some morphometric characteristics were measured and meristic characteristics were counted (Figure 2) and then compared with previous studies.

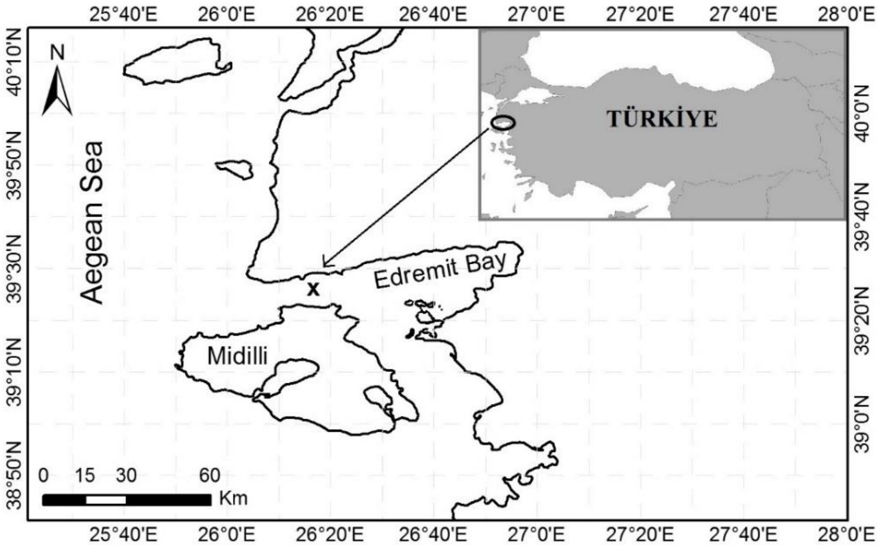

Figure 1. Map of sampling area (x showing the catch location of *N. ductor* on Edremit Bay)

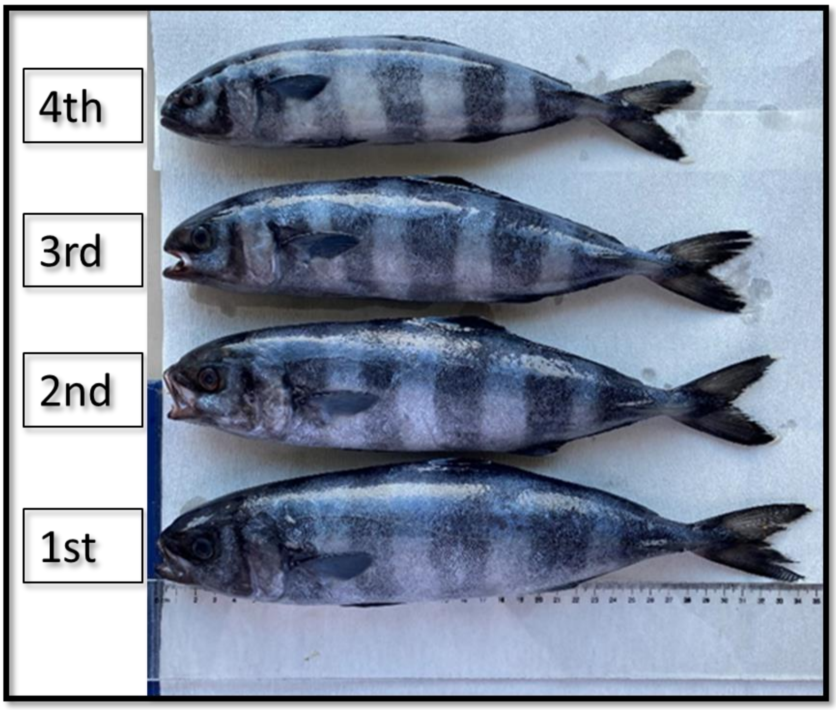

Figure 2. Individuals of *N. ductor*

The morphometric measurements were performed to the nearest millimeter (mm) using a measuring board. The total body and gonads were recorded to the nearest 0.0001 gram (g). The length-weight relationship was estimated using the equation $W = a * L^b$, where, W = total weight (g), L = total length (cm), a = intercept and b = regression coefficient (Ricker, 1975). Following log transformation, the constants a and b were estimated by least square linear regressions. Growth type was determined by the value “b” which reflects allometry of the growth and it was tested by t-test (Pauly,

1984) at the 0.05 significance level to verify the significant difference from the isometric growth (b = 3). The statistical significance level of the coefficient of determination (r^2) and 95% confidence intervals (95% CI) of b were also estimated (Zar, 1999).

Only 1 out of 4 otoliths were successfully recovered intact and therefore, otolith measurements were based on a single otolith. Age was estimated by interpreting the annual growth rings of the otolith according to Iglesias and Dery's (1981) method using an image analysis software (Q-capture). Readings were made by three independent researchers.

Sex and maturity stages were determined by macroscopic observation of gonads. The sexual maturity stages were determined according to the method described by Holden and Raitt (1975) as;

Stage I (Immature), ovary and testis about 1/3rd length of body cavity, ovaries pinkish, testis whitish.

Stage II (Maturing), ovary and testis about 1/2 length of body cavity.

Stage III (Ripening), ovary and testis is about 2/3rds length of body cavity.

Stage IV (Ripe), ovary and testis from 2/3rds to full length of body cavity, ovary orange-pink in colour with conspicuous superficial blood vessels, testis whitish-creamy, soft.

Stage V (Spent), ovary and testis shrunken to about 1/2 length of body cavity, ovary contain remnants, testis bloodshot and flabby.

The stomach was dissected, dried on blotting paper and all materials inside the stomach were sorted and identified (Fischer, 1973; Murduchay-Boltouski, 1969).

Results

Morphometric measurements, meristic counts, total body, gonad weights, and otolith measurements of the pilot fish were summarized and compared with previous studies in Table 1. The total length and weight was highly correlated (r^2:0.99) and the b value was found as 2.88 indicating a negative allometric growth.

Gonad weights for the 1st, 2nd, 3rd and 4th pilot fish were recorded as 1.1421 g, 0.7366 g, 3.3952 g and 0.3777 g, respectively. The 4th individual was determined as a male and the rest of the specimens were female. Only a single ripe gonad (4th stage) was found (3rd individual) covering more than 2/3 of the body cavity, having an orange color and surrounded by blood vessels (Figure 3a, 3b). The gonads of the remaining three individuals were immature (1st stage) with a tube-shaped, thin and transparent appearance (Figure 3c, 3d).

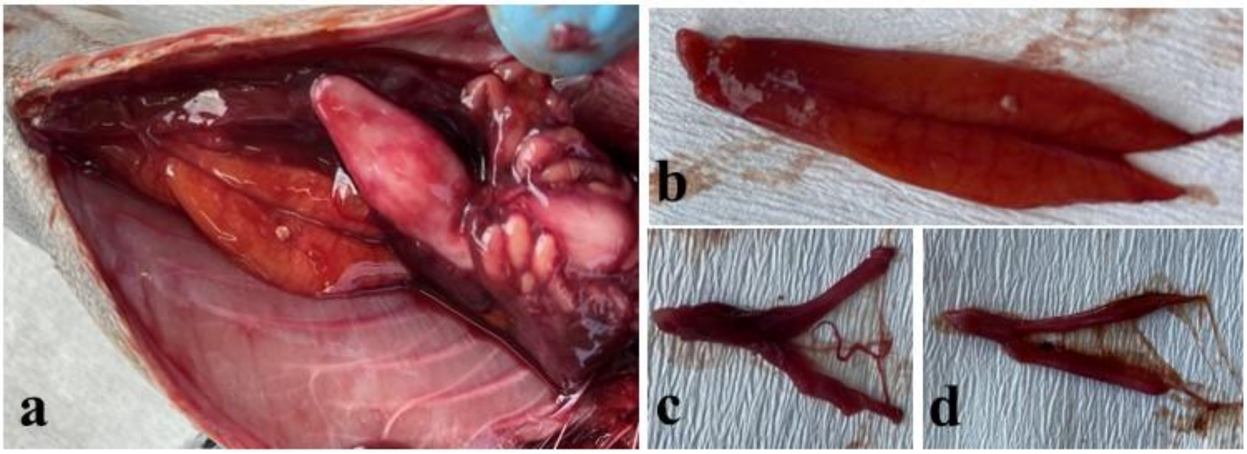

Figure 3. a) Dissected individual with gonads (3rd individual), **b)** 3rd individual with 4th stage gonad, **c)** Female with 1st stage gonad (1st individual), **d)** Male with 1st stage testis (4th individual)

The length, width and weight of the right otolith of 4th individual were measured as 0.944 mm, 1.699 mm, and 0.0001 g, respectively. The nucleus was clearly visible but there was no valid opaque zone on the otolith. Thus, the age was determined to be 0 due to the invisible annual age mark (Figure 4).

The stomachs of three individuals were empty. The stomach of 4th individual contained two prey items belonging to two different prey types. One of them was identified as a seaweed, with a length of 11 mm (Figure 5a) and the second item was identified as cuttlefish (*Sepia officinalis* Linnaeus, 1758) having a total length of 70 mm, and a mantle length of 45 mm (Figure 5b).

Table 1. Morphometric measurements, meristic counts, sex, maturity stages and otolith measurements recorded and compared with previous studies of *N. ductor*

N. ductor	**In this study**				**Akyol (2019)**	**Ali-Basha et al., (2021)**
	North Aegean Sea				**Aegean Sea**	**Syrian waters**
Fish numbers	**1st**	**2nd**	**3rd**	**4th**	**1st**	**1st**
Sex	Female	Female	Female	Male	-	-
Maturity stages	I	I	IV	I	-	-
Morphometric measurements (mm)						
Total length (TL)	346	324	311	276	275	300
Standart length	271	256	243	222	247	261
Fork length (FL)	312	292	281	251	224	267
Head length	71	66	64	56	58	61
Interorbital space	31	27	25	23	-	-
Predorsal length	117	98	96	87	84	118
Preanal length	185	166	165	136	134	163
Prepectoral length	78	71	65	57	62	66
Prepelvic length	8	82	81	68	-	-
Max. Body depth	67	63	55	47	-	65
Eye diameter	12	11	10	9	11	11
Preorbital length	23	22	21	19	18	34
Total weight	468.66	385.37	326.56	244.75	-	294.29
Gonad weight	1.1421	0.7366	3.3952	0.3777	-	-
Stomach weight	7.4452	5.142	4.9193	8.1304	-	-
Meristic counts						
Dorsal fin rays	IV+I+27	IV+I+27	IV+I+27	IV+I+27	IV+I+28	IV+I+27
Anal fin rays	II+16	II+16	II+16	II+16	III+15	II+ I+15
Pectoral fin rays	18	18	18	18	18	20
Caudal fin rays	12	12	12	12	-	-
Pelvic fin rays	I+5	I+5	I+5	I+5	I+5	I+5
Gillrakers	23	23	24	24	-	-
Linea lateralis scale	148	144	145	142	-	-
Operculum rays	8	8	8	8	-	-
Otolith measurements				**Right**		
Width (mm)	-	-	-	1.699	-	-
Length (mm)	-	-	-	0.944	-	-
Weight (g)	-	-	-	0.0001	-	-

Figure 4. Right sagittal otolith of 4th individual

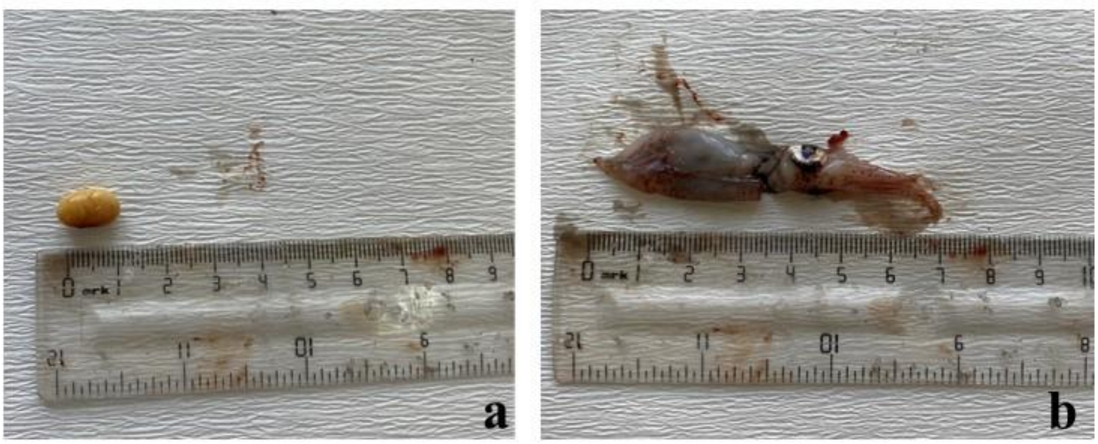

Figure 5. Stomach contents of 4th individual **a)** Seaweed, **b)** Cuttlefish

Discussion

Although considered as a common species in some areas such as the Indian Ocean (Eschmeyer, 1983), the pilot fish is a rarely seen fish species in the Mediterranean. Recent new records from the western Mediterranean to the Irish coasts and from the Syrian coasts to the Northeastern part of the Aegean Sea indicated a broad range distribution (Corsini-Foka et al., 2015; Quigley, 2019; Ali-Basha et al., 2021). However, large stocks of the pilot fish have not been reported.

Reñones et al. (1998) stated that sex determination of individuals below 10 cm FL was not possible. Individuals between 10 - 31.2 cm FL were sexed as 0 years old and the largest specimens were five month old. Vassilopoulou et al. (2014) reported that individuals between 18.9 - 30.5 cm were aged as 0. In the present study, all individuals were sexed and one female specimen had mature gonads but none of them reached the age of 1 year. Thus, the findings of this study support those of Reñones et al. (1998). Jardas (1996) reported that pilot fish reach sexual maturity at total length of approximately 25 cm. In the present study, the smallest individual with a 27.6 cm TL was found to be sexually mature. Vassilopoulou et al. (2014) stated that TL>250 mm was at stage III. This finding supports that of Jardas (1996). In the present study, however, gonads with more advanced stages were not observed in the larger 3rd individual due possibly to spawning. Reñones et al. (1998) stated that pilot fish spawning occurred in two different peaks in July and December. The ichthyoplankton results showed that pilot fish spawned in October in the Aegean Sea (Hossucu and Taylan, 2015; Mater and Coker, 2002) and between December to March around the Dardanelles Strait (Daban and Yüksek, 2017). Since all fish examined in the present study were caught in December, the ichthyoplanktonic findings supported the possibility of earlier spawning.

Reñones et al. (1998) and Vassilopoulou et al. (2014) estimated the age of an individual with 31.2 cm TL as 0 years-old. These authors also stated that this species can reach 26.1 cm FL in four months. The findings in this study are in accordance with those of Reñones et al. (1998) and Vassilopoulou et al. (2014) and suggested a rapid growth during the first year.

The morphometric measurements and meristic counts of pilot fish and the comparative results in previous studies are shown in Table 1. Differences between studies may be related to the fish size and habitat.

Whitehead et al. (1986) and Cervigón (1993) reported pilot fish lengths of 35 cm and 40 cm TL, respectively, whereas the maximum length was reported as 70 cm TL (Edwards, 1990). In previous studies, total length measurements ranged between 4 -8 cm (Özgül, 2015) and 27.5 mm (Akyol, 2019) in İzmir Bay, Aegean Sea, between 21 -39 cm (mean: 30.3 cm) in the Irish coasts (Quigley, 2019), 28.7 cm in Adriatic coasts (Glamuzina et al., 2017), 30 cm TL in Syrian coasts (Ali-Basha, 2021), and between 15 - 31.2 cm fork length in the Mallorca coasts, western Mediterranean (Reñones et al., 1999). The length measurements of *N. ductor* from the Mediterranean Sea were relatively lower than those reported by Whitehead et al. (1986) and Cervigón (1993). Lower reported values may stem from foraging by dolphins or overfishing by purse seine fishing.

In the previous studies, members of Arthropoda, Annelida, Mollusca and Tunicata were found in the stomach contents of pilot fish (Reñones et al., 1998; Pipitone et al., 2000; Vassilopoulou et al., 2004; Glamuzina et al., 2017). Jellyfish and seaweeds were also reported as prey items for pilot fish (Golani et al., 2006; Froese and Pauly, 2018) along with *Octopus* sp. and *Onychoteuthis banksi* reported from pilot fish caught in Mallorca coasts (Reñones et al., 1998). Cuttlefish (*Sepia officinalis*), on the other hand, was reported for the first time as a prey item for the pilot fish.

With increasing sea water temperatures due to global climate change pilot fish can expand its distribution range in the near future to other regions such as the Marmara Sea and the Black Sea. FAD locations may prove successful sampling areas to study pilot fish biology for this rare species in the Mediterranean. Although ichthyoplankton and gonad findings indicate reproduction potential of pilot fish in the Aegean Sea, a better understanding of its rarity is required with respect to conservation efforts as rare species are more prone to overfishing

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Conflicts of Interest

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

Author Contributions

Yusuf Şen: Designing of the study, writing-original draft preparation, submission, writing-review and editing, visualization. İsmail Burak Daban: Designing of the study, sorting into taxonomic groups, identification of species, data analysis, checking-original draft preparation. Ceyda Kalemli: Supported the laboratory study, checking-original draft preparation.

Ethics Approval

Ethics committee approval isn't required for this study.

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RESEARCH ARTICLE

Assessment of Potentially Toxic Element Pollution in Tributaries of Mogan Lake, Türkiye

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Creeks
Mogan Lake

Abstract: In this study, four tributaries (Başpınar, Gölcük, Sukesen and Yavrucak Creeks) of Mogan lake under anthropogenic pressure and declared as a Special Environmental Protection Area were investigated with the following goals: a) Determination of the variations in potentially toxic element concentrations (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) in the creek waters between different months (December 2002, February 2023, April 2023), b) Calculation of potentially toxic elements indexes (Heavy Metal Pollution Index-HPI and Heavy Metal Evaluation Index-HEI) in terms of irrigation water quality, based on international and national values, c) Determination of the potential ecological risk (PER) status of the creeks in terms of irrigation water quality, d) Comparison of potentially toxic element concentrations with the environmental quality standard values specified in the current "Turkish Surface Water Quality Regulation". According to the findings; a) In terms of overall potentially toxic element levels, the creeks are ranked as follows: Başpınar Creek> Sukesen Creek>Gölcük Creek>Yavrucak Creek. The heavy metal As was identified as having the highest contribution to metal pollution and the potentially toxic element concentrations in all creeks in February and April were found to be higher compared to that in December, b) The HPI value was found to be less than 15 for all months and creeks, with the highest HPI value of 13.21 calculated in Başpınar Creek in February, c) The HEI values were found to be less than 10 (ranging from 0.02 to 0.84), indicating a low level of potentially toxic element pollution according to this index, d) In terms of irrigation water quality, all creeks exhibited a low level of PER, with the highest PER value of 13.54 belonging to Başpınar Creek, e) The detected potentially toxic element concentrations in all four creeks did not exceed the maximum EQS values provided. In this context, it has been determined that the potentially toxic element levels in creek waters, due to anthropogenic activities, are not currently causing significant pollution. However, it is noted that Başpınar Creek is at a higher risk compared to other creeks. Furthermore, because of the ongoing anthropogenic activities in the basin, long-term metal monitoring studies are important in terms of the sustainability of Lake Mogan.

Anahtar kelimeler:

Potansiyel toksik elementler
Kirlenme
Yüzey suları
Dereler
Mogan Gölü

Mogan Gölü'nü (Türkiye) Besleyen Derelerde Potansiyel Toksik Element Kirliliğinin Belirlenmesi

Öz: Bu çalışmada. antropojenik baskı altındaki bir havzada konumlanan ve Özel Çevre Koruma Bölgesi olarak ilan edilen Mogan Gölü'nü besleyen dere (Başpınar, Gölcük, Sukesen ve Yavrucak Deresi) sularında; a) Potansiyel toksik elementlerin (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) dereler ve aylar (Aralık-2002, Şubat 2023, Nisan-2023) arası farklılığının belirlenmesi, b) Sulama suyu kalitesi açısından ağır metal indekslerinin (Heavy Metal Pollution Index-HPI and Heavy Metal Evaluation Index-HEI) uluslararası ve ulusal değerler esas alınarak hesaplanması, c) Derelerin sulama suyu kalitesi açısından potansiyel ekolojik risk (PER) durumunun tespit edilmesi, d) Potansiyel toksik element konsantrasyonlarının yürürlükte olan -Yerüstü Su Kalitesi Yönetmeliği- kapsamında belirtilen çevresel kalite standard değerleri ile karşılaştırılması amaçlanmıştır. Bulgular doğrultusunda, a) Tüm potansiyel toksik element düzeyleri açısından dereler; Başpınar Creek>Sukesen Creek>Gölcük Creek>Yavrucak Creek olarak sıralanmıştır. Derelerdeki metal kirliliğinde payı en çok olan ağır metal As iken, tüm derelerde şubat-nisan ayı ağır metal konsantrasyonları, aralık ayına göre daha yüksek bulunmuştur, b) Tüm ay ve derelerde HPI değeri 15'den küçük bulunurken, en yüksek HPI değeri 13.21 olarak Başpınar Deresi'nde şubat ayında hesaplanmıştır, c) HEI değerleri ise 10'dan küçük (0.02-0.84) saptandığından bu indis açısından da düşük düzeyde ağır metal kirliliği belirlenmiştir, d) Tüm derelerde sulama suyu kalitesi açısından düşük düzeyde PER söz konusu olup, en yüksek PER değeri 13.54 ile yine Başpınar Deresi'ne aittir, e) Dört derede de tespit edilen ağır metal konsantrasyonları, Max.-EQS için verilen sınır değerleri aşmamıştır. Bu bağlamda, antropojenik faaliyetlerden dolayı dere sularındaki ağır metal seviyelerinin şu an için ciddi boyutlarda kirlilik yaratmadığı ancak özellikle Başpınar Deresi'nin diğer derelere göre daha fazla risk altında olduğu belirlenmiştir. Ayrıca havzadaki antropojenik faaliyetlerin süregelmesi nedeniyle, uzun-dönemli metal izleme çalışmaları Mogan Gölü'nün sürdürülebilirliği açısından önem arz etmektedir.

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Introduction

Anthropogenic activities limit the use of surface water resources for irrigation, domestic fishing, and industrial purposes by deteriorating water quality. The main causes of potentially toxic element pollution in water and sediment include discharge of untreated waste from the mining industry and other industrial activities. Additionally, the contamination of water bodies can occur through various pathways, such as the introduction of heavy metal-containing chemical pesticides used in agricultural practices (Pulatsü et al., 2014). In this context, recommended thresholds for potentially toxic element concentrations in water used for drinking, irrigation, domestic use, as well as for the protection of aquatic life have been provided by different sources (FAO, 1994; Goher et al., 2014; Mollo et al., 2022).

Determining various pollution indices is one of the most common methods for assessing environmental pollution in surface waters, soils and sediments. The Heavy Metal Pollution Index (HPI) is widely used to assess the cumulative impact of various metals on the quality of surface waters. On the other hand, the Heavy Metal Evaluation Index (HEI) is extensively employed to indicate the overall surface water quality in terms of metal levels. HPI is considered a reliable technique for assessing water quality based on potentially heavy metal concentrations. On the other hand, HEI is defined as a rating that reflects the compound effect of different dissolved heavy metals (Edet and Offiong, 2002; Rahman et al., 2014). Moreover, the potential ecological risk (PER) index can be evaluated considering the toxicity factor of a specific metal, making it possible to enumerate the contamination status of any given metal in an ecosystem (Hakanson, 1980).

The increasing number of industrial facilities (such as aluminum coating factories, brick factories, and machinery factories) due to its proximity to Ankara and efficient road transportation are among the main anthropogenic factors in the Mogan Lake basin (Karaaslan, 2009). Additionally, unplanned urbanization due to increasing human population around the lake, increasing number of hobby gardens and the transportation of waste from andesite and stone quarries to the lake through tributaries can also be counted as contributing factors. In this context, certain rehabilitation efforts have been conducted within the lake basin (Anonymous, 2022). It appears inevitable that negative interventions on tributaries will lead to further reduction in the already limited water flows and increase pollution.

The "Regulation on Surface Water Quality" in effect in Turkey establishes priority substances and environmental quality standards for surface water resources. Within this framework, the arithmetic mean of one year's monitoring results of specific pollutants and priority substances for each water body category (rivers/lakes, coastal and transitional waters) is compared to the annual average environmental quality standard (Mean-EQS). Alternatively, for any specific pollutant and/or priority substance, the individual monitoring data is compared to the maximum allowable environmental quality standard (Maximum-EQS). As a result of the assessment, if the monitoring data is lower than both the Mean-EQS and the Maximum-EQS values, the environmental quality standard values for the receiving environment are considered to be met (TSWQR, 2016). In the Gölbaşı Special Environmental Protection Area Management Plan, covering the period from 2015 to 2019 and issued by the General Directorate of Protection of Natural Assets under the Ministry of Environment and Urbanization, the objective of achieving compliance with the standard values of the Surface Water Quality Regulation (Water Framework Directive) is set for Mogan-Eymir Lakes and the creeks that feed these lakes by the end of the fifth year (Anonymous, 2022).

Despite the potentially toxic element studies conducted on the water-sediment of Lake Mogan (Yüksel and Arica, 2018; Küçükosmanoğlu and Filazi, 2020; Binici et al., 2021; Binici and Pulatsü, 2022a, 2022b), no previous studies has been found regarding the determination of potentially toxic element levels in tributaries of Lake Mogan. The aim of this research is to assess the following aspects in tributaries receiving both point and non-point sources of pollution that reach Lake Mogan: a) Determination of variations in potentially toxic element levels (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) among different creeks and months, b) Calculation of metal pollution indices such as Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) in the context of irrigation water quality, c) Identification of the potential ecological risk (PER) status of the creeks in terms of irrigation water quality, d) Comparison of potentially toxic element concentrations with the environmental quality standard values specified in the "Turkish Surface Water Quality Regulation", e) Statistical evaluation of possible sources of heavy metals. The findings of this study are important for revealing the current status of heavy metals in the surface waters of tributaries and for establishing a preliminary database in this regard.

Material and Methods

Study area

Lake Mogan is located within the boundaries of the Gölbaşı Special Environmental Protection Area and is also considered as one of the significant wetlands in our country nominated for Ramsar designation. The groundwater recharge of Lake Mogan is relatively low and water input during summers primarily occurs through irregularly flowing creeks that tend to dry up. The most important creeks are Sukesen, Başpınar, Gölova, Yavrucak, Çolakpınar, Tatlım, Kaldırım, and Gölcük, located in the eastern-northwestern parts of the basin (Anonymous, 2017). In this study, water samples were taken from four selected creeks that represent the sources of pollution for Lake Mogan and contribute to its inflow (Figure 1). The coordinates of the creeks and potential pollutant sources related to them are presented in Table 1.

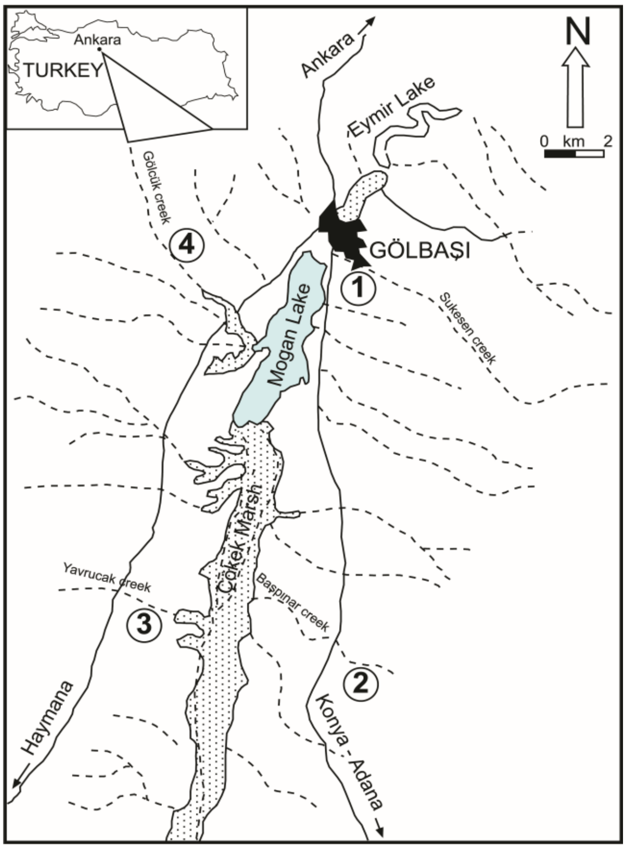

Figure 1. Map of the study area and selected creeks

Table 1. Characteristics of selected creeks

Creeks	Pollution sources	Coordinates
Sukesen Creek	Quarries and settlements	39°47,350"N, 32°48,266"E
Başpınar Creek	Settlements and agricultural activities	39°46,616"N, 32°48,149"E
Yavrucak Creek	Agricultural activities	39°47,542"N, 32°48,205"E
Gölcük Creek	Agricultural activities	39°45,436"N, 32°45,568"E

Methods

In this study, three sampling periods (December 2022, February 2023, and April 2023) were conducted on the selected creeks that feed Lake Mogan. . The sampling periods were chosen due to the increased likelihood of heavy rainfall based on previous meteorological data. The water samples were transported to the laboratory in a dark and cool environment. Potentially toxic element analyses (Hg, As, Cd, Cr, Pb, Ni, Cu, Zn) were performed on the water samples in an accredited laboratory according to TS EN ISO 17294-1.24 standards, with four replicates for each analysis.

a) Pollution evaluation indices

In the present study, eight metals (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) were assessed for the calculation of HPI, HEI and PER. Regarding HPI, HEI and PER values; Data I: FAO (1994), Data II: TSWQR (2015) were calculated separately (Table 2) based on the potentially toxic element concentration values reported for the irrigation water class regarding the quality of creek surface waters are also presented.

Heavy Metal Pollution Index (HPI)

Heavy Metal Pollution Index (HPI), which characterizes the pollution status of the creeks in terms of heavy metals, is calculated according to the following formula:

$HMPI=\sum(Q_i \times W_i)/\sum W_i$

$Q_i = C_i / S_i \text{ x}100$

Where, Qi is the sub-index of the ith parameter; Wi is the unit weightage of the ith parameter (=k/ Si); k: Proportionality constant for metal (=1); Ci is the monitored value of potentially toxic elementsof ith parameter; Si is the standard value of the ith parameter (µg/L).

To assess the contamination level, HPI values are divided into three groups: (i) Low (HMPI value < 15), (ii)

Moderate (HMPI value = 15-30), and (iii) High (HMPI value > 30) (Edet and Offiong, 2002).

Heavy Metal Evaluation Index (HEI)

The HEI value, which provides information about the overall water quality in terms of heavy metals, is calculated using the following equation (Zakir et al., 2020):

$HEI = \sum_{i=1}^{n} M_i / MAC_i$

M_i: The monitored value of the ith metal (μg/L)

MACi: Maximum admissible concentration of the ith metal (μg/L) (The standard permissible value of the ith metal (μg/L)

The classifications of surface water quality based on HEI are as follows: <10 for low pollution, 10-20 for moderate pollution, and >20 for high pollution (Kumar et al., 2019).

b) Risk assessment

Potential ecological risk (PER) assessment

The potential ecological risk assessment of potentially toxic element contamination was proposed as a diagnostic tool for identifying and managing water pollution problems associated with increasing levels of heavy metals in surface water (Rahman et al., 2014). Based on Hakanson (1980) method, the potential ecological risk index has been calculated:

$PER = \sum_{i=1}^{n} Ti \times Ci/Si$

Ci: The monitored value of the ith metal (μg/L)

Si: The standard permissible value of the ith metal (μg/L) (Table 2)

Ti = The individual metal's biological toxicity factor.

The toxicity factor values are: 5 for Ni, Cu, Pb; 2 for Cr; 1 for Zn; 10 for As; 30 for Cd; 40 for Hg (Hakanson, 1980).

The classifications of surface water quality based on PER are as follows: <110 for low risk, 110-200 for moderate risk, 200-400 for considerable risk, and >400 for very high risk. (Sharafi et al., 2016).

c) Comparison based on EQS values

The Mean-EQS and Max. EQS values specified under the "Turkish Surface Water Quality Regulation" (TSWQR, 2016) for heavy metals in Table 2.

Table 2. Standard permissible value (S_i) or maximum admissible concentrations (MAC_i) of metals for irrigation water and national surface water quality standard values (EQS)

Metals	Concentrations (μg/L)			
	Irrigation water		EQS (TSWQR, 2016)	
	FAO (1994)	TSWQR (2015)	Mean-EQS	Max. EQS
Cr	100	50	1.6	142
Ni	200	50	4	34
Cu	200	50	1.6	3.1
Zn	2000	500	5.9	231
As	100	50	53	53
Cd	10	5	0.08	0.45
Hg	2	0.5	1.2	0.07
Pb	5000	20	1.2	14

d) Statistical analyses

The Kruskal-Wallis test was used to determine if there was a significant difference in the concentration values of heavy metals among different months. The Tukey test, a post hoc multiple comparison test, was used to identify which months had significant differences. The Spearman correlation coefficient was used to determine whether there was a significant relationship among the concentration values of heavy metals. In this study, Principal Component Analysis/Factor Analysis (PCA/FA) with varimax rotation was conducted to identify potential sources of heavy metals. To determine the appropriate dataset for PCA, the Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests were performed. The KMO is a measure of the acceptability of sampling since it indicates the common variance that might be induced by underlying factors. If the KMO value is close to 1, it suggests that the samples are acceptable or PCA might be more effective (Kolassa, 2020). All statistical analyses were performed by using SPSS 22.

Results

Potentially toxic element concentrations in creeks In

Table 3, the variations of the eight heavy metals (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) for each creek according to the months, as well as the variations of each potentially toxic element across the creeks for each month are presented. It was determined that the concentrations of all heavy metals vary according to the months for each creek. Cr, Ni, and As levels in Başpınar Creek in December showed a statistically significant difference compared to those of other two months ($p<0.05$). In Gölcük Creek, while the levels of Ni, Cd, and Hg did not show a statistically significant difference between February and April ($p>0.05$), there was a significant difference ($p<0.05$) between the values of Cr, Cu, Zn, As, and Pb for the same months. For Sukesen Creek, the As values showed a statistically significant difference across all months. In December, the Cr value differed significantly from those of February and April, while the Ni concentrations in April showed a significant difference compared to those of December and February ($p>0.05$). In Yavrucak Creek, there were no statistically significant differences ($p>0.05$) between the concentrations of As and Hg during the months of February and April. In all creeks, the concentrations of Cu, Cd, Hg, and Pb were below the detection limits in December, while the Cr concentrations were below the detection limits in Gölcük and Yavrucak Creeks (Table 3).

As seen in Table 2, the monthly concentration values of each potentially toxic element also varied depending on the creeks. In Başpınar Creek, the concentration of Cr reached its maximum value in April (2.41 µg/L), while the concentration of Zn reached its maximum value in February (1.64 µg/L), showing a statistically significant difference compared to other creeks ($p<0.05$). In Sukesen Creek, it was determined that the concentration of Ni in February (3.51 µg/L) and the concentration of Cu in April (1.54 µg/L) showed a statistically significant difference compared to those of other creeks ($p<0.05$). The concentration of As was found to be high in Başpınar Creek in all months. Based on the concentration data of As, the ranking of the creeks was as follows: Başpınar Creek > Sukesen Creek > Gölcük Creek > Yavrucak Creek. The difference in As levels between Gölcük and Sukesen Creeks in February was not statistically significant ($p>0.05$). In Başpınar Creek, while the Cd (0.39 µg/L) and Hg (0.07 µg/L) concentrations showed a statistically significant difference compared to other creeks in February ($p<0.05$), the difference in Pb concentration between February and April among the creeks was not statistically significant ($p>0.05$).

Potentially toxic element pollution status in creeks

The variations of the calculated HPI-I and HPI-II and HEI-I and HEI-II values based on the months and creeks are shown in Figure 2, according to the criteria reported by the International (FAO, 1994) and national (TSWQR, 2015) standards, respectively. In this study, when HPI-I and HPI-II values are considered together, the range of variations were as follows: for Başpınar Creek: 0.29-13.21; for Gölcük Creek: 0.03-3.94; for Sukesen Creek: 0.01-1.60; and for Yavrucak Creek: 0.01-0.91 (Figure 2). As seen in the figure, the HPI value was less than 15 for all months and creeks, indicating a low level of pollution. The maximum HPI value was determined for Başpınar Creek (13.21) and the main contributors to pollution, as evaluated by HPI, were As, Cr, Ni, and Cu. When considering the HEI values within the scope of the study, it was found that they were less than 10 (ranging from 0.02 to 0.84) for all creeks on a monthly basis (Figure 2). Therefore, with respect to HEI,, there is a low level of potentially toxic element pollution in the irrigation water.

Ecological risks of potentially toxic elements in creeks

When considering the PER-I and PER-II values together for the creeks, the range of variations are determined as follows: for Başpınar Creek: 1.81-13.54; for Gölcük Creek: 0.53-6.46; for Sukesen Creek: 0.91-5.52; and for Yavrucak Creek: 0.16-2.64. Although the PER values for February and April were found to be higher compared to that in December, overall, there was a low risk in terms of irrigation water quality for all creeks (Figure 2-5). According to the potential ecological risk values, the creeks were ranked as follows: Başpınar Creek > Sukesen Creek > Gölcük Creek > Yavrucak Creek.

Within the scope of the study, for all creeks examined, As, Ni, and Cr were identified as significant components of potential ecological risk in December, while Hg, As, and Cd were identified as important heavy metals in February and April, which are key contributors to a potential ecological risk. Moreover, the highest PER value of 13.54 belonged to Başpınar Creek in February.

Possible sources of potentially toxic elements in creeks

Principal component analysis was performed to identify the types and contributions of heavy metals from pollution sources in selected tributaries. Başpınar Creek had two factors with eigenvalues greater than 1. In the first factor, Ni, Cr, As, Pb, and Cu metals were grouped together, explaining 56% of the variance. In the second factor, Cd, Zn, and Hg were grouped, explaining 29% of the variance (Table 4). It was determined that the concentration values of the 5 heavy metals that contributed significantly to the pollution in Başpınar Creek were generally higher than those of other creeks (Table 3). In this context, the proximity of the tributary to residential areas and intensive agricultural activities supported the varying levels of contribution and high concentrations of heavy metals. In Gölcük Creek, it was determined that the first principal component, which explained 53% of the total variance, exhibited stronger positive loadings for Cr (0.987), Cu (0.981), Zn (0.957), Hg (0.751), and Ni (0.700) compared to other metals, while the second factor grouped Pb (0.941), As (0.919), and Cd (0.884), explaining 40% of the variance (Table 4). It was observed that particularly in February, the concentrations of Cu and Ni in these creek waters were higher compared to the other two months. The waters of both creeks, influenced by residential and agricultural activities, seemed to be responsible for the increase in potentially toxic element concentrations, especially with respect to Ni and Cr.

Table 3. Levels of potentially toxic element concentrations according to months and creeks

Creeks	Metals	December Mean ± SD (µg/L)	February Mean ± SD (µg/L)	April Mean ± SD (µg/L)
Başpınar	Cr	$0.29 \pm 0.04^{aA*}$	2.18 ± 0.23^{bA}	2.41 ± 0.18^{bA}
	Ni	0.37 ± 0.28^{aA}	1.57 ± 0.00^{bA}	2.00 ± 0.68^{bA}
	Cu	-	0.63 ± 0.21^{aAB}	0.49 ± 0.31^{aA}
	Zn	0.53 ± 0.24^{aA}	1.64 ± 0.42^{bA}	0.33 ± 0.05^{aA}
	As	17.94 ± 1.45^{aA}	26.39 ± 1.85^{bA}	27.41 ± 2.13^{bA}
	Cd	-	0.39 ± 0.06^{aA}	0.01 ± 0.01^{bA}
	Hg	-	0.07 ± 0.02^{aA}	0.04 ± 0.04^{bA}
	Pb	-	0.08 ± 0.03^{aA}	0.55 ± 0.19^{bA}
Gölcük	Cr	-	0.25 ± 0.00^{aB}	0.06 ± 0.02^{bB}
	Ni	0.80 ± 0.14^{aB}	3.06 ± 0.00^{bB}	2.98 ± 0.15^{bB}
	Cu	-	0.75 ± 0.07^{aA}	0.04 ± 0.01^{bB}
	Zn	-	0.50 ± 0.07^{aB}	0.02 ± 0.01^{bB}
	As	5.11 ± 0.10^{aB}	4.22 ± 0.38^{aB}	12.02 ± 0.91^{bB}
	Cd	-	0.2 ± 0.07^{bB}	0.31 ± 0.07^{bB}
	Hg	-	0.02 ± 0.01^{aB}	0.01 ± 0.01^{aA}
	Pb	-	0.06 ± 0.04^{aA}	0.95 ± 0.12^{bA}
Sukesen	Cr	0.36 ± 0.02^{aB}	0.64 ± 0.10^{bC}	0.76 ± 0.07^{bC}
	Ni	3.45 ± 0.04^{aC}	3.51 ± 0.66^{aB}	2.34 ± 0.30^{bAB}
	Cu	-	0.87 ± 0.09^{aB}	1.54 ± 0.00^{bC}
	Zn	0.21 ± 0.07^{aB}	0.71 ± 0.04^{bB}	0.55 ± 0.19^{bB}
	As	8.16 ± 0.63^{aC}	11.18 ± 1.13^{bC}	14.14 ± 0.0^{cB}
	Cd	-	0.18 ± 0.08^{aB}	0.10 ± 0.02^{bC}
	Hg	-	0.02 ± 0.01^{aB}	0.03 ± 0.02^{bA}
	Pb	-	0.08 ± 0.03^{aA}	0.87 ± 0.15^{bA}
Yavrucak	Cr	-	0.16 ± 0.03^{aB}	0.58 ± 0.06^{bC}
	Ni	0.71 ± 0.21^{aAB}	0.45 ± 0.16^{aC}	2.50 ± 0.11^{bB}
	Cu	-	0.40 ± 0.09^{aB}	0.05 ± 0.02^{bB}
	Zn	-	0.36 ± 0.11^{aB}	0.18 ± 0.04^{bAB}
	As	1.28 ± 0.31^{aD}	1.90 ± 0.29^{bB}	2.00 ± 0.17^{bC}
	Cd	-	0.05 ± 0.03^{aB}	0.02 ± 0.01^{bA}
	Hg	-	0.02 ± 0.01^{aB}	0.02 ± 0.01^{aA}
	Pb	-	0.02 ± 0.02^{aA}	0.95 ± 0.12^{bA}

*: The different lower-case letters in the same row show the differences between months in the same creek, while the different capital letters in the same column show the differences between the creeks at the same month ($p < 0.05$)

- : Below detection limit

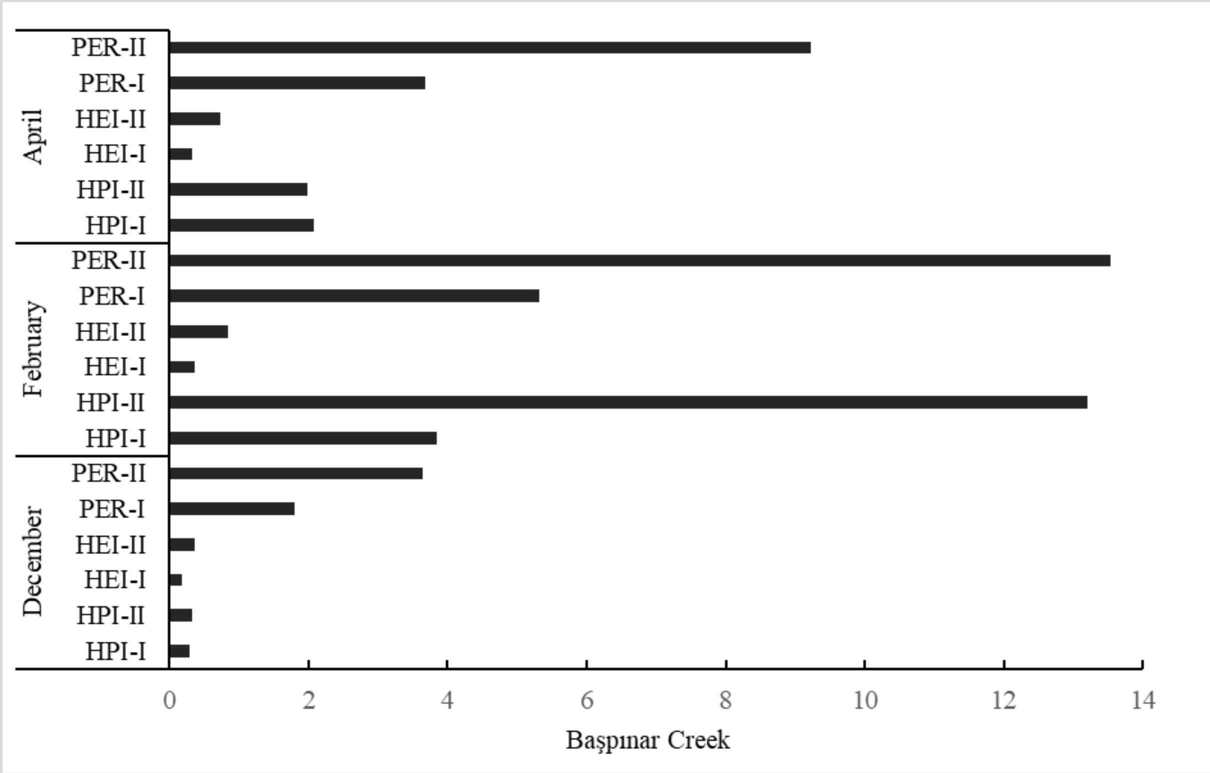

Figure 2. Variations of Heavy Metal Pollution Index (HPI), Heavy Metal Evaluation Index (HEI) and Potential Ecological Risk (PER) values according to months in Başpınar Creek

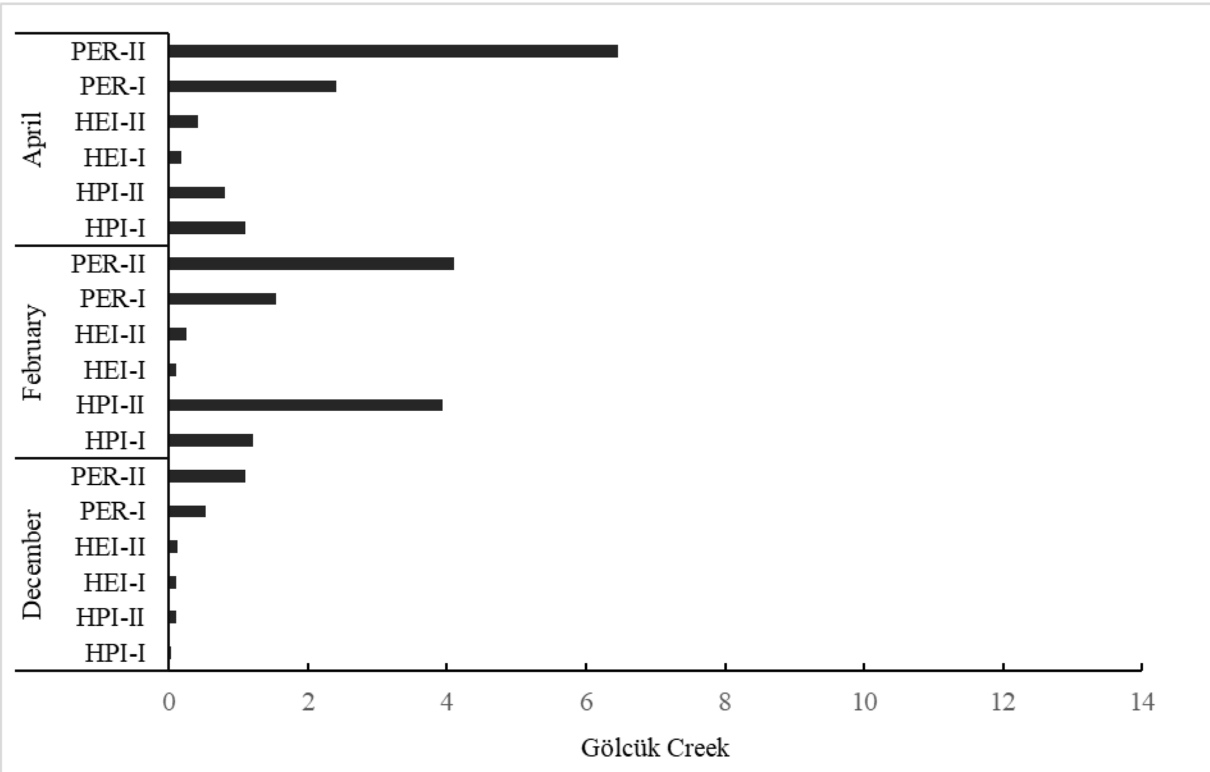

Figure 3. Variations of Heavy Metal Pollution Index (HPI), Heavy Metal Evaluation Index (HEI) and Potential Ecological Risk (PER) values according to months in Gölcük Creek

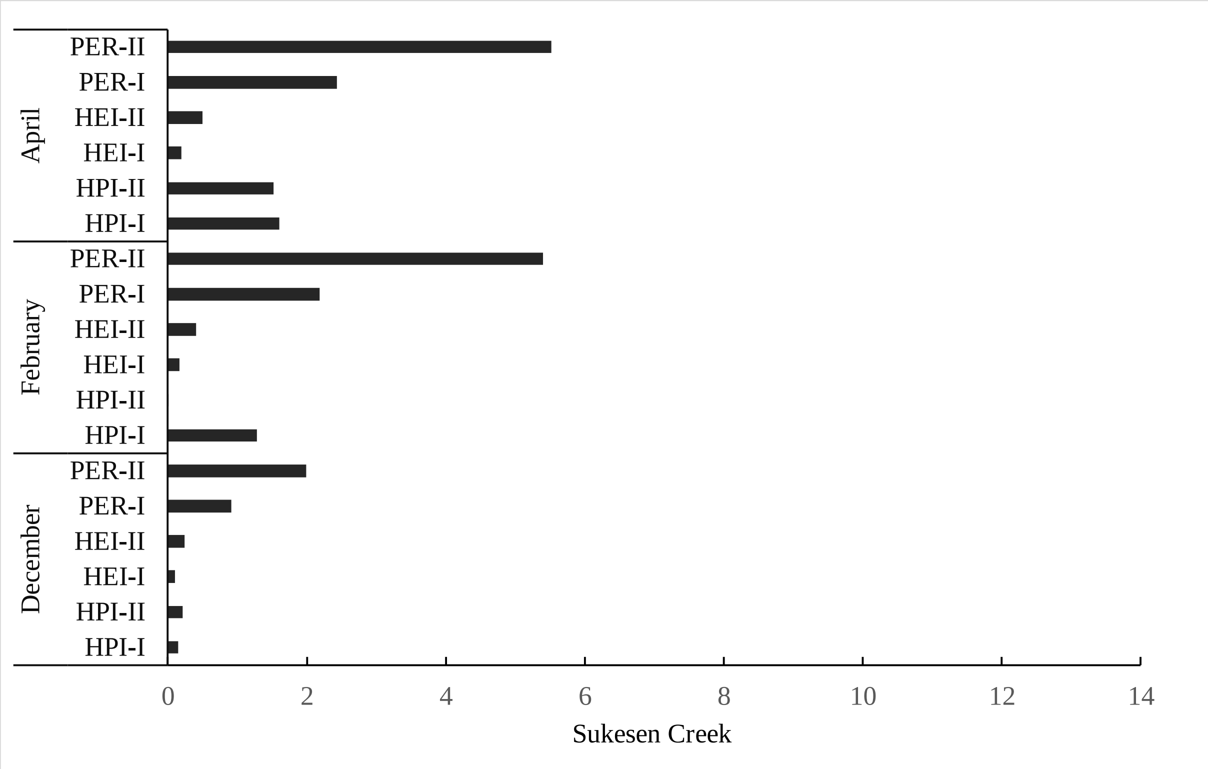

Figure 4. Variations of Heavy Metal Pollution Index (HPI), Heavy Metal Evaluation Index (HEI) and Potential Ecological Risk (PER) values according to months in Sukesen Creek

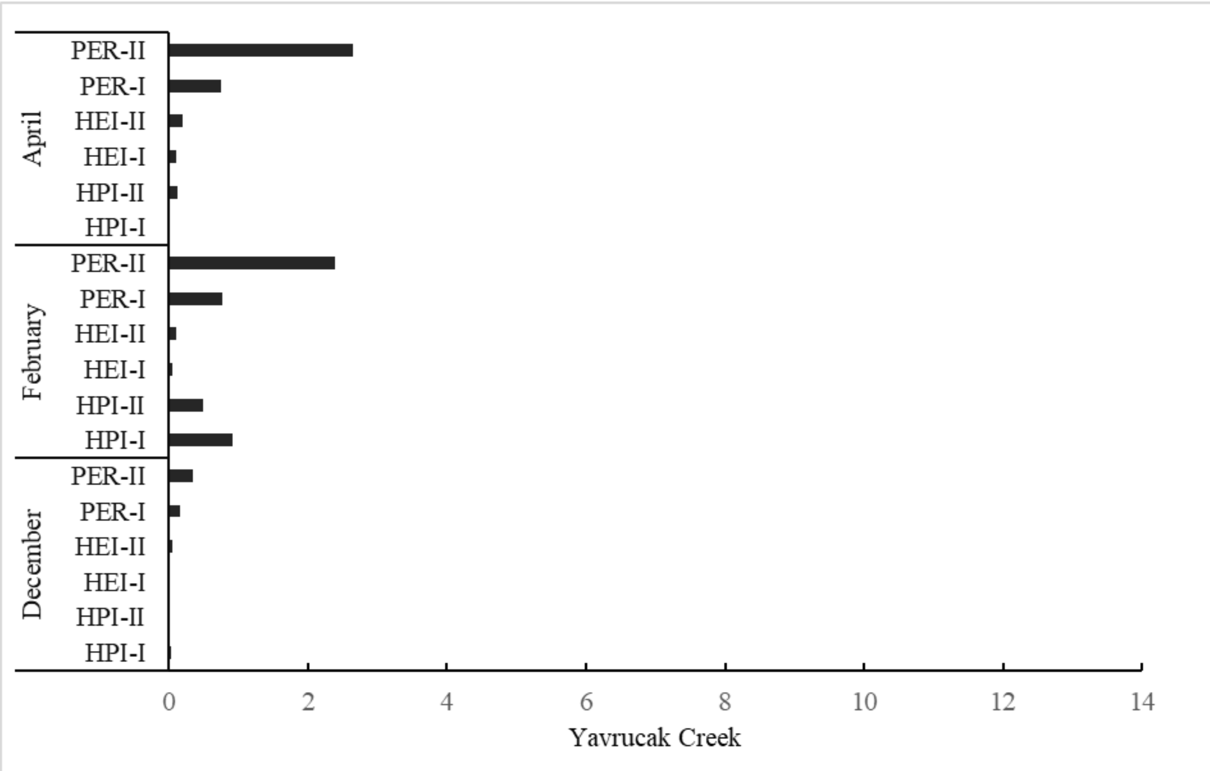

Figure 5. Variations of Heavy Metal Pollution Index (HPI), Heavy Metal Evaluation Index (HEI) and Potential Ecological Risk (PER) values according to months in Yavrucak Creek

Table 4. Total variance explained and component matrixes for potentially toxic elements in Başpınar and Gölcük Creek

Başpınar Creek						
Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	4.443	55.535	55.535	Ni	**.931**	
2	2.328	29.097	84.633	Cr	**.929**	.305
3	.694	8.671	93.303	As	**.881**	
4	.235	2.939	96.243	Pb	**.838**	-.506
5	.136	1.702	97.944	Cu	**.732**	.361
6	.095	1.181	99.125	Cd		**.945**
7	.046	.571	99.696	Zn		**.943**
8	.024	.304	100.000	Hg	.391	**.695**
Gölcük Creek						
Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	4.269	53.362	53.362	Cr	**.987**	
2	3.165	39.560	92.922	Cu	**.981**	
3	.382	4.772	97.694	Zn	**.957**	
4	.116	1.454	99.148	Hg	**.751**	.376
5	.043	.540	99.689	Ni	**.700**	.679
6	.017	.214	99.903	Pb		**.941**
7	.006	.076	99.979	As	-.369	**.919**
8	.002	.021	100.000	Cd	.402	**.884**

In Sukesen Creek, the first principal component, which explains 49% of the total variance, was characterized by stronger loadings for Pb (0.947), Ni (-0.904), As (0.856), Cu (0.837), and Cr (0.773) compared to other metals. In the second factor, Zn, Cd, Cu, Hg, and As were grouped together, explaining 39% of the variance (Table 5). For Yavrucak Creek, there were also two factors with eigenvalues greater than 1. In the first factor, where Pb, Ni, and Cr metals were grouped, the variance accounted for by these metals was 49%. In the second factor, Zn, Cd, Cu, Hg, and As were grouped, explaining 39% of the variance (Table 5). The lead concentration in the creek receiving agricultural wastewater in April showed similarities with that in Gölcük Creek, which was also affected by agricultural wastewater, and the lead concentrations were found to be higher compared to Başpınar and Sukesen Creek waters.

Correlation analysis

The Spearman correlation coefficient was used to determine whether there was a significant relationship between the concentration values of heavy metals (Table 6). The correlation between heavy metals provides some information about their sources and transport pathways. The identified positive values suggested that the he heavy metals examined had a common source, co-occured during transport, and exhibited similar behaviors. On the other hand, the identified negative values are indicated that these elements originated from different sources (Binici et al., 2021). According to the results of the Spearman correlation analysis ($p<0.05$), strong positive relationships were observed between Cd-Hg, Cu-Cd, and Cu-Hg (Table 6). The significant variations of these metals among the creeks (Table 3) indicated that there were similar and significant pollution sources for these heavy metals in the vicinity of the creeks.

Table 5. Total variance explaind and component matrixes for potentially toxic elements in Sukesen and Yavrucak Creek

Sukesen Creek						
Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	5.236	65.444	65.444	Pb	**.947**	
2	1.595	19.937	85.382	Ni	**-.904**	
3	.609	7.610	92.992	As	**.856**	.430
4	.278	3.472	96.463	Cu	**.837**	.522
5	.162	2.030	98.494	Cr	**.773**	.540
6	.091	1.140	99.633	Hg	.546	**.535**
7	.029	.359	99.992	Cd		**.929**
8	.001	.008	100.000	Zn		**.924**
Yavrucak Creek						
Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	3.922	49.027	49.027	Pb	**.992**	
2	3.133	39.168	88.195	Ni	**.971**	
3	.579	7.233	95.428	Cr	**.968**	
4	.193	2.407	97.835	Zn		**.980**
5	.129	1.608	99.443	Cd		**.875**
6	.036	.453	99.896	Cu	-.354	**.833**
7	.007	.084	99.980	Hg	.515	**.724**
8	.002	.020	100.000	As	.550	**.693**

Assessment of Creek Water Quality within the Scope of EQS

Under the currently effective "Turkish Surface Water Quality Regulation," priority substances and environmental quality standards have been established for surface water resources (Table 2). In Başpınar Creek, the average value (Mean-EQS) of Cr in February (2.18 µg/L) and April (2.41 µg/L) exceeded the threshold of 1.6 and the level of Cd (0.39 µg/L) in February also exceeded the average value of 0.08. In Gölcük Creek, it was found that only Cd exceeded the average value of 0.08 in February (0.20 µg/L) and April (0.31 µg/L). Similarly, in Sukesen Creek, it was observed that Cd exceeded the average value of 0.08 in February (0.18 µg/L) and April (0.10 µg/L). Based on the Max-EQS values reported in TSWQR (2016), the potentially toxic element concentrations observed in all four creeks did not exceed the limit values set for Max-EQS. However, in Başpınar Creek, the Hg concentration value in February (0.07 µg/L) was found to be equal to the Max-EQS reported for Hg.

Table 6. Pearson correlation matrix analysis for all potentially toxic elements in the studied creeks

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb								
Cr	r	-.184	-.011	.395*	.007	.051	.364*	-.115	1.000							
	p	.211	.943	.005	.962	.732	.011	.438								
Ni	r	.263	.285*	.333*	.164	.173	.436*	.068	.286*	1.000						
	p	.071	.049	.021	.266	.239	.002	.646	.049							
Cu	r	-.270	.126	.033	.100	.181	.699*	.224	.601*	.321*	1.000					
	p	.063	.395	.825	.498	.219	.000	.126	.000	.026						
Zn	r	-.525*	.158	.248	.127	.249	.442*	.374*	.661*	.143	.665*	1.000				
	p	.000	.283	.090	.389	.088	.002	.009	.000	.332	.000					
As	r	-.114	-.233	.378*	.528*	.408*	.409*	-.173	.658*	.089	.303*	.538*	1.000			
	p	.439	.111	.008	.000	.004	.004	.240	.000	.549	.036	.000				
Cd	r	-.053	-.077	-.126	.272	.492*	.913*	.364*	.365*	.387*	.750*	.539*	.308*	1.000		
	p	.719	.604	.392	.062	.000	.000	.011	.011	.007	.000	.000	.033			
Hg	r	-.060	-.162	-.149	.044	.263	.718*	.246	.594*	.219	.746*	.570*	.319*	.784*	1.000	
	p	.683	.273	.314	.769	.071	.000	.092	.000	.135	.000	.000	.027	.000		
Pb	r	.306*	-.095	.061	.176	.129	.653*	-.250	.488*	.383*	.626*	.205	.251	.645*	.623*	1.000
	p	.035	.519	.682	.231	.383	.000	.087	.000	.007	.000	.162	.085	.000	.000	

*p<0.05

Discussion

The tributaries of Mogan Lake receive domestic, agricultural, and industrial waste.. Studies have revealed that most of the heavy metals, which constitute a significant component of pollutants, originate from various anthropogenic sources. In this study, it is known that the heavy metals (Hg, Zn, Pb, Cd, Cr, Pb, Cu, As) considered are primarily accumulated in agricultural soils, and the contamination with heavy metals is also associated with anthropogenic sources such as mining, industrial activities, agricultural practices and traffic emissions (Pulatsü et al. 2014; Xiao et al., 2019).

It was stated that mining activities from anthropogenic sources caused significant pollution in heavy metal levels in wastewater and it was indicated by examining the Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) values that surface and groundwater were highly contaminated with heavy metals (Custodio et al., 2020; Moldovan et al., 2022; Pan et al., 2022). It was reported that in the Mogan Lake Basin, there were factories and facilities processing andesite stone and in earlier inspections, it was found that many facilities were discharging their wastewater directly into Sukesen Creek, without any treatment. It was also reported that high levels of heavy metals were detected in samples taken from the sludge of these facilities (Anonymous, 2013). It is believed that this activity particularly triggered the higher concentrations of Ni and As detected in Sukesen Creek compared to other creeks. The contamination of heavy metals in surface waters can have a detrimental impact on aquatic life and fishing activities, in addition to being unsuitable for drinking water and non-potable domestic and agricultural uses (Goher et al., 2014; Mollo et al., 2022; Varol and Tokatlı, 2023). For this purpose, potentially toxic element pollution indices (HPI and HEI) were calculated based on international and national standards and it was found that the index results based on national values (TSWQR, 2015) were higher compared to the international standards. However, in both cases, the index values indicated a low level of heavy metal pollution. Similar findings have been reported by Varol et al. (2021) for the Karasu River and by Kutlu and Sarıgül (2023) for the Munzur Stream. Yüksel et al. (2021), in a study investigating the effects of the garbage disposal facility built near Çavuşlu Stream (Giresun, Turkey, reported that the 1st station closest to the facility had moderate pollution, while the other stations and tap waters indicated low pollution. Based on the potential ecological risk values (PER) identified in this study, it is currently unlikely to mention a risk. Furthermore, our findings are consistent with other studies, indicating that As has thehighest contribution to low HPI and HEI values observed in our research (Tokatlı and Varol 2021; Zhang et al. 2021).

Çapar et al. (2016) stated that based on the salinity and alkalinity values they observed in water samples collected from wells and springs in some villages of Gölbaşı district (Ankara), a group of water samples were deemed unsuitable for irrigation under normal conditions. Within the scope of this study, Sukesen Creek, which runs through the Gölbaşı district, was determined to be the creek with the highest levels of potentially toxic element contamination following Başpınar Creek. According to Leventeli and Yalçın (2019), an increase in HPI values was observed in the locations between Alakır Dam and Alakır Bridge (Antalya, Turkey), indicating the influence of the dam in the upper region and agricultural activities in the lower region. In this study, it is believed that the increase in certain potentially toxic element concentrations (As, Ni, Cr) in the creeks, particularly in April, can be attributed to increased agricultural activities.

Wuhan by Zhang et al. (2021), reported that natural sources control Mn, Fe, Co, Ni, and Mo, while As was predominantly derived from the combined input of urban and agricultural activities. In this study, high levels of As were detected in Başpınar and Sukesen Creeks, where waste waters from the residential areas and agricultural activities were discharged. In this study, metals such as Cd, Hg, Zn, and Cu were detected at lower levels compared to other heavy metals. However, the high correlation values suggested the likelihood of similar anthropogenic sources. Furthermore, heavy metals such as chromium, nickel, and lead, which were identified as having a high contribution to the total variance in the PCA analysis, are associated with anthropogenic sources such as domestic-agricultural waste and motor-vehicle pollution (Wang et al., 2017; Saleem et al., 2019). The above-mentioned heavy metals align with the finding that they are the primary pollutants from both point and non-point sources, to which all the creeks are exposed.

In a study by Karaouzas et al. (2021) where potentially toxic element pollution indices were calculated in surface waters for a period covering 1999-2019 in Greece, potentially toxic element concentrations were found to be below the Environmental Quality Standards (EQS) and categorized as good quality according to surface water pollution indices. In the present study, it was determined that heavy metal concentrations in the majority of samples did not exceed the Environmental Quality Standards (EQS) for heavy metals, except for Cd, which exceeded the Mean-EQS in the 3 out of 4 creeks examined. Long-term studies monitoring EQS will enhance the accuracy of assessments regarding surface water quality.

Conclusion

The contamination of surface waters by heavy metals is widely recognized as a significant environmental issue. The transportation of domestic and agricultural waste through creeks and the uncontrolled discharge of waste into lakes occur in many parts of the world. In the Mogan Lake Basin, despite intensive urbanization, agricultural activities still exist. In this study, it was determined that the irrigation water qualities of the four significant tributaries (Başpınar, Gölcük, Sukesen, and Yavrucak) of Lake Mogan were moderately contaminated with heavy metals. However, according to the findings, it was revealed that the Başpınar Creek is more threatened compared to the other creeks. Furthermore, the effects of pollutants can vary through physicochemical interactions and this study provides only preliminary results. In this context, regular monitoring of potentially toxic element levels in creek waters by local authorities and taking necessary environmental interventions, if required, are of great importance. The findings are believed to provide a quantitative reference for future studies on the subject.

Conflict of Interest

The authors declare that they have no conflict of interest

Author Contributions

All authors took part in designing the research, collecting, and writing the manuscript. All authors took part in a part of the article.

Ethics Approval

There are no ethical issues with the publication of this manuscript.

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RESEARCH ARTICLE

The Relationships Between Blood Parameters of *Liocarcinus depurator* (Linnaeus, 1758) and Environmental Conditions in Çardak Lagoon (Çanakkale, Türkiye)

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Key words:

Liocarcinus depurator
Blood biochemistry
Environmental variables
Çardak Lagoon

Abstract: In this study, the effects of environmental variables such as temperature, salinity, pH, and oxygen on blood variables of the portinid crab, *Liocarcinus depurator*, were determined. A total of 50 crabs were collected in Çardak Lagoon in 4 different periods; February, May, July, and November 2020. Crabs were first anesthetized on ice for 10 minutes and a 500 µL blood sample from each crab was collected from the coxae of the walking legs, using a 1 mL plastic syringe. The collected hemolymphs were then centrifuged and ELISA (Enzyme-Linked ImmunoSorbent Assay) analysis was performed. The effects of seasonal variables on blood variables were evaluated using Pearson's correlation analysis. A negative relationship was found between temperature and ALB (albumin), while a negative relationship was found between saturated oxygen and GLU (glucose) and CHOL (cholesterol). The relationship between pH and CHOL was also negative.

Anahtar kelimeler:

Liocarcinus depurator
Kan biyokimyası
Çevresel değişkenler
Çardak Lagünü

Çardak Lagünü'nde (Çanakkale, Türkiye) *Liocarcinus depurator* (Linnaeus, 1758)'un Kan Parametreleri ile Çevresel Değişkenler Arasındaki İlişkisi

Öz: Bu çalışmada, portunid yengeç *Liocarcinus depurator* 'un kan değişkenleri üzerine sıcaklık, tuzluluk, pH ve doymuş oksijen gibi çevresel değişkenlerin etkileri belirlenmiştir. Çardak Lagünü'nde 4 farklı dönemde; Şubat 2020, Mayıs 2020, Temmuz 2020 ve Kasım 2020' de toplam 50 yengeç bireyi toplanmıştır. Yengeçlere ilk olarak buzda 10 dk anestezi uygulandı ve 1mL'lik plastik bir şırınga kullanılarak her bir yengeçten yürüme bacakların koksalarından 500 µL kan örnekleri alınmıştır. Toplanan hemolenfler daha sonra santrifüj edilerek ELISA (enzime bağlı immünosorbent testi) analizi yapılmıştır. Mevsimsel değişkenlerin kan değişkenleri üzerindeki etkisi Pearson korelasyon analizi kullanılarak değerlendirilmiştir. Sıcaklık ile albümin (ALB) arasında negatif bir ilişki bulunurken, doymuş oksijen ile glukoz (GLU) ve kolesterol (CHOL) arasında negatif ilişki bulunmuştur. pH ile CHOL arasındaki ilişki de negatifti.

Giriş

Yakın zamanlarda yapılan çalışmalar ile okyanus ısınması, asitlenmesi gibi çeşitli olaylar, deniz ekosistemi ve biyolojik çeşitlilik üzerinde önemli tehditler oluştuğunu göstermiştir (Gattuso vd., 2011). Lagünler deniz ve kara arasında bir bağlantı niteliğindeki deniz ve tatlı su ortamları arasındaki geçiş bölgeleridir (Healy vd., 1997). Lagüner alanlar birçok denizel tür için beslenme ve barınma alanları olmalarının yanısıra, bu özel ekotonlarda derinlik, tuzluluk, sıcaklık ve benzeri çevresel faktörler önemli değişkenlerdendir. Lagüner alanlardaki yengeçlerin fizyolojisi çevresel değişkenlerden negatif olarak etkilenmektedir (Rewitz vd., 2004). Su sıcaklığı, bulanıklık, oksijen, pH, rüzgar hızı gibi çevresel faktörler popülasyon yoğunluklarını doğrudan etkilemektedir (Ansari vd., 2003; Cyrus ve Blaber, 1992). Bu değişkenlerdeki değişimler sonucu lagünlerde türlerin adaptasyon geliştirmeleri, canlının bölgeden uzaklaşması gibi etkiler ile karşımıza çıkabilmektedir.

Dekapod krustaselerde hematolojik ve biyokimyasal değişiklikler, organizma içinde homeostatik kontrol sağlamak için çevresel faktörlere verdiği yanıt ile popülasyonların sağlığını değerlendirmek için teşhis araçları olma niteliğini kazanmışlardır (Velisek vd., 2009). Kan kimyası çalışmaları, çeşitli elektrolitler, enzimler ve hormonları içeren kanın hücresel olmayan kısmı olan serum veya plazmadaki kimyasal bileşenlerini ifade etmekte kullanılırlar (Petri vd., 2006). Kan parametrelerindeki değişiklikler, değişen fizyolojik ve enerjik gereksinimlere verilen yanıtlara bağlanmıştır (Kutz vd., 2004; Ochang vd., 2007; Adeogun, 2011).

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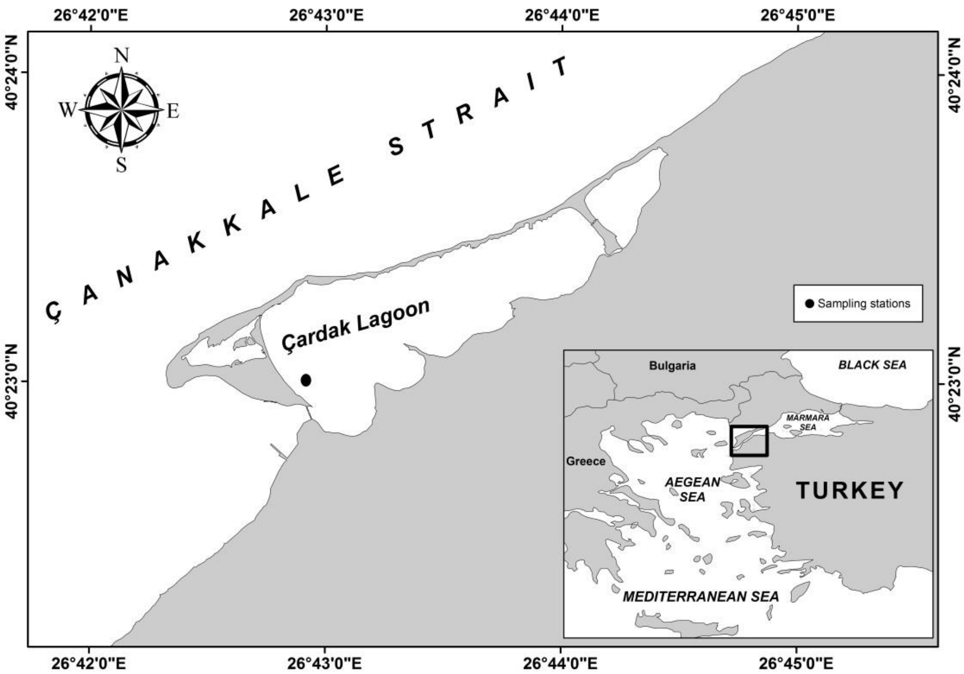
Daha önce *L. depurator* üzerine yapılan birçok çalışma, kan hücreleri ve kandaki birkaç kan değişkenindeki farklılık üzerinedir (Huntingford vd., 1995; Fragkiadakis, 2000; Bergmann ve Moore, 2001; Mattiello vd., 2004). Bu çalışma ile 10 farklı kan değişkeni ele alınmıştır. *L. depurator* üzerine yapılan çalışmalar, diğer yengeç türlerinin aksine, oldukça kısıtlı ve az sayıdadır. Özellikle ülkemizde ve dünyada, ticari trol balıkçılığında ıskarta olarak yakalanan bu yengeç konusunda yapılan çalışmaların kısıtlı olması ve şimdiye kadar yapılan araştırmaların stres, tür tayini, üreme biyolojisi vb. üzerine olması konuyla ilgili literatürdeki boşluğu göstermektedir. Bu çalışmada Çardak Lagünü'nde bulunan portunid yengeç, *L. depurator*'un çevresel değişkenlere bağlı kan biyokimyası kompozisyonu incelenmiştir. Bu kapsamda çevresel değişkenlerden sıcaklık, tuzluluk, pH ve doymuş oksijen ölçümlenmiş, kan değişkenlerinden glikoz (GLU), total protein (TP), albümin (ALB), globulin (GLB), kolesterol (CHOL), trigliserit (TG), alanin aminotransaminaz (ALT), aspartat aminotransferaz (AST), alkalen fosfataz (ALP) ve laktat dehidrogenaz (LDH) düzeyleri incelenmiştir.

Materyal ve Yöntem

Yengeç bireylerinin örneklemesi

Çardak Lagünü yapısal olarak çukurlaşmış lagün özelliğine sahip olup dar bir kanal ile Çanakkale Boğazı'na bağlantısı bulunmaktadır (Şekil 1). Yerleşim yerlerine oldukça yakın olduğundan antropojenik kaynaklı kirlenme lagünün kara tarafı kıyılarını etkilemektedir.

Çardak Lagünü sınırları içerisinde belirlenen bir noktaya bırakılan, genellikle kerevit avı için kullanılan 36 mm göz açıklığına sahip ve ağız açıklığı 38 cm olan 4 m boyundaki tek girişli kerevit pinterleri kullanılarak yengeçler yakalanmıştır. Yakalanan yengeçler taze deniz suyu bulunan kutular içerisinde laboratuvara transfer edilmiştir. Çardak Lagünü'nde 50 adet portunid yengeç, *L. depurator* yakalanmıştır (Şekil 2).



Şekil 1. Çardak Lagünü'nün Türkiye'deki konumu ve örnekleme alanını gösteren harita

Şekil 2. *L. depurator*'un dorsal ve ventral görünümü (Fotoğraf H.A. Küçükballı)

Fizikokimyasal parametrelerin ölçümü

Lagün suyuna dair ölçümler, örneklemelerin yapıldığı noktada 1-1,5 metre derinliğe salınan YSI 556 model prob ile ölçülmüştür. Yengeçlerin sudan çıkarılacağı vakit suyun fizikokimyasal parametreleri (sıcaklık, tuzluluk, pH ve doymuş oksijen) ölçülmüştür. Ölçümler tüm dönemlerde aynı yöntemle yapılmıştır.

Kan örneklerinin alınması

Laboratuvara getirilen yengeçler, buzda 10 dk anestezi edildikten sonra 1mL'lik plastik şırıngalarla ortalama her yengeçten 500 µL olacak şekilde yürüme bacaklarının koksalarından kan alınmıştır. Alınan kan örnekleri eppendorf tüplerde 1:1 oranında citrate buffer/EDTA solüsyonuyla (sodyum klorür 0,45 mol L^{-1}, glikoz 0,1 M, sodyum sitrat 30 mM, sitrik asit 26 mM, EDTA 10 mM, pH 4,6, 4°C'de saklandı) seyreltilmiştir (Smith ve Söderhäll, 1983). Ependorf tüplerine alınan hemolenfler, 4000 g devirde 10 dakika santrifüj edilmiş, hemolenfin serumu ayrıldıktan sonra 96 kuyucuklu mikro plakalara aktarılmıştır. Reaktif ve serum koyulan mikro plakalar ile ELISA testi yapılarak kan prametrelerine ait sonuçlar alınmıştır.

Veri analizleri

Çevresel ve kan değişkenleri arasındaki ilişki Pearson korelasyonuna (*r*) göre ilişkilendirilmiştir. Çalışmalar sonucu elde edilen tüm veriler MS Office Excel ile tablolaştırılmıştır. İstatistiksel analizler SPSS25 ve PAST 3 ile yapılmıştır.

Bulgular

Şubat 2020 ve Kasım 2020 tarihleri arasında, çevresel değişkenlerin (sıcaklık, tuzluluk, pH ve doymuş oksijen) *L. depurator* bireylerinin kan değişkenlerine parametrelerine etkileri incelenmiştir. Sıcaklık 7,85-22,73 °C, tuzluluk ‰22,89-24,72, pH 6,63-7,87 ve doymuş oksijen 6,52-11,52 mg L^{-1} aralıklarında tespit edilmişlerdir (Tablo 1).

Tablo 1. Lagün suyunda ölçülen çevresel değişkenlerin mevsimsel değişimleri

Çevresel Değişkenler	Örnekleme Dönemi			
	Şubat 2020	**Mayıs 2020**	**Temmuz 2020**	**Kasım 2020**
Sıcaklık (°C)	7,85	17,48	22,73	11,78
Tuzluluk (‰)	22,89	23,85	23,1	24,72
pH	7,06	7,86	7,87	6,63
Doymuş Oksijen (mg L^{-1})	11,52	10,39	7,2	6,52

L. depurator bireylerindeki kan değişkenlerinin mevsimler bazında genel ortalama değerleri kaydedilmiştir (Tablo 2). ALB (1,73±0,27 gdL^{-1}) ve ALT (11,61±1,56 U/L) en yüksek değerlere kış mevsiminde ulaşmıştır. TP (1,7±0,5 gdL^{-1}), ALB (0,03±0,01 gdL^{-1}), ALT (6,93±1,23U/L), AST (4,71±0,68 U/L) ve ALP (8,31±3U/L) değerleri ilkbahar mevsiminde en düşük seviyelerdedir. TP (4,38±0,78 gdL^{-1}), GLB (4,33±0,77 gdL^{-1}), GLU (792,84±12,25 mgL^{-1}), TG (15,82±2,29 $mgdL^{-1}$) ve LDH (60,69±3,72 U/L) en yüksek değerlere yaz mevsiminde, CHOL (19,8±3,79 $mgdL^{-1}$), AST (13,82±3,68 U/L) ve ALP (60,95±10,84 U/L) en yüksek değerlerine sonbahar aylarında ulaşmışlardır.

Kan çevresel değişkenleri arasındaki ilişkiler Pearson korelasyonuna göre analiz edilmiştir. ALB ile doymuş oksijen (r=0,64, p<0,05) arasında güçlü bir ilişki olduğu bulunmuştur. GLB ile sıcaklık arasında (r=0,33, p<0,05) orta derecede bir ilişki olduğu bulunmuştur. GLU ile sıcaklık (r=0,48, p<0,01) ve tuzluluk (r=0,35, p<0,01) arasında orta derecede bir ilişki vardır. Yine TG ile sıcaklık arasında (r=0,37, $p<0,05$) orta derecede bir ilişki olduğu görülmektedir. CHOL ile tuzluluk (r=0,66, p<0,01) arasında bir ilişki vardır. LDH ile sıcaklık (r=0,70, p<0,01) ve pH (r=0,64, p<0,01) bir ilişki olduğu görülmektedir. ALP ile tuzluluk (r=0,65, p<0,01) arasında güçlü bir ilişki vardır (Tablo 3). Sıcaklık ile ALB arasında negatif bir ilişki vardır. Doymuş oksijen ile GLU ve CHOL arasında negatif ilişki bulunmakla birlikte, yine pH ile CHOL arasında negatif bir ilişki vardır.

PCA analizi sonucunda Component 1'in modelin %52,8'ini, Component 2'nin ise modelin %34,6'sını toplamda analizin %87,4'ünün açıklandığı görülmüştür (Şekil 3). Yapılan analiz sonucunda tuzluluğun CHOL, ALP ve TP ile ilişkili olduğu görülmüştür. Mevsim değişkeninin ise TG ve GLU üzerinde etkili olduğu yapılan analizde görülmüştür.

Tablo 2. *L. depurator* bireylerindeki kan değişkenlerinin mevsimlere göre ortalama değerleri

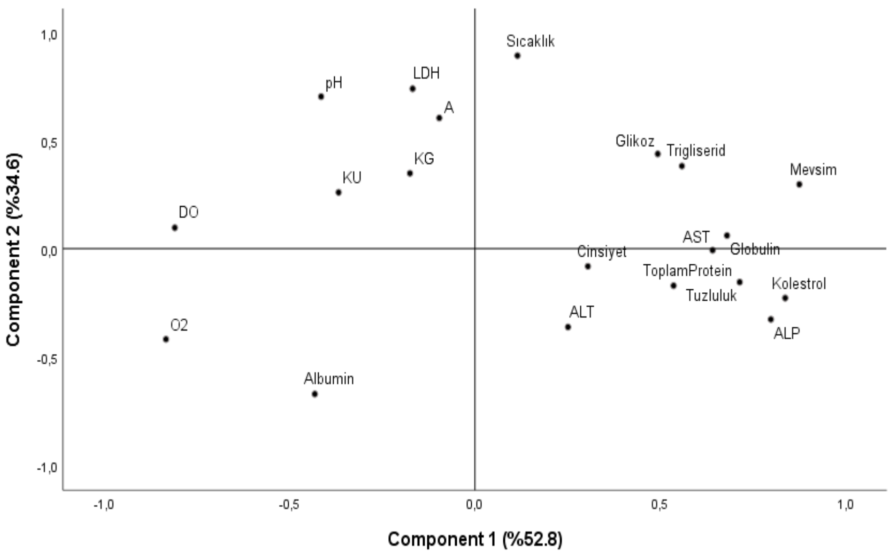
Örnekleme Periyodu	Birey sayısı	TP (gdL^{-1})	ALB (gdL^{-1})	GLB (gdL^{-1})	GLU (mgL^{-1})	TG ($mgdL^{-1}$)	CHOL ($mgdL^{-1}$)	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)
Kış 2020	12	3,02±0,56	1,73±0,27	1,28±0,34	581,49±56,34	0,3±0,08	0,32±0,09	5,46±0,95	11,61±1,56	31,12±3,08	10,75±0,85
İlkbahar 2020	9	1,7±0,5	0,03±0,01	1,67±0,5	755,46±8,74	7,38±5,73	1,66±0,42	4,71±0,68	6,93±1,23	45,53±4,26	8,31±3
Yaz 2020	14	4,38±0,78	0,05±0,01	4,33±0,77	792,84±12,25	15,82±2,29	3,13±0,61	11,91±1,85	9,89±1,14	60,69±3,72	9,26±1,04
Sonbahar 2020	15	3,75±0,62	0,11±0,02	3,64±0,61	779,2±9,5	13,82±2,5	19,8±3,79	13,82±3,68	9,22±1,74	30,8±3,46	60,95±10,84
Ortalama	-	3,38±0,34	0,47±0,12	2,91±0,34	731,3±18,43	9,97±1,63	7,19±1,63	9,64±1,33	9,57±0,76	41,9±2,54	24,95±4,66

GLU: Glikoz, TP: Total Protein, ALB: Albümin, GLB: Globulin, CHOL: Kolesterol, TG: Trigliserit, ALT: Alanin Aminotransferaz, AST: Aspartat Aminotransferaz, ALP: Alkalen fosfataz, LDH: Laktat dehidrogenaz, N= 50.

Tablo 3. Tüm mevsimlerde elde edilen bireylere ait kan ve çevresel değişkenler arasındaki korelasyonlar

	M	sD	TP	ALB	GLB	GLU	TG	CHOL	AST	ALT	LDH	ALP	Sıcaklık	Tuzluluk	pH	DO
TP	3,38	2,45	1,00	0,15	0,94**	0,17	0,28*	0,37**	0,467**	0,62**	-0,06	0,42**	0,12	-0,02	-0,05	-0,28
ALB	0,47	0,85	0,15	1,00	-0,20	-,42**	-0,38**	-0,23	-0,15	0,40**	-0,39**	-0,15	-0,60**	-0,47**	-0,24	0,64**
GLB	2,91	2,47	0,94**	-0,20	1,00	0,31*	0,41**	0,45**	0,51**	0,48**	0,07	0,46**	0,33*	0,15	0,04	-0,49**
GLU	731,30	130,38	0,17	-0,42**	0,31*	1,00	0,39**	0,24	0,24	-0,10	0,14	0,15	0,48**	0,35*	0,16	-0,55**
TG	9,97	11,53	0,28*	-0,38**	0,41**	0,38**	1,00	0,42**	0,43**	0,14	0,11	0,46**	0,37**	0,23	0,07	-0,51**
CHOL	7,19	11,57	0,37**	-0,23	0,45**	0,24	0,4**	1,00	0,71**	0,32*	-0,38**	0,88**	-0,18	0,66**	-0,54**	-0,53**
AST	9,64	9,45	0,47**	-0,15	0,51**	0,24	0,43**	0,70**	1,00	0,45**	-0,10	0,54**	0,11	0,22	-0,14	-0,41**
ALT	9,57	5,39	0,62**	0,40**	0,48**	-0,10	0,14	0,31*	0,45**	1,00	-0,16	0,35*	-0,12	-0,16	-0,09	0,06
LDH	41,90	18,01	-0,06	-0,389**	0,07	0,14	0,11	-0,38**	-0,10	-0,16	1,00	-0,49**	0,7**	-0,32*	0,64**	-0,18
ALP	24,95	33,02	0,42**	-0,15	0,46**	0,15	0,46**	0,88**	0,54**	0,35*	-0,49**	1,00	-0,28	0,65**	-0,60**	-0,46**
Sıcaklık (ºC)	14,93	5,82	0,12	-0,60**	0,33*	0,48**	0,37**	-0,18	0,11	-0,12	0,70**	-0,28	1,00	-0,19	0,81**	-0,43**
Tuzluluk (‰)	23,67	0,77	-0,02	-0,47**	0,15	0,35*	0,23	0,66**	0,22	-0,16	-0,32*	0,65**	-0,19	1,00	-0,58**	-0,58**
pH	7,30	0,55	-0,05	-0,24	0,04	0,16	0,07	-0,54**	-0,14	-0,09	0,64**	-0,60**	0,81**	-0,58**	1,00	0,18
DO	8,61	2,14	-0,28	0,64**	-0,49**	-0,55**	-0,51**	-0,53**	-0,41**	0,06	-0,18	-0,46**	-0,43**	-0,58**	0,18	1,00

**Korelasyon istatistiksel açıdan önemlidir (0,01) $p < 0,01$, *Korelasyon istatistiksel açıdan önemlidir (0,05) $p<0,05$, N=50



Şekil 3. Çardak Lagünü çevresel değişkenlerinin *L. depurator* bireylerinin kan değişkenleri için temel bileşen analizi

Tartışma

Ülkemizde ve dünyada, ticari trol balıkçılığında ıskarta olarak bilinen *L. depurator* üzerine yapılan çalışmaların az sayıda ve bunların stresin tür davranışına ve kan biyokimyasına etkisi, tür tayini, üreme dönemleri vb. üzerinedir. Çevresel değişkenlerin türün kan biyokimyası üzerine etkileri detaylı olarak çalışılmamıştır. Burada *L. depurator* bireylerinin kan biyokimyası değişkenlerinin çevresel değişkenlerle olan ilişkisi değerlendirilmiştir.

Bu çalışma sonucunda, doymuş oksijen ile ALB arasında pozitif ve güçlü, GLU ve CHOL arasında negatif ilişki kaydedilmiştir. Önceki yıllarda, Adeogun vd. (2015), Lagos Lagünü'nde GLB ile doymuş oksijen arasında negatif bir korelasyon bulmuştur. Doymuş oksijenin etkileri üzerine çalışmalar genelde hepatopankreas, solungaç vb. dokulardan alınan örnekler (Li vd., 2016), beslenme fizyolojisi (Paschke vd., 2010) gibi konularda yapılmıştır. Düşük çözünmüş oksijene (hipoksi), yüksek karbondioksite (hiperkapni) maruz kalma, deniz organizmalarında birçok davranışsal, fizyolojik, biyokimyasal ve genetik tepkileri tetiklediği bilinmektedir (Burnett ve Stickle, 2001). Çalışmada pH ile LDH arasında pozitif ve anlamlı, CHOL arasında negatif anlamda bir ilişki vardır. Dekapod krustaselerde pH'ın beslenme davranışlarını, metabolizmayı, fizyolojiyi ve olgunlaşma sürecini etkilelediği (Muthu ve Laxminarayana, 1977), yüksek değerlerin ve pH'daki ani dalgalanmaların kabuklu larvalarında hastalık salgınlarının yanısıra strese neden olduğu da bilinmektedir (Cheng ve Chen, 1998; Thi Hoang Oanh vd., 2023).

Bu çalışmada sıcaklık ile GLB, GLU, LDH ve TG arasında zayıf ve orta düzeyde ilişkiler kaydedilmiştir. Ayrıca sıcaklık ile ALB arasında orta düzeyde negatif bir ilişki bulunmuştur. PCA analizinde mevsim değişkeninin TG ve GLU üzerinde etkili olduğu görülmüştür (Şekil 3). Adeogun vd. (2015), *Callinectes amnicola* üzerine Lagoos ve Epe Lagünlerinde yaptıkları çalışmada, bu çalışmada bulunan *L. depurator* bireylerindeki TP değerine oranla, yaz aylarında daha düşük TP değerleri bulmuşlardır. Yaz mevsiminde ALB değeri Lagoos Lagünü'nden elde edilen bireylerde daha yüksek iken, Çardak Lagünü'nden elde edilen bireylerin hemolenf GLB değerleri daha yüksektir. Çardak Lagünü'nde en düşük hemolenf TP değeri ilkbaharda ve en yüksek değer yazın ölçülmştür. Sonbahar ve kış mevsimlerinde ise birbirine yakın değerdedir. Matozzo vd. (2011), yüksek sıcaklıkta (30°C) düşük sıcaklıklara göre *Carcinus aestuarii*'de hemolenf TP seviyelerinde düşüş kaydetmiştir. Dekapod krustaselerde birincil enerji kaynağı proteinler olduğundan *C. aestuarii*'de yüksek sıcaklık stresi sırasında proteinlerin acil bir enerji kaynağı olarak kullandığı bilinmekle birlikte, daha sonra kullanılmak üzere hemolenfte glikoz biriktirdiği söylenebilmektedir (Helland vd., 2003; Sanchez-Paz vd., 2007).

Çardak Lagünü'nde *L. depurator* bireylerinde sıcaklık artışına bağlı olarak hemolimf GLU değerlerinde artış gözlenmiştir. Tersine, Matozzo vd. (2011), diğer bir portunid yengeç, *C. aestuarii*'de aşırı sıcaklıkların hemolimf GLU konsantrasyonlarında önemli değişikliklere yol açmadığını bildirmişlerdir. Sıcaklık artışlarının *Uca minax*'ta hemolenf GLU seviyelerinde bir düşüş (Dean ve Vernberg, 1965), *Panulirus interruptus*'ta (Ocampo vd., 2003) hemolenf GLU seviyelerinde artış bildirilmiştir. Vinagre vd. (2007), *Ocypode quadrata*'da kış (19.23±0.28 °C) ve yaz mevsimlerinde (22.90±2.0°C) yüksek hemolimf GLU değerleri kaydetmişlerdir. Giomi vd. (2008), yaz aylarında aşırı egzersiz ve düşük oksijenin etkileri sonucunda hemolenf GLU konsantrasyonlarında önemli artışlar gözlemlemiştir. Hemolimf GLU seviyelerindeki artışların, stres altındaki yengeçlerde hepatopankreastan ve/veya kaslardan glikozun mobilizasyonuna bağlı olabileceği öne sürülmektedir (Powell ve Rowley, 2008).

Bu çalışmada sıcaklık ile LDH ve TG arasında bir ilişki olduğu görülmektedir. Vinagre vd. (2007), *O. quadrata*'da ilkbaharda hemolenf TG düzeyleri ve CHOL seviyelerinde önemli bir azalma olduğunu bildirmiştir. Kışın ise toplam CHOL seviyelerinde (p<0,05) önemli bir artış bulmuştur. *O. quadrata*'da ilkbaharda hemolimf toplam CHOLve TG seviyeleri önemli ölçüde azalmasının nedeni olarak; lipid rezervlerinin spermatogenez süreci ve gonad sayısı ile ilişkili olduğu bilinmektedir (Komatsu ve Ando, 1992).

Yahia ve Selmi (2019), yaz aylarında kirlenmiş bir bölgeden gelen *C. aestuarii* yengecinde, hemolenf LDH aktivite seviyesinde artış gözlemlemiş, ALP, AST ve ALT seviyelerinin oldukça yüksek olduğunu bildirmişlerdir.

Tuzluluk ile CHOL, GLU ve ALP arasında güçlü bir ilişki bulunmuştur. Yapılan PCA analizi sonucunda tuzluluğun CHOL, ALP ve TP ile ilişkili olduğu görülmüştür. Adeogun vd. (2015), Epe Lagünü yengeçlerinde TP ve ALB'ni daha yüksek rapor etmişken, GLB'nin Lagos Lagünü'ndekilerden daha yüksek olduğunu bildirilmiştir. Lagos Lagünü'nde tuzluluk ile GLB arasında güçlü bir pozitif korelasyon kaydetmişlerdir. Çardak Lagünü'nde ise kışın ‰22,89'den sonbaharda ‰24,72'e yükselen tuzluluğa karşın, kışın 3,02±0,56 gdL^{-1} olan TP sonbaharda 3,75±0,62gdL^{-1}' e yükselmiştir ve en yüksek TP değeri (4,38±0,78) ise tuzluluğun ‰23,1 olduğu yaz mevsiminde ve bulunmuştur. Çardak Lagünü kış periyodu TG ve CHOL değerleri sırasıyla 0,3±0,08 $mgdL^{-1}$ ve 0,32±0,09 $mgdL^{-1}$ olarak ölçülmüştür. En yüksek TG değeri (15,82±2,29 $mgdL^{-1)}$ ise yaz mevsiminde ve en yüksek CHOL değeri (19,8±3,79 $mgdL^{-1}$)'de sonbaharda kaydedilmiştir. Kabuklularda çevresel tuzluluk ve hemolenf parametreleri arasında doğrudan bir ilişki olduğu bilinmektedir (Santos ve Nery, 1987; Spaargaren ve Haefner, 1987; Da Silava ve Kucharski, 1992). Deniz suyu tuzluluğunun yengeç türlerinde lipit sınıfı bileşimi ve sentezi üzerindeki etkisi önceki çalışmalarda raporlanmıştır (Chapelle vd., 1982; Chapelle ve Zwingelstein, 1984; Zwingelstein vd., 1998).

Sonuç

Bu çalışmada, *L. depurator*'un kan değişkenleri ile mevsimsel çevresel değişkenler (sıcaklık, tuzluluk, pH ve doymuş oksijen) arasındaki ilişkiler araştırılmıştır. Önemli bir çevresel değişken olan sıcaklık ile LDH, GLU, TG, GLB arasında pozitif, ALB ile negatif bir ilişki bulunmuştur. Ayrıca tuzluluk ile ALP arasında güçlü bir pozitif ilişki vardır. Doymuş oksijen ile ALB ve CHOL arasında güçlü ve negatif ilişki bulunmuşken, doymuş oksijen ile GLU arasında negatif ilişki kaydedilmiştir. Ayrıca pH ile CHOL arasında negatif anlam vardır. Sonuç olarak Çardak Lagünü'nde *L. depurator'*un serum biyokimyası için oluşturulan bu çalışmanın sonuçları bu türe ve diğer portunid türlerine özgü temel kan değişkeni değerlerinin farklı çevresel değişkenlere verdiği tepkileri karşılaştırmak açısından bir referans olabilir.

Teşekkür

Bu çalışma "Çardak Lagününde Dağılım Gösteren Portunid Yengeç, *Liocarcinus depurator* (Linnaeus, 1758)'un Çevresel Değişkenlere Bağlı Kan Biyokimyası Kompozisyonu" adlı Doktora tezinin bir parçasıdır. Yazar, bu çalışmada yengeç bireylerinin toplanmasındaki yardımları dolayısıyla Çardak Lagünü balıkçılarından Sayın Hasan Şenses TÜLÜMEN'e, laboratuvar analizlerindeki destekleri için Sayın Sevdan YILMAZ ve Sayın Seçil ACAR'a teşekkür eder.

Çıkar Çatışması

Yazar herhangi bir çıkar çatışması olmadığını beyan eder.

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RESEARCH ARTICLE

Evaluation of Illegal, Unreported and Unregulated (IUU) Trawl Infringements Committed by Turkish Fishing Vessels

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Trawl fishery
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Trawl violation

Abstract: This study analyses a highly damaging and prevalent fishing practice of trawl infringements, which is one of the crucial components of IUU fishing in Turkish waters. The raw data gathered from the Turkish Coast Guard Command (TURCG), covered 2012 – 2014 period. Data covered a total of 1040 trawl infringements, with considerable differences in trawling violations among the seas surrounding Türkiye; the Black Sea, the Sea of Marmara, the Aegean Sea, and the Mediterranean Sea. With respect to trawling infringements, the Sea of Marmara (37%) and Istanbul Strait (15%) were the hot points. The most common infringement type was trawling in a closed area (43%) and when combined with the rate of infringements on trawling during the closed season (10%), these violations accounted for 53% of spatio-temporal infringements. Regarding illegal trawling by vessel type, trawl vessels had a 44% share and non-trawlers had a share of 56%, of which included infringements by other type of fishing vessels (46%) and non-fishing vessels (10%). It was also found that infringement by vessels smaller than 12 meters in length had a share of 46%. These vessels (known as "**şebeke**" in Turkish), trawl illegally using small types of nets and gears in coastal waters without giving any obvious indication of fishing activity. The results of this study will be useful to policy-makers, practitioners and scientists to successfully combat trawling infringements by providing information on distribution, intensity and methods of trawl violations.

Anahtar kelimeler:

Trol balıkçılığı
Türk Denizleri
Yasadışı avcılık
Trol ihlalleri

Türk Balıkçı Gemileri Tarafından İşlenen Yasa Dışı, Kayıt Dışı, Kural Dışı (YKK) Trol İhlallerinin Değerlendirilmesi

Öz: Bu çalışma, Türkiye Denizlerinde YKK (Yasa Dışı, Kayıt Dışı, Kural Dışı) balıkçılığın en zararlı ve yaygın bileşenlerinden biri olan trol ihlallerini analiz etmektedir. Çalışmaya ait ham veriler, Sahil Güvenlik Komutanlığı'ndan 2012-2014 döneminde temin edilmiştir. Toplam 1040 trol ihlaline ait veriler incelendiğinde, Türkiye'yi çevreleyen Karadeniz, Marmara Denizi, Ege Denizi ve Akdeniz arasında trol ihlalleri bakımından dikkate değer farklılıklar olduğu tespit edilmiştir. Trol ihlalleri bakımından Marmara Denizi (%37) ve İstanbul Boğazı (%15) kendi ölçeklerinde en sıcak noktalar olarak bulunmuştur. En yaygın ihlal türü %43 ile yasak sahada trol çekmek olup, yasak zamanda trol çekme ihlalleri ilave edildiğinde zamansal-mekansal ihlallerin %53'e ulaştığı gözlemlenmiştir. Yasadışı trol avcılığı gemi türü bakımından incelendiğinde ise trol gemileri %44'lük bir paya sahipken, diğer balıkçı gemileri (%46) ve balıkçılık ruhsatına sahip olmayan gemilerin (% 10) oluşturduğu trol ruhsatına sahip olmayan gemiler %56 paya sahip olmuştur. Ayrıca boyu 12 metreden küçük olan gemilerin ihlaller içinde % 46 gibi önemli bir paya sahip olduğu tespit edilmiştir. Bu gemiler (Türkçede "şebeke" olarak adlandırılmaktadır), dışarıya hiçbir emare vermeden küçük boyuttaki ağlar ve takımlarla kıyı sularında gizlice trol çekebilmektedir. Bu çalışmanın sonuçları, trol ihlallerinin dağılımı, yoğunluğu ve metotları hakkında bilgi vererek, trol ihlalleriyle etkin mücadele kapsamında uygulayıcılara, politika yapıcılara ve bilim insanlarına faydalı olacaktır.

Introduction

The widely accepted and adopted definition of IUU fishing has three distinct dimensions: illegal, unreported, and unregulated activities. Illegal fishing is conducted by vessels of countries that are party to a fisheries organization. Unregulated fishing is normally conducted by vessels flying the flag of countries that are not parties of or participants in relevant fisheries organizations implementing such activities as fishing without a license, fishing in a closed area or marine protected area (MPA), fishing with prohibited gear, fishing over a quota, or the fishing of prohibited species. Unreported fishing refers to fishing activities, which have not been reported, or have

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been misreported, to the relevant national authority, in contravention of national laws and regulations, or similar lack of reporting or misreporting to regional fisheries management organizations (FAO, 2001; Sumaila et al., 2006; Hosch, 2006; Polacheck, 2012; Phelps Bondaroff et al., 2015). In this study, the criteria mentioned above will be dealt with to assess situations of trawl infringements in Turkish Waters due to IUU fishing.

Türkiye is by far the largest producer accounting for 27.5% of the total fishing in the Black Sea, with 81.6% of capture fisheries coming from the Black Sea, and has the greatest fishing capacity (17.4%) in the GFCM area of application (FAO, 2022). With regard to IUU fishing, Türkiye has an index of 2.34, which is close to the world average (2.29), and ranks 54th place among 152 countries. Türkiye also ranks 8th in the GFCM area among 28 countries (Macfadyen et al., 2019). Data suggests that Türkiye does not make significant contribution to the IUU fishing, when compared to its proportionately great fishing capacity. Nevertheless, Türkiye's marine capture production decreased by 30% in the last decade (GDFA 2022). It is strongly considered that Türkiye's large-scale fishery could be responsible for this decline. As one of the main gear types of large-scale fisheries, trawl fishery (bottom and mid-water trawls) constitutes 32% of total marine capture production (Anonymous 2018) and represents 5.7% of the fishing fleet with 786 trawl-licensed vessels (TURKSTAT, 2020). Therefore, trawling infringements in Turkish waters have substantial significance to combat IUU fishing with respect to developing solutions and updating regulations (GFCM, 2017; EU, 2017).

The present study has two major goals; i) the analysis of infringements by fishing vessels in Turkish Waters with or without a bottom or mid-water trawl permit as the primary or secondary fishing gear (LOA ≥12 m) (6 m < LOA <12 m), ii) providing information and giving recommendations to fisheries policymakers, and scientists in order to combat trawl infringements.

Material and Methods

Data for a total of 29 303 fishery infringements were collected from the Regional Commands (n: 10 079) and Central Database (n: 19 224) of TURCG. The raw data sets which include "all fisheries infringements", "multiple duplications " and "separate records for each person involved in the same infringement incidence" were subjected to a detailed examination process. Two worksheets (1: Fleet segments based on gear type and length classes, Table 1 and 2: Type of infringements, Table 2) were prepared by analysing 1040 incidences within the scope of this study using the method described below:

Fishing vessels and fleet segments

The vessels that committed the infringements were classified according to their equipment, commercial fishing or commercial trawling licenses, and length segments modified from Appendix B (GFCM, 2018) (Table 1).

Place and time data

Location data were based on the sea, province, district and geographical positioning, and time data were based on the year and month in which the infringements occurred.

Types of infringement

Infringement types were examined in 3 main groups (illegal, unreported, and illegal beyond the EEZ) and 12 subgroups and have been detailed and presented in Table 2. Although rarely occurred, each infringement of the vessel that was found to have been committed more than one infringement at the same time was considered a separate incidence.

Fine data

In this section, administrative fines imposed on vessels were assessed based on infringement incidences. That is, fines imposed on more than one person on board in the same infringement were collected and the total amount of fines for the infringement was used. The fines imposed in Turkish Liras were converted to USD by using the parity of 1.80 TL = 1 USD.

Confiscation data

One of the legal actions that were imposed on the vessels for violating the fisheries legislation was the seizure of catch, gear or vessel.

Applicable legislation

In this study, the legal basis of the reported infringements included: *i*) Fisheries Law No. 1380 (Anonymous, 1971) *ii*) Fisheries Regulation No. 22223 (Anonymous, 1995), and *iii*) Communiqué 2012/65 no 3/1 regulating commercial fishing (Anonymous, 2012). Due to protection of personal information, no data were given on the identities of the fishermen and vessels that violated the national fisheries regulations as well as the coast guard boats that had reported the incidences.

Results

In total, 1040 trawl infringements were reported during three years from 2012 to 2014 in the Black Sea, the Sea of Marmara, the Aegean Sea, and the Mediterranean Sea and were evaluated under six different titles.

Place and time

The shares of 1040 trawl infringements according to the seas over a period of three years were 424, 350 and 266, respectively (Figure 1). In addition, 36.6% of these infringements took place in the Sea of Marmara (including Istanbul and Çanakkale Straits), where all kinds of trawling were prohibited throughout the year.

30.3% of infringements took place in the Aegean Sea, along with 21.8% in the Black Sea and 11.3% in the Mediterranean Sea (Figure 1).

Table 1. The codes of reported fleet segments according to gear type and length classes (modified from GFCM (2018)). (LL: Longlines, GEN: Gillnets and entangling nets, GTR: Trammel nets, GNC: Encircling gillnets, DRB: Boat dredges)

		Fleet segments						
		Vessel groups			Gear code	Length classes (LOA)		
						6<12 m	12-24 m	>24 m
Commercial fishing licensed (CFL)		1	$\text{Polyvalent}_{Small}$ (P_S)	: The small-scale vessels that use passive or active gears	LL, GEN, GTR, GNC, DRB	P_S-01	P_S-02	P_S-03
		2	Seiner (S)		PSS (Purse seines)		S-02	S-03
	Trawl fishing	3	Trawler (T)		TB (Bottom trawl)		T-02	T-03
					PTM (Mid-water pair trawl)		T-04	T-05
		4	Polyvalent_{Big} (P_B)	: The vessels that trawl and/or purse seine fishing licensed using a secondary or a tertiary gear.	TB/PSS		P_B-02	P_B-03
					TB/PTM		P_B-04	P_B-05
					PTM/PSS		P_B-06	P_B-07
					TB/PTM/PSS			
							P_B-08	P_B-09
No CFL		5	Other	Y	: Carrier vessels	Y-01	Y-02	Y-03
				O	: Diving, service, shipping, pleasure, excursion boats etc.	O-01	O-02	O-03
				Prv	: Private boats	Prv -01	Prv -02	Prv-03

Table 2. Type of infringements

Infringements			**Description of the infringements**
Main group	**Codes**	**Sub-groups**	
Illegal	1.1	Fishing in a closed area	**:** Fishing in a closed area, regardless of whether the vessel has the appropriate license.
	1.2	Fishing during a closed season	**:** Fishing in a closed season, regardless of whether the vessel has the appropriate license.
	1.3	Fishing beyond a closed depth	**:** Using a mid-water pair trawl in shallow waters less than 24 m, although it has the appropriate license.
	2	Possessing improperly stowed trawling equipment on the deck when transiting a closed area or during seasonal closure	
	3	Inappropriate gear features	**:** Minimum mesh size, mesh shape, etc...
	4.1	Lack of Fisheries license (Vessels)	
	4.2	Lack of Fisheries license (Fisherman)	
	4.3	Lack of License plate of the Vessels	
	4.4	Lack of Fishing permit for the Mid-water pair trawl fishing	
	5	Minimum landing sizes	
Unreported	6	No logbook or no product registration	
Illegal (Beyond EEZ)	7	Illegal Fishing in Foreign States Exclusive Economic Zones (EEZ)	

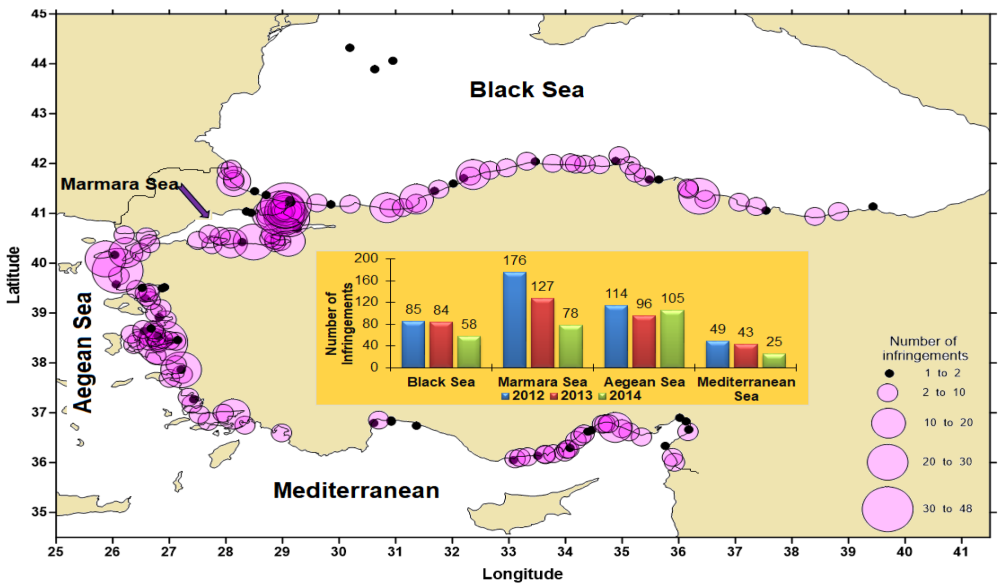

Figure 1. The distribution of trawl infringements in Turkish Waters (2012-2014)

Provinces with high infringement rates were Istanbul (27%), Izmir (14%), Çanakkale (10%), Mersin (7%), Balıkesir (5%), Samsun (5%), and Bursa (4%). Lower infregement rates were observed in Artvin, Rize, Trabzon with a near-zero rate and Ordu, Giresun, Kocaeli, Düzce, Edirne, and Antalya below 1%. Although the violator's vessels were registered in 59 different ports, 23% of the infringements were committed by the vessels registered in Istanbul, 14% were from Bandırma and 11% were from İzmir ports. Istanbul Strait is the primary hotspot with respect to violated areas in the Sea of Marmara (41%) and in all of the Turkish Waters (15%). The second hotspot in the Sea of Marmara was the Adalar-Kadıköy region (12.6%) near the Istanbul Strait. Except for the Eastern Black Sea, which is closed to trawling, trawl infringements were encountered on all other coasts of the Black Sea; Samsun (22%) in the Central Black Sea and Kırklareli (13%) in the Western Black Sea were determined as hotspots in the Turkish Coasts of the Black Sea. Izmir Bay (34%) and the vicinity of Çanakkale Strait (26%) were two significant violated areas on the Turkish coasts of the Aegean Sea. Mersin Bay, where 63% of infringements on the Turkish Coasts of the Mediterranean Sea occurred, was another hot spot. It seems that trawl infringements occurred mostly in January (17%), followed by February (13%) and March (11%). With respect to seasons, most were determined in the winter (41%) and autumn (28%). While the proportion of the trawl infringements was 10% during the closed season, which was between April 15th and September 15th, cases related to engaging in illegal trawling activity had a 22% share, consisting of 80% by non-trawlers in the same period.

Fishing vessels and fleet segments

The LOA of the violator's vessels varied between 6.30 and 40.85 m with an average of 15.1 ±5.8 m. The lowest average length was in the Sea of Marmara (12.8 ±5.3 m) and the largest in the Black Sea (18.1 ±5.7 m). While 57% of all infringements were committed by the vessels with a commercial trawl license (T and PB), small vessels and unlicensed vessels (LOA < 12 m; PS-01, Y-01, O-01, and Prv-01) were responsible for 36% of the infringements.

Although the rate of infringements by vessels without a trawl license and are smaller than 12 m was 56% in the Sea of Marmara, it was only 17% in the Black Sea. The fleet segments with the highest infringement rates were T-01 (25%), P_S-01 (20.6%), and P_B-02 (16.6%), respectively (Table 3, Figure 2).

Infringements

The vast majority of infringements determined was "fishing in a closed area" and had a share of 42.7% of total infringements. Other reasons for infringements were "possessing improperly stowed trawling equipment on the deck when transiting a closed area or during seasonal closure" (15.3%), "lack of fishing license" (11.4%), "trawling during closed season" (10%), "unreported catch" (8%), "minimum landing size" (7%), and "use of prohibited fishing gear/equipment" (2%) (Figure 3e). In Polyvalent (P_S) (Figure 3a) and Other (Y, O, and Prv) vessels (Figure 3d) that did not have commercial trawl fishing licenses, 97% and 93% of infringements consisted of these three infringements, respectively. Spatio-temporal infringement's share was determined as 52.7% and the infringements related to gear were found to be 17,3% in total.

Although fishing in a closed area was the most common infringement (33.4%) for trawlers (T), these vessels also committed 10 other types of infringements (Figure 3b). "Employing unlicensed fishermen" and "unreported catch" were other common infringements. The three most common infringements in Polyvalent (P_B) vessels with a commercial trawl fishing license were identical to those of the trawlers group and had a share of 70% (Figure 3c).

Table 3. The distribution of the infringements according to seas, vessel groups, and length segments (see descriptions of vessel groups and gear codes in Table 1)

Vessel groups	Gear code	Sea of Marmara			Black Sea			Aegean Sea			Mediterranean			All Seas		
		LOA (m)														
		6-<12	12-24	≥24	6- <12	12-24	≥24	6- <12	12-24	≥24	6- <12	12-24	≥24	6- <12	12-24	≥24
	P_S	94	13		23	1		83	9		14	1	-	214	24	
	S		1			1			1			1	-		4	
	T		37	14		43	3		103	14		77	2		260	33
	TB/PS		59	7		62	7		42	10		10	4		173	28
P_B	TB/PTM		1	2		45	19		4	2		2			52	23
	PTM/PS		1				1								1	1
	TB/PTM/PS		3	1		14	2		1	1					18	4
	Y	93	21		1	4		2	5		-			96	30	
Oth.	O	17	4			1		8	2		4			29	7	
	Pr	11	2					24	4		2			37	6	
Σ=		215	142	24	24	171	32	117	171	27	20	92	6	376	575	89
			381			227			315			117			1040	

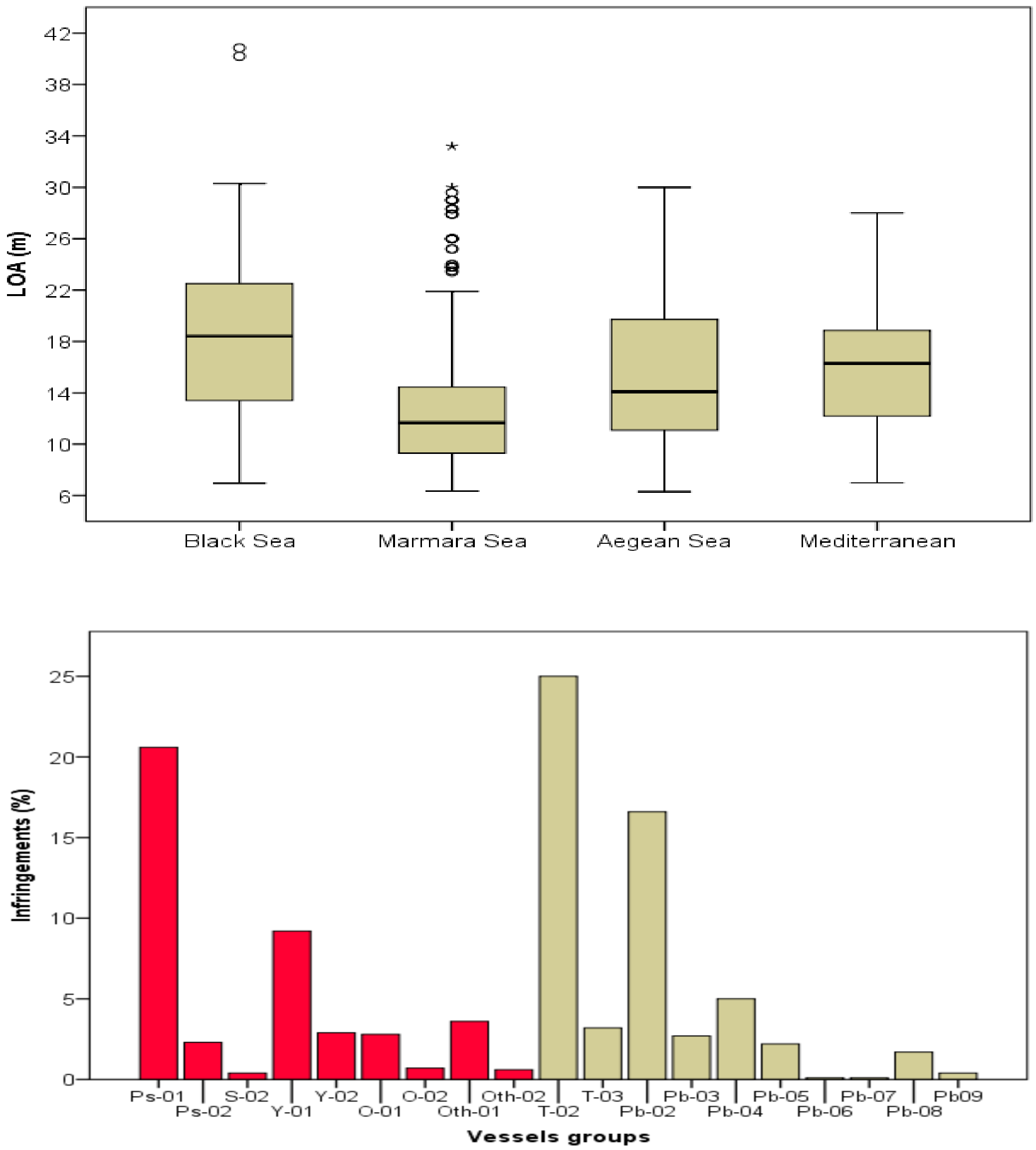

Figure 2. a: Total length (LOA) distribution of vessels in four different seas (n), b**:** infringement distributions (%) according to vessel segments (Red bars: Not licensed for trawl fishing)

Mid-water trawl infringements had a share of 3.5% of all infringements committed by mid-water trawlers (PTM) and consisted of "fishing in shallower waters" (39%), "documental infringement" (33%), and "minimum landing size" (28%).

Fines

During the study period, 96.1% of the total amount of fines (5.97 million USD) for IUU trawl fishing was issued due to illegal fishing infringements, whereas 85.5% of the fines were issued for "fishing in a closed area and fishing during a closed season". Nearly half of the fines (46.4%) were issued to vessels without a trawling licence that were engaged in trawling activities. The highest total fines were issued on the groups of $\mathbf{P_B}$ (30.2%) and vessels in the other segments (26.1%), without a commercial fishing license.

Confiscation

In addition to fines issued to fishing vessels, confiscation was used as a means of penalty. The proportion of seized vessels with a commercial trawl license was only 17%. Interestingly, 156 of 157 seized boats were in the Sea of Marmara and the remaining one was in the Black Sea. With respect to vessel size, 105 of them were 6-12 m, 46 were 12-24 m, and 6 were larger than 24 m. (Fig. 4)

i). Of the 238 infringements committed by the P_S group vessels, 51 were imposed as seizure of fishing vessel, 21 were imposed as seizure of the trawl net, and one infringement was imposed as seizure of both the vessel and trawl net; ii) Out of 293 infringements committed by the vessels in the Trawler group, 8 were imposed as seizure of the vessels, 68 were imposed as seizure of the trawl net and 2 of the infringements were imposed as seizure of both the vessel and trawl net; iii) Out of the 300 infringements committed by the vessels with P_B license 16 were imposed as seizure of the vessels and 35 were imposed as seizure of the trawl net; iv) Out of 205 infringements committed by vessels in the Other group, 74 were issued as seizure of the vessel, 19 were issued as seizure of the trawl net and 5 infringements were imposed as confiscation of both the vessel and trawl net. With respect to species composition of the 16.4 tons of catch that was seized, the majority of the pelagic species were horse mackerel and bluefish whereas the majority of the benthic species were whiting and red mullets (Fig. 5).

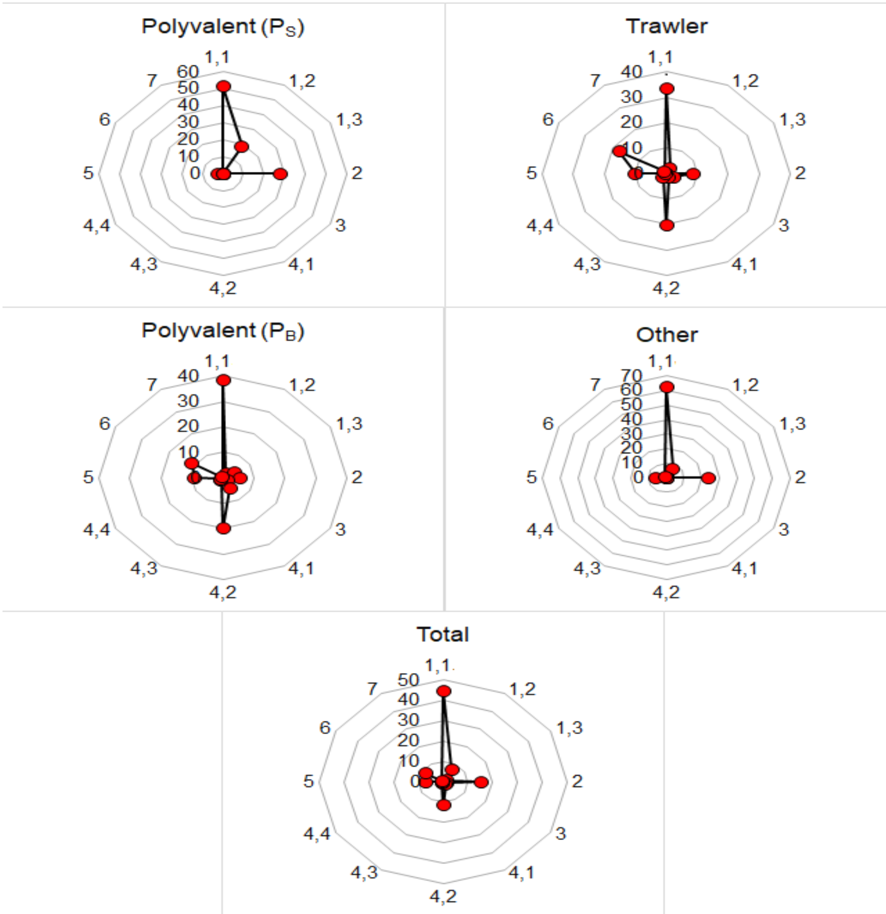

Figure 3. The distribution (%) of trawl infringements according to vessel segments (**a**: Polyvalent, P_s; **b**: Trawler, T; **c**: Polyvalent, P_B; **d**: Other; **e**: Total)

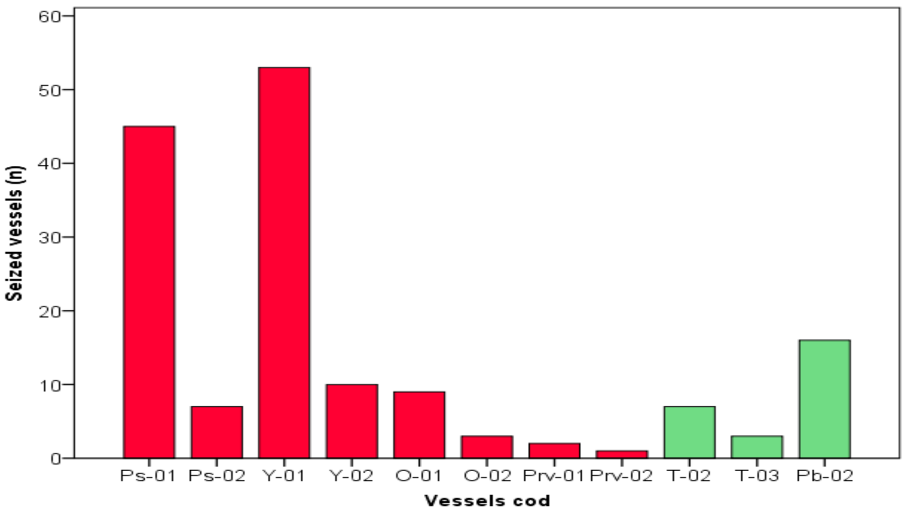

Figure 4. Seized vessel distribution (n) according to the vessel segments (red bars: Not licensed for trawl fishing)

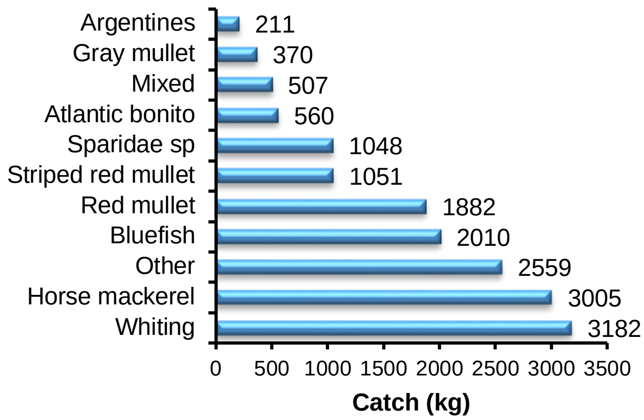

Figure 5. Confiscated species and quantities (kg)

Fisheries in the foreign state's exclusive economic zones (EEZ)

Among 19 cases reported in foreign EEZ, fishing infringements committed by three vessels were in the T-02 segment. One in each Pb-02, Y-02, and Oth-02 segment in the Ukrainian and Romanian Territorial Waters was included in the Black Sea in calculations by trawl vessels.

Discussion

The findings of this study indicated that illegal and unreported trawl fishing is prevalent in Turkish waters. Trawling infringements, which were examined in detail, had different characteristics in different regions of Turkish waters. Therefore, precautions to combat trawling infringements should be considered based on the geographic and socio-economic characteristics of each region as described below.

The Black Sea

In the Black Sea, trawling infringements were highest in Samsun followed by Kırklareli. Tanış (2013) studied the Black Sea coasts of Türkiye from Sakarya to the east during the period of September 2008-August 2012. According to this, trawlers committed 238 infringements (60 per year) which were mostly spatial (55%), documental (21%), and temporal (12%). In addition, trawling infringements were highest in Samsun (29%), followed by Kastamonu (17%), Bartın (13%) and Ordu (12%), and reached to near-zero levels towards the east such as in Trabzon, Rize and Artvin provinces. In the present study, 151 infringements by trawlers and 25 cases by non-trawlers were committed. Trawling infringements were mostly spatial (57%), documental (26%), and minimum landing size (14%). The regions with high rates of infringements were Samsun (29%), Bartın (25%), Kastamonu (23%), and Sinop (19%) respectively. Juxtaposing that study with ours, there seems to be a decrease (17%) in trawler infringements during 2012-2014. Infringements were concentrated in the Central Black Sea (Samsun, Kastamonu, and Bartın), with almost no cases in the eastern region (Trabzon, Rize, and Artvin). Besides, a substantial decrease in Ordu and Giresun was also noticed. Ayaz and Mazlum (2019) examined the fishery violations at the Black Sea coasts of Türkiye from Giresun to the east during the period of 2009-2014. A total of 11 illegal trawling (around 2 per year) were reported in this region, mostly observed in Giresun (80%) in May and September (80%). Our results in the same region during 2012-2014 were similar to those reported by Ayaz and Mazlum (2019). A total of 7 illegal trawling cases were reported, all practiced by non-trawlers, accounting for 2 per year, mostly in Giresun (86%) and in May, August, and September (in total 86%). Hence, it can be concluded that a minor decrease was observed in trawl infringements between 2012 and 2014. Furthermore, non-trawler's contribution to trawling violations is remarkable. Since trawling in the eastern Black Sea is banned in all seasons, violations are decreasing and presumably, legal trawlers are being converted to non-trawler vessels, which are harder to detect. However, in the rest of the Black Sea coasts of Türkiye, trawling ban within 3-miles from the coast has led to comparatively higher infringement rates as it is practically almost impossible to control illegal trawling activities due to the lack of strong MCS Systems. Therefore, not only is it essential to reconsider range restriction regulations for the Black Sea, but the use of small vessels for illegal fishing activities should be monitored in all regions within the fishing zone of Turkish Waters.

Beyond the Turkish EEZ in the Black Sea, Öztürk (2013) reported 65 illegal fishing cases committed by Turkish vessels in the Black Sea between 1992 -2012, accounting for 3.1 incidents per year. In our study, a relative increase was observed with 19 cases (4 cases belonging to trawlers) during 2012-2014, corresponding to 6,3 cases annually. However, Türkiye, which has the greatest fleet capacity and ranked 8th given the IUU fishing index in the GFCM area of application (Macfadyen et al., 2019), in comparison, appears to make only a limited contribution to IUU fishing.

The Sea of Marmara

Although the Sea of Marmara has a much smaller area than other seas, fishery infringements are relatively high. The Sea of Marmara and Istanbul Strait are hot spots for trawling infringements. The other provinces that have high infringement rates are Çanakkale, Balıkesir, and Bursa. Interestingly, a majority of trawl infringements (51%) were from Istanbul, Izmir, and Çanakkale. This may be due to the high demand for seafood and heavy maritime traffic in this region.

The present study reveals that the percentage of small-size vessels (<12 m) is the highest in the Sea of Marmara (56%), followed by the Aegean Sea, the Mediterranean, and the Black Sea. Besides, the confiscation rate of small-size vessels (67%) was much higher than that of other types of fishing vessels. In practice, small-size vessels are notorious with respect to a specific type of mini-trawl gear towed from the stern or sides of the boat, which is commonly known as "şebeke" in Turkish. Even boats with 6-7 meters in length can tow a trawl net without any visual sign of the trawling activity. In addition, when a suspicious activity is detected by the coast guards and engaged, “şebeke” can easily be cut loose by the fishermen. This also explains lower confiscation rates of trawl nets which account for only 10% of all illegal trawling activities. The “şebeke” can later be retrieved from the bottom and used again for illegal near-shore trawl operations for high-value demersal species. For this reason, it is very important that these small-size “şebeke” trawl vessels should be effectively monitored.

The Aegean Sea

Findings of the present study have shown that the Aegean Sea ranks second with respect to trawling infringement rate and İzmir has the second highest rate of trawling violations. İzmir Bay and the vicinity of Çanakkale Strait had high infringement rates. Small-size vessels engaged in illegal trawling activity were also common in this region. Moutopoulos et al. (2016) stated in their study conducted in Greek waters of the Aegean Sea between 1999-2013 that trawl infringements were more common in the winter, in bays and gulfs, and in areas where fishing is prohibited. In addition, trawlers turned off their VMS devices when fishing illegally within a protected area leading to a relatively higher number of fishing violations in prohibited areas compared to those committed during closed periods. Similarly, in Turkish waters, trawling infringements were common in the winter period (41%) and the number of violations (43%) in bays and gulfs were higher than those committed during closed seasons (10%). In Greek Waters most common types of trawl infringements were spatio-temporal (45.5%), prohibited fishing gear (37.7%), and minimum landing size (2%), whereas, in Turkish Waters, spatio-temporal (53.2%), infringements related to gear (17,3%), and minimum landing size (7.1%) were more common. In both countries, spatio-temporal infringements were the most common type of trawling infringements.

The Mediterranean Sea

Our findings showed that the Mediterranean coasts of Türkiye had the lowest rate of trawl infringement. Trawl fisheries are more common on the eastern coasts and Mersin Bay is by far the most prevalent region for trawling infringements. Yağcılar (2009) reported 33 illegal trawling cases per year, mostly in October, November, and January with a rate of 71% in total, between 2008 -2012. In our study, in the same region in 2012-2014, the mean number of annual cases was 30, and more cases were reported in November, December, and January (47% in total) during 2012-2014, in the same region a 10% reduction in illegal cases were observed during 2012-2014 compared to 2008-2012.

General issues

Although spatially based fisheries management is crucial to sustainability, its implementation is challenging due to conceptual and technical difficulties (Russo et al., 2014). Lack of surveillance and control systems (MCS) together with VMS (Vessel Monitoring System) and AIS (Automatic Identification System) present difficulties in combatting illegal trawling particularly when small-size vessels are used extensively for trawling. Therefore, instead of establishing a distance restriction of 3 miles in the coastal zone for trawl fishing, establishing MPA (Marine Protected Area), or NFZ (No-Fishing Zone) zones will be a better monitoring approach in combatting trawling until effective MCS measures are implemented. In this regard, studies carried out in Sicily, Italy and Gökova Bay, Türkiye are successful examples (Pipitone et al, 2000; Ünal and Kızılkaya, 2019).

Temporal management of trawl fisheries seems more effective than spatial management as it contains only 10% of total infringements. Moreover, for specific infringement types, engagement in illegal trawling cases (related to fishing methods, catch, and gear infringements) was observed with a share of 22%, which is mostly caused by non-trawlers (80%). In infringements concerning time-closure for trawl fisheries, the majority of violations were due to small-size (<12m) illegal trawlers without valid licenses. Likewise, while the percentage of the vessels without a trawl license was 43%, the share of trawl infringements by vessels smaller than 12 meters was 37%. When engaged in illegal trawling, infringement rates surged up to 56%, and 47% respectively, so it can be concluded that in Turkish Waters, most of the infringements were related to engaging in illegal trawling by non-trawlers, and 84% of the non-trawlers consisted of vessels <12 meters. These non-trawlers without a trawl license consisted of fishing or auxiliary fishing vessels (81%) and non-fishing vessels (19%) such as private vessels (11%) and other types of vessels including diving, service, excursion, and tug boats (8%).

The share of trawling infringements on minimum landing size and unreported catch had a share of 7% and 8%, respectively. Since small-size vessels are not required to register, it is expected that when adequate enforcements are implemented, the share of unreported catch will likely increase. In addition, although mid-water trawl infringements had a share of 3.5% of all infringements, higher rates of violations such as “fishing in shallower waters” (39%) and “minimum landing size” (28%) are critical considering their negative effect on fish stocks (Göktürk & Deniz 2017). With respect to confiscated species, whiting, horse mackerel, bluefish and red mullet accounted for 62% of the total and indicated that the economic value of the target fish is an important factor for trawling violations.

Recommendation

Gallic and Cox (2006) suggested that permanently reducing fishing capacities or preventing further development of capacities are alternative and less costly measures for preventing IUU fishing. The size of the Turkish fishing fleet should be reduced as the capacity of the Turkish fishing fleet and the potential of fish stocks is not balanced (Anonymous, 2014). Despite five different decommissioning programs between 2013-2018, there is no significant change in the fishing power of the large-scale Turkish fleet. (Yılmaz et al., 2017). For example, from a total of 15352 powered vessels in 2008, a 13% reduction was achieved in 2018, but large-size vessels still accounted for 10.2% of the total capacity (Ünal & Göncüoğlu-Bodur., 2018). Therefore, in order to ensure sustainable fishing, the total capacity of the Turkish fishing fleet should be reduced by 50% by means of an effective buyback program.

Since most of the IUU vessels engaging in illegal trawling (e.g. şebeke) have lower running costs (inexpensive to buy, fewer personnel, low fare, no licenses, no documents, etc.) than registered vessels, it is important to propose harsher penalties for combating IUU fishing activities. It is expected that increasing fines and deploying MCS systems could directly affect IUU fishing activities. Strict monitoring of IUU fisherman, also serves as a deterrent (Phelps Bondaroff et al., 2015) and the countries that have strong fisheries management use effective MCS. Despite being expensive, it can be observed that fish stocks are recovered by tighter inspection and control measures performed by competent authorities both on the land and at the sea. Trade measures and sanctions are also very important to combat IUU fishing, i.e. strict inspections on landing ports, local markets, and even restaurants to prevent the consumption of undersized, prohibited, and illegally caught species. Supporting legitimate fishermen as “guardians of the sea” and collaborating with related fishery management units will also contribute to increasing costs for combating illegal activities. However, it is important to protect legitimate fishermen from unfair competition with illegal fishermen so that they can remain legitimate, and refrain from IUU activities.

As mentioned in the EU (2017) and GFCM (2017) related to having taken measures by coastal states to eliminate IUU fishing mainly include conducting effective MCS systems, not undertaking fishing activities without valid authorization and maintaining a logbook while fishing. In addition, preventing trading or importing the illegally caught catch, developing a national control and sanctioning system, improving fisheries by declaring restricted or marine protected areas are the other crucial precautions on this issue. The regulations put into force in 2020 in Türkiye are an important step in preventing IUU fishing in Turkish Waters, and they serve to harmonize EU rules consisting of critical confiscation, punishment, and jail sentence regulations against violator fishers and vessels. Moreover, the MCS system, which is an ongoing project called “coast surveillance radar system” includes radar, electro-optic, thermal, and data link systems surrounding all seas and including relevant partner authorities for information sharing, and it is expected to run completely within a few years which can be another major development. Locally used drones are an alternative and effective solution in the extent of MCS for combatting illegal fishery since air vessels have a significant advantage of speed and secrecy.

Conclusion

As experienced historically, Mediterranean community-based fishery management was particularly effective in fostering not only social cohesion but also sustainable utilization of coastal resources (Raicevic et al 2018). Similarly in Türkiye, community-based solutions together with ecosystem-based and species-based solutions should be preferred principally in fishery management, which can also contribute to reducing trawl infringements and IUU fishing at the same time. Effective measures such as declaring NFZ and/or MPA in overexploited or extensively violated areas and defining quotas for high-value species or overexploited stocks are significant measures in the fight against IUU. Within this scope, strengthened regulations and the implementation of the MCS system will be a significant milestone in controlling IUU fishing. In this context, reliable field data and case studies on IUU fishing are critical to defining priorities and developing effective solutions for sustainable fisheries.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

G.S. Karabacak: Conceptualization, data curation, review and editing, investigation, resources. M.C. Deval: Conceptualization, data curation, methodology, visualization, and writing -original draft.

Ethics Approval

No ethical permissions are required.

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RESEARCH ARTICLE

Some Biological Characteristics of the Invasive Moon Crab *Matuta victor* (Fabricius, 1781) (Crustacea: Decapoda: Matutidae) in Karataş Coasts (Adana, Türkiye)

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Abstract: In this study, some biological characteristics of the invasive moon crab, *Matuta victor*, which has recently entered the waters of the northeastern Mediterranean, Türkiye, were investigated for the first time. The study was carried out seasonally on sandy bottoms at 0.5-3 m depths along Karataş (Adana, Türkiye) coasts between 2020 -2021. A total of 364 crabs were obtained, 93 females (27 ovigerous), 270 males and one juvenile. The carapace width of female crabs ranged between 27.49-49.30 mm with a mean of 41.55±4.01 mm. The carapace width of male crabs ranged between 21.28-66.70 mm with a mean of 49.15±8.97 mm. Mean carapace length measurements in females and males were 26.83±2.52 mm and 32.71±5.85 mm, and the mean total weights were 8.18±1.99 g and 16.27±7.30 g, respectively. The male:female ratio was 2.16:1. Morphometric relationships indicated negative allometric growth for females, males, and both sexes. Ovigerous females were obtained in all seasons except the winter. The female crabs with carapace widths that ranged between 35.31-48.84 mm and a mean of 41.97±3.29 mm had a mean fecundity of 36822.04±29745.9 eggs.

Anahtar kelimeler:

İstilacı tür
Matuta victor
Oransal büyüme
Morfometrik ilişkiler
Yumurta verimliliği
Akdeniz

Karataş Sahillerindeki (Adana, Türkiye) İstilaci Tür Ay Yengeci *Matuta victor* (Fabricius, 1781) (Crustacea: Decapoda: Matutidae)'un Bazı Biyolojik Özellikleri

Öz: Bu çalışmada kuzeydoğu Akdeniz'e son zamanlarda giriş yapmış istilacı Ay Yengeci *Matuta victor*'un bulunurluğu ve bazı biyolojik özellikleri ilk kez incelenmiştir. Çalışma Karataş (Adana) sahillerinde, 2020-2021 yılları arasında mevsimsel olarak 0,5-3 m derinliklerdeki kumlu zeminde gerçekleştirilmiştir. Çalışmada 93 dişi (27'si yumurtalı) 270'i erkek ve 1 tanesi juvenil olmak üzere toplam 364 birey elde edilmiştir. Dişi bireylerde 27,49-49,30 mm arasında değişen karapas genişliği ortalama 41,55±4,01 mm olarak hesaplanmıştır. Erkek bireylerde 21,28-66,70 mm aralığında değişen karapas genişliğinin ortalaması 49,15±8,97 mm olarak ölçülmüştür. Ortalama karapas uzunluk ölçümleri dişilerde ve erkeklerde sırasıyla; 26,83±2,52 mm ve 32,71±5,85 mm; ortalama total ağırlıkları ise 8,18±1,99 g ve 16,27±7,30 gr bulunmuştur. Erkek:dişi oranı 2,16:1 olarak hesaplanmıştır. Dişi, erkek ve tüm bireylerde hesaplanan morfometrik ilişkiler incelendiğinde negatif allometrik büyüme saptanmıştır. Kış ayları dışında diğer mevsimlerde yumurtalı dişiler elde edilmiştir. Karapas genişlikleri 35,31-48,84 mm ve ortalama 41,97±3,29 mm olan yumurtalı dişilerin, yumurta verimlilikleri ortalama 36822,04±29745,9 yumurta olarak hesaplanmıştır.

Introduction

With the opening of the Suez Canal, many organisms of Indo-Pacific origin migrated into the Mediterranean. From 1980 onwards, the coastal communities of the Levantine coast differed significantly from communities elsewhere in the Mediterranean due to populations of species migrating through the Suez Canal. The Suez Canal is the most important transit route for Decapods and Stomatopods alien to the Mediterranean, but the relative importance of vectors/pathways varies between countries. Almost all of the alien species recorded on the Levantine coast are found to be introduced via the Suez Canal, while ship transportation and aquaculture are major introduction pathways on the coasts of Italy, France, and Spain (Galil et al., 2015). The number of alien species has doubled since 1970 (Katsanevakis et al., 2014). In the Mediterranean Sea, 900 alien species were detected in December 2020

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(Zenetos and Galanidi, 2020). In Turkish seas, 79 of 539 alien species were reported to be Crustacean species. The majority of the alien species along the Turkish coasts are species introduced from the Red Sea (58%) (Çınar et al., 2021). Many Lessepsian species have already crossed the Levantine coast, some of them spreading towards the western Mediterranean. Alien species content is increasing in the Levantine upper continental shelf biota (Galil, 2012). While some Penaeid and Portunid species establish economic populations that contribute to the Levantine fisheries, some species are represented by single individuals (Galil and Mendelson, 2013). Approximately 60% of the alien Brachyuran crab species distributed in the world have been reported from the Mediterranean Sea. This makes the Mediterranean Sea the richest sea in terms of invasive crabs (Brockerhoff and McLay, 2011, Moussa et al., 2016). Regarding alien invasive crab species, 39 species of the Red Sea/Indo-Pacific origin belonging to 19 families have been reported in the Mediterranean (Levitt et al., 2014; Corsini-Foka and Kondylatos, 2015; Galil et al., 2015). In Turkish waters, 15 of the 86 Brachyuran crab species, mostly found in the Aegean and Levantine seas, were reported to be foreign species (foreign/native ratio= 21%) (Bakir et al., 2014)

The natural distribution area of the moon crab (*Matuta victor*) is the Indo-West Pacific, including the Suez Canal, Red Sea, Gulf of Oman, East African coasts, Madagascar, Bay of Bengal, Andaman Sea, Pakistan, India, Malaysia, Indonesia, South China Sea, Japan, Australia and the Mediterranean Sea (Galil and Mendelson, 2013). It is also found off the coasts of the Philippines, Fiji (Ng, 1998), Taiwan (Ng and Huang, 1997; Ng et al., 2002), and southern Thailand (Fazrul et al., 2015). It is an aggressive, omnivorous species and was first reported from Haifa Bay (Israel) in the eastern Mediterranean Sea in 2012 (Galil and Mendelson, 2013). Afterward, the first reports were made from the Lebanese coast of Batroun in 2013, from the coasts of Tyr and Saida in 2014 (Crocetta and Bariche, 2015), from the Mediterranean coast of Syria in 2017 (Zeini and Hasan, 2017), and most recently from the island of Rhodes in 2018 (Kondylatos et al., 2018).

On the coasts of our country, reports were made from the Gulf of Antalya in 2015 (Gökoğlu et al., 2016), Iz Tuzu coast, Muğla (Ateş et al., 2017), and Yumurtalık Cove İskenderun Bay (Yeşilyurt et al., 2019). *M. victor* (Matutidae) is the only known species of the genus *Matuta* in the Mediterranean (Kondylatos et al., 2018).

In its natural habitat, the ecology of Matutid crabs is little known. This active, voracious and omnivorous predator exhibiting intraspecific feeding competition is known for its reproductive flexibility and high egg productivity (Perez, 1990; Innocenti et al., 2017). These are characteristics of a successful disperser species, but the potential impacts of this disperser species are not yet known (Hänfling et al., 2011; Innocenti et al., 2017). Some of the invasive species can establish large populations in the Levantine basin, becoming more dominant than their endemic distribution range. Since brachyuran crabs have an important role in regulating prey populations, the possibility that *M. victor* establishing large populations on the Levantine beaches should not be ruled out. The diet of moon crabs consists mainly of crustaceans and molluscs. Small crabs feed on small soft-shelled species, while larger crabs feed on sessile crustacean invertebrates such as Anomuran crabs, Bivalves, and Gastropods or slow-moving species (Perez and Bellwood, 1988). *M. victor* is an entrant to the Mediterranean and it is thought that it may settle and establish populations in the eastern Mediterranean due to suitable conditions. In this study, we determined the status, abundance, and some biological characteristics (morphometric characteristics, length-weight relationship, fecundity, etc.) of *M. victor* collected from Karataş, Adana, Türkiye. This is the first detailed bio-ecological study on this alien species on the Mediterranean coast of Türkiye. This study also aims to collect data that will help to determine the potential impacts of *M.victor* and to contribute to future plans for its management.

Material and Methods

The coast of Karataş, where the study was conducted, is located in the northwest of the Iskenderun Bay (Fig. 1). Sampling was carried out seasonally between 2020-2021 in Akyatan Beach, Karataş district (Adana), in shallow areas with sandy bottom at depths of 0.5-3 m, using a hand grab and a beach seine with a wing length of 10 m and a mesh size of 1 mm (Figure 1).

The samples obtained were kept in freezers and brought to the laboratory. The crabs were then identified according to Galil and Clark (1994), Ateş et al. (2017), and Behera et al. (2018) (Figure 2).

Biometric characters; total weight (TW), carapace width (CW), and carapace length (CL) were measured. The total weight (TW) of each specimen was measured using a precision balance sensitive to 0.1 g, and carapace measurements were made with the help of a digital caliper sensitive to 0.1 mm.

Carapace width-carapace length and total weight-carapace width relationships were determined using regression analysis in SPSS 13 package program. The morphometric relationship was calculated for females, males, and both sexes using the formula $Y=aX^b$ (Ricker, 1975).

The allometric coefficient was determined from the b value in the linear regression equation. Accordingly, b=3 is considered as isometric growth, b<3 as negative allometric growth and b>3 as positive allometric growth (Zar, 2010).

Egg mass from ovigerous females was weighed and stored to determine egg production. Eggs were preserved in Bouin's solution (picric acid 75cc, formaldehyde 40% 25cc, glacial acetic acid 5cc). For egg counting, 3 subsamples of 1-10 mg were taken from each specimen. The subsamples were weighed on a balance, placed on a glass slide and wetted with 30% glycerin before counting using a stereo microscope (Olympus, SZX16). Egg productivity was determined according to Türeli (1999).

Figure 1. Sampling Area

A B C

Figure 2. *M. victor* A: Dorsal View, B: Ventral View (male and female), C: Ovigerous Female (with Orange Eggs) (Original)

Fecundity (Egg Productivity) = (total egg weight x number of sub-sample eggs) / sub-sample egg weight

The fecundity of each female crab was calculated using the mean egg count from three subsamples.

Statistical equality of the morphometric relationship between sexes was tested using One Way ANOVA at a significance level of $p \leq 0.05$. Sex ratios were calculated using the Chi-Square (x^2) test against an expected frequency of 1:1 (Zar, 1999). All statistical analyses were performed using SPSS for Windows (Version 13).

Results

In the present study, a total of 364 *M. victor* specimens, including 66 adult females, 27 ovigerous females, 270 males, and 1 juvenile were captured. The mean ± standard deviation and min-max values are given in Table 1.

The differences between carapace width, carapace length, and total weight of male and female crabs were found to be statistically significant ($p<0.05$). The distribution of carapace widths of all specimens was 21.28-66.70 mm and the mean carapace width was 40.77±9.41 mm.

During this study, most crabs (n: 146) were caught in spring season. This was followed by the fall (n: 90) and the winter (n: 73) seasons. The least number of crabs were caught in summer (n: 55). During the whole sampling period, male crabs were caught in higher numbers in each season. It was determined that 25% of *M. victor* specimens were female and 75% were male. When the capture rates of male and female crabs were analyzed, this difference was found to be statistically significant ($p<0.05$). The calculated male to female sex ratio was 2.9:1. The highest female ratio was in the fall (43%) and the lowest was in the spring (8%). The highest male ratio was observed in the spring with a ratio of 11.16:1. The difference in the male to female ratio in the spring and the autumn was significant ($p<0.05$).

The linear relationship between carapace length (CL) and carapace width (CW) was CW= 7.0698 + 1.2864 CL (R^2 = 0.67) in females, CW= 1.6713 + 1.4514 CL (R^2 = 0.89) in males, and CW= 3.1327 +1.4125 CL (R^2 = 0.89) in both males and females (Figure 3). The relationship between carapace width and the total weight was TW= 0.0017 CW 2.2862 (R^2 = 0.55) in females, TW= 0.0004 CW 2.7187 (R^2 = 0.88) in males, and TW= 0.0003 CW 2.7767 (R^2 = 0.87) in total (Figure 4).

Table 1. Mean length and weight values calculated from specimens of *M. victor* (mean±std. deviation, min.-max.)

Sex	Number of Sp.	Total Weight (g)	Carapace Length (mm)	Carapace Width (mm)
Female	66	8.18±1.99 (2.84-13.75)	26.83±2.52 (16.16-33.20)	41.55±4.01 (27.49-49.30)
Ovigerous Female	27	9.77±2.53 (5.58-15.11)	27.15±2.12 (22.9-31.82)	42,09±3,17 (35.31-48.84)
Male	270	16.27±7.30 (1.62-41.40)	32.71±5.85 (15.37±46.63)	49.15±8.97 (21.28-66.70)
Juvenile	1	2.4	19.27	26.95
Total	364	14.39±7.45 (1.62-41.40)	31.19±5.79 (15.37-46.63)	40.77±9.41 (21.28-66.70)

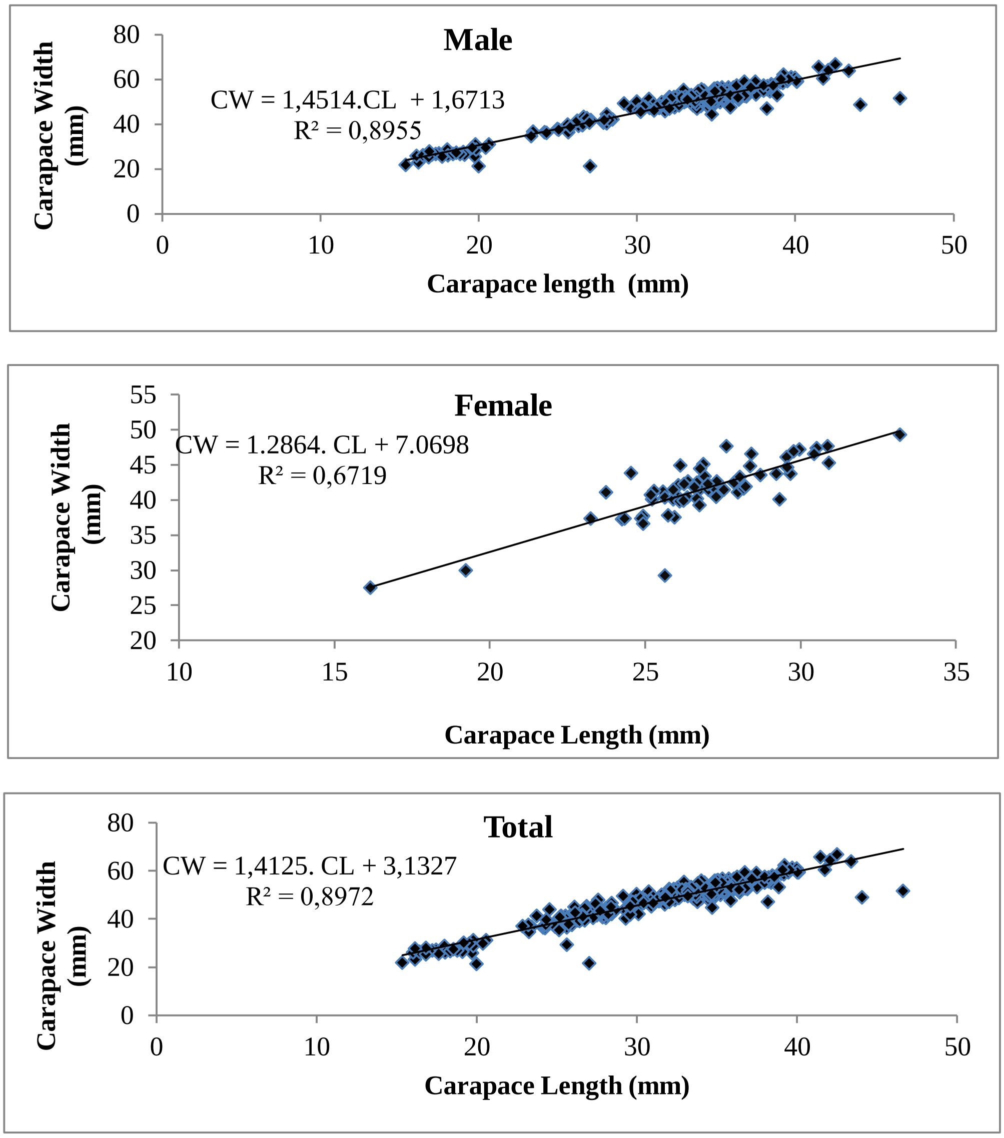

Figure 3. Relationship between the carapace length (CL) and carapace width (CW)

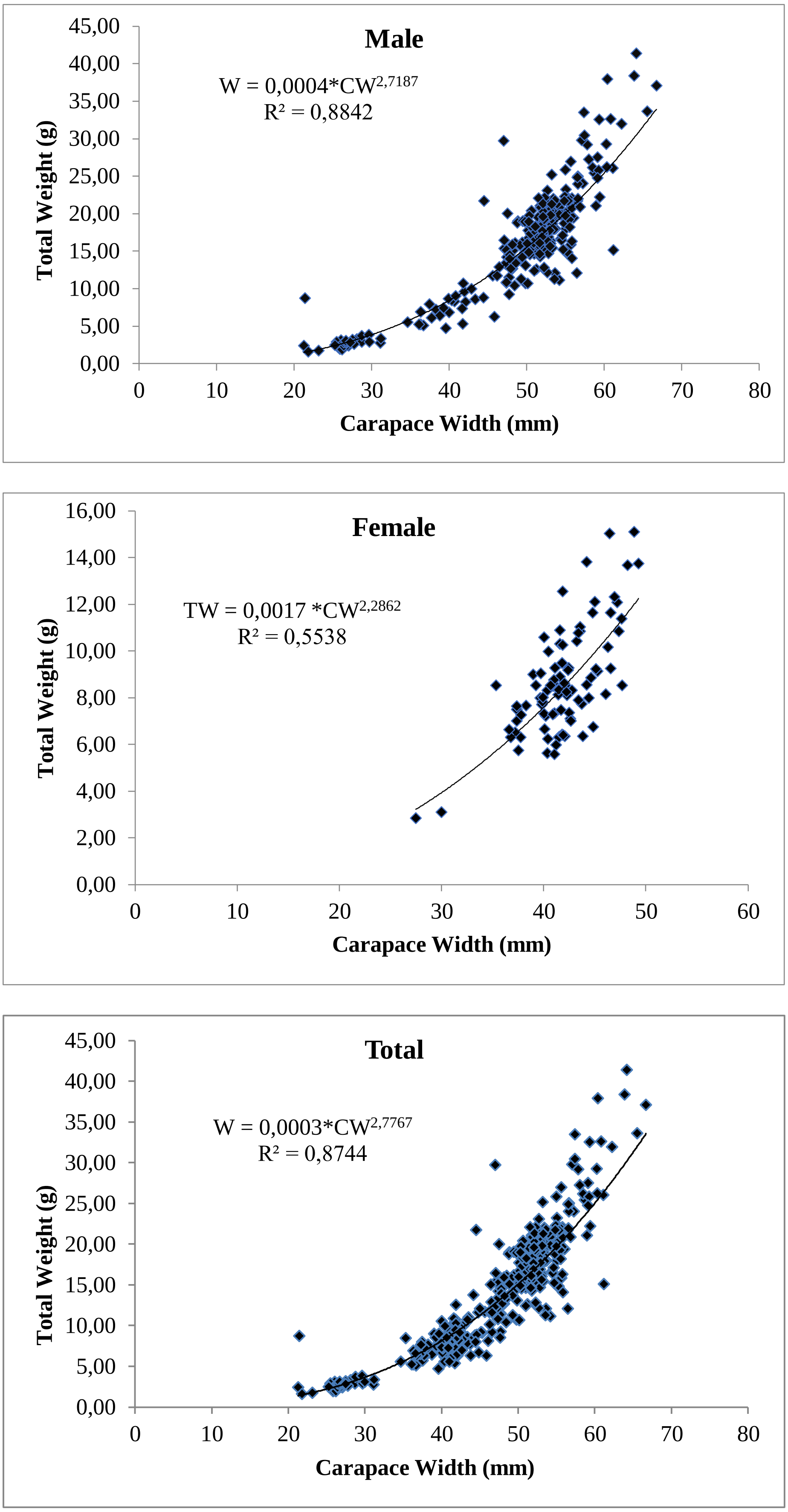

Figure 4. Relationships between the carapace width (CW) and total weight (TW)

A negative allometric growth was observed for both sexes and all specimens since b values were less than 3. A strong relationship was observed between proportional growth (carapace width - carapace length; weight - carapace width) in *M. victor* females, males, and all crabs (R^2 ranged between 0.67 to 0.89).

Carapace width was used to determine the length-frequency distribution (Figure 5) . In males, 120 crabs were observed in the 56.0-60.9 mm range, followed by 64 specimens in the 46.0-50.9 mm range. The highest number of *M. victor* females was in the 46.0-50.9 mm range with 47 crabs range. While 28 females were found in the 51.0-55.9 mm length range, only one female was found in the 36.0-40.9 mm range. With respect to all crabs collected in this study, the maximum number of crabs was found in the 56.0-60.9 mm range, and the minimum number of specimens was in the of 71.0-75.9 mm range (Figure 5).

Ovigerous females (OF) were obtained in all seasons except in the winter. The highest number of ovigerous females was in the fall with 15 specimens, followed by the summer with 9 females. The lowest number of ovigerous females were caught in the spring (n= 3). In terms of developmental stages, eggs in the early developmental stage, indicated by the typical orange-color, were represented by the highest numbers. Eggs in the developing stage were brown in colour and those in the final development stage were black (Figure 6). The carapace widths of females with orange-colored eggs ranged between 36.78-46.27 mm, those with brown eggs ranged between 38.99-48.84 mm, and those with black eggs ranged between 35.31-44.23 mm.

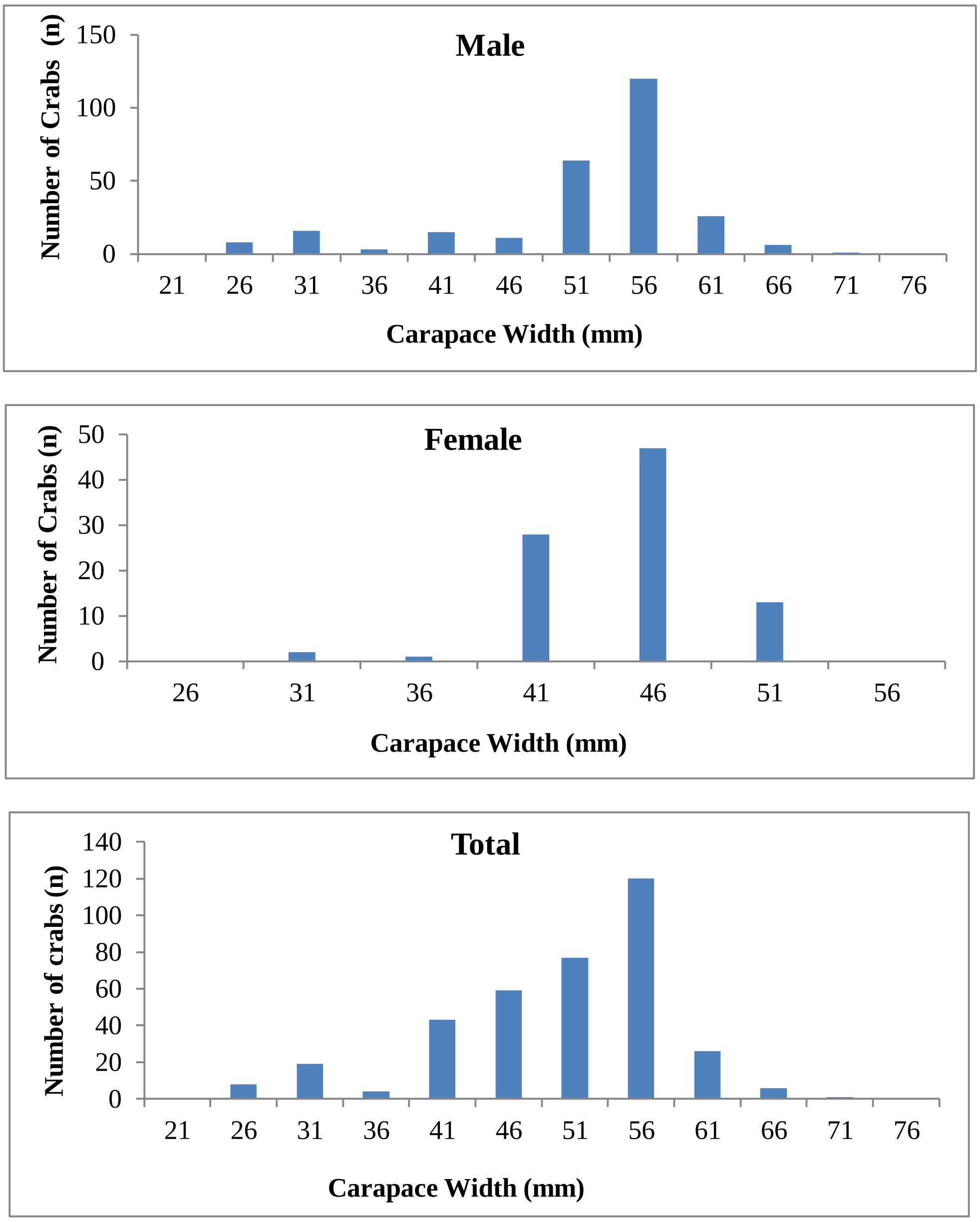

Figure 5. Length-frequency (CW) distribution of *M. victor* a) males, b) female, and c) males and females

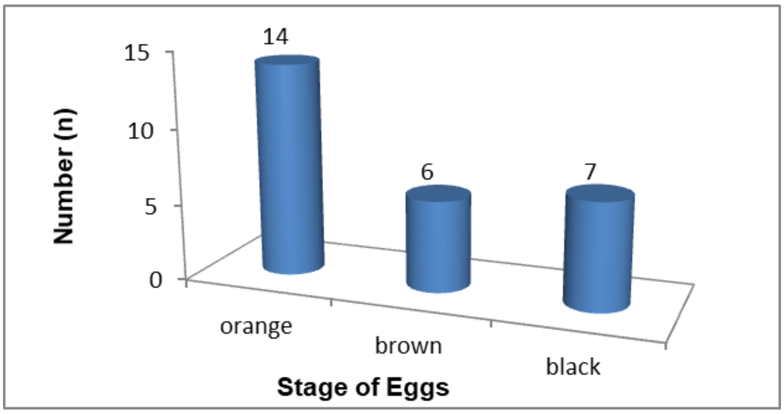

Figure 6. Developmental stages of eggs as indicated by their coloration.

Fecundities were calculated for 21 individuals with proper egg mass out of a total of 27 ovigerous females. Carapace lengths of ovigerous females ranged between 22.9 and 31.82 mm with a mean of 27.01±2.22 mm, carapace widths between 35.31-48.84 mm with a mean of 41.97±3.29 mm (Figure 7), total weights between 6.32 -15.11 g with a mean of 10.01±2.46 g, and egg weights between 0.13-2.37 g with a mean of 0.97±0.66 g. The mean egg productivity was 36822.04±29745.9. The highest number of eggs was 123419.27 from an individual with 48.84 mm in CW and 15.11 g in weight, and the lowest number of eggs was 5334 from an individual with 43.39 mm in CW and 10.78 g in weight.

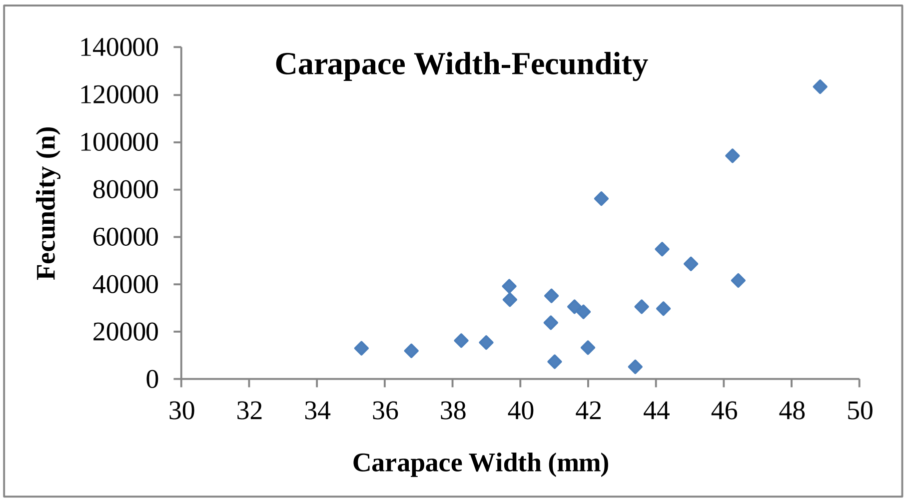

Figure 7. Relationship between the carapace width (CW) and fecundity.

Discussion

In its natural habitat, the ecology of Matutid crabs is poorly known and data presented in this study contributes to the existing literature. With respect to *M. victor* densities in the wild, different results were reported. In Haifa Bay (Israel) near Kiryat Yam in the fall of 2013, 0.1-1 crab/m^2 were reported and the majority of crabs were caught from depths of less than 0.3 m. Near Na'aman Lagoon, Zevulun Plain Beach in 2013, a large number of crabs were caught corresponding to a density of 27 crabs/m^2 whereas in May 2017 in Kiryat Yam, the density was reported to be 2 crabs/m^2 (Innocenti et al., 2017). Others reported considerably lower number of crabs. For example, Zeini and Hasan (2017) captured a total of 11 specimens, including one female, at a depth of 8-10 m with a sandy bottom off the Mediterranean coast of Syria. Kondylatos et al. (2018) reported *M. victor* from the island of Rhodes in the Gulf of Zefyros in the Mediterranean. They caught a single male by snorkeling at a depth of 1.5-2 m on a sandy bottom. Galil and Mendelson, (2013) captured an adult male with a carapace length of 3.25 cm from a depth of 10 m in Haifa Bay (Israel) and 1 adult female with a carapace length of 2.95 cm from a depth of 5 m in Kiryat Yam. Behera et al. (2018) caught 10 individuals of *M. victor* consisting of 5 males and 5 females off the coast of Gopalpur Odisha (India). They calculated the mean carapace width as 4.84±0.5 cm for males and 4.46±0.2 cm for females. Yeşilyurt et al. (2019) caught 4 males, 1 female, and 1 juvenile at a depth of 30-50 cm with a sandy bottom in Kokar locality, Yumurtalık Bay (Türkiye). The carapace width of male crabs ranged between 54.65 to 62.43 mm and was 44.47 mm in the female specimen; the carapace length was 29.52 mm in the female and ranged between 35.56-40.01 mm in male crabs. The carapace width and the carapace length of the juvenile crab were 46.90 mm and 30.23 mm, respectively. Although the carapace widths of the individuals caught by Behera et al. (2018) from the shores of Gopalpur Odisha are similar to those in our study, the range of carapace widths of males in this study was larger than that reported in Yumurtalık Bay (Türkiye) (Yeşilyurt et al., 2019). The carapace width of the juvenile crab was considerably smaller than that of the single specimen reported from Yumurtalık Bay. Naderi and Mahigir (2022) reported 181 males and 129 females from the coasts of Gehrdo in the Oman Sea (Iran). The carapace width of male and female crabs ranged between 16.4-44.3 mm and 19.3-36.9 mm, respectively. The range of carapace width and weight values reported in the Oman Sea was lower compared to our results. These differences are thought to be due to the differences in the regional and bio-ecological conditions. The mean carapace width and weight were found to be higher in males than in females.

In this study, the ratio of male:female crabs was 2.9:1 and this is the first data on the sex distribution of the species for our region and Türkiye. Naderi et al. (2021) reported a sex ratio of 1.4:1 (M:F) from the coast of Gehrdo (Sea of Oman) which was lower than that reported in the present study. In this study, the majority of males were caught in the spring. Conversely, the lowest number of females (8) was found in the same season. Throughout the study period, the sex ratio of *M. victor* was different than 1:1. This difference in the sex ratio may be due to depth preferences of females for reproduction. Since there is limited information on the bio-ecology of this newly introduced species in our seas, further studies on the reproduction period should be carried out.

In the present study, the minimum and maximum number of eggs ranged between 5334- 123419.27. Naderi et al., 2021, reported a maximum of 43423 eggs (CW: 26.7 mm; TW: 6.25 g) and a minimum of 11635 eggs (CW: 28.55 mm; TW: 8.79 g) from the Arabian Sea. These authors also reported an inverse relationship between fecundity and carapace width and weight. On the contrary, our findings indicated that fecundity of *M. victor* increased as a factor of carapace width. It has been reported that ovigerous females were mostly caught in the spring and the summer (Naderi et al., 2021). The spawning season of the Genkai-nada Sea (Japan) moon crab was in July and September (Kobayashi, 2013). In our study, most ovigerous females were caught in the fall and summer.

In our study, the morphometric relationship and proportional growth of female, male and all specimens were calculated as negative allometric growth. In the study conducted in the Oman Sea, while males showed positive allometry in accordance with our study, females had negative allometry (Naderi and Mahigir, 2022). Reported b values for males and females were 3.11 and 2.75, respectively, and these values were lower than reported for *M. victor* in its natural distribution area in the Oman Sea. According to Gökçe et al. (2016), b values vary depending on many factors affecting growth in crustaceans such as salinity, temperature, nutrition, sex, maturity and season. It should be kept in mind that the species only provides information about the region studied during the study. Since the length-weight study of the species was not available, our findings could not be compared with the studies conducted in our country and in endemic distribution regions.

The species diversity of the Mediterranean is changing with the introduction of endemic and exotic species and a new Lessepsian or species of Indo-Pacific and Atlantic origin. *M. victor* is an entrant to the Mediterranean and it is expected that it will settle and establish populations in the eastern Mediterranean due to suitable environmental conditions. In this study, distribution and some biological characteristics of *M. victor* are reported for the first time. Ovigerous individuals were obtained in all seasons except winter and the majority of ovigerous females were caught in the fall. More comprehensive studies should be carried out to fully reveal the biological characteristics of *M. victor*, which has recently entered the coastal waters of our region and established populations.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Author Contributions

The study was planned and designed by Canan Türeli and Tamer Leba. Tamer Leba performed to collected samples from the field and analysed them in the laboratory. All authors contributed to the writing of the final manuscript.

Ethics Approval

The material used in this article is invertebrate species therefore ethics committee approval is not required for this study.

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RESEARCH ARTICLE

Chasing Light: How Dichromatic LEDs Affect the Elemental Profile of *Gongolaria barbata*

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Dichromatic LED

Abstract: This study aims to investigate the influence of three different dichromatic LED light sources and varying photoperiod durations on the mineral content and trace element compositions in cultivated *Gongolaria barbata* under controlled culture conditions. During the experiment, red-blue (RB), blue-green (BG), red-green (RG) and fluorescent lights were examined at 16:16, 12:12, and 8:16 Light: Dark (L:D) photoperiods, and at 150 µmol photon m^{-2} s^{-1} intensity of light in all treatments. The elemental compositions of the thallus samples were analyzed for Mg, Ca, K, Na, P, Zn, Mo, Cu, Mn, Cr, Co, Cd, Fe, and As.. Our results showed that macro element and trace element compositions significantly varied among different experimental groups. Regarding the order of abundance, macroelements were ranked as follows: K > Na > Ca > Mg > P. Meanwhile, trace elements followed this order: As > Zn > Mn > Cr > Co > Cu > Cd > Mo > Fe. Among the experiment groups, the highest value of the macro elements was recorded as 1041.3±22.2 mg kg^{-1} for K, and the lowest value was 26.61±0.02 mg kg^{-1} for the P. Among the trace elements, for As, the highest value was recorded as 1339.86±5.27 µg kg^{-1}, and the lowest was determined at 1.93±0.04 mg kg^{-1} for the Fe. The findings highlight that LED lighting conditions can significantly influence the elemental composition of *G. barbata*.

Anahtar kelimeler:

Makro elementler
İz elementler
Kahverengi deniz yosunu
Gongolaria barbata
Dikromatik LED

Işığın peşinde: Dikromatik LED'ler *Gongolaria barbata*'nın Elementel Profilini Nasıl Etkiler?

Öz: Bu çalışmanın amacı, kontrol koşullarında yetiştirilen *Gongolaria barbata*'nın mineral içeriği ve iz element değişimleri üzerinde üç farklı dikromatik LED ışık ve değişen fotoperiyot sürelerinin etkisini araştırmaktır. Deneme aşamasında, kırmızı-mavi (RB), kırmızı-yeşil (RG), mavi-yeşil (BG) ve floresan (F) LEDler, sırasıyla 16:16, 12:12 ve 8:16 Aydınlık:Karanlık (L:D) fotoperiyotlarında, her biri 150 µmol photon m^{-2} s^{-1} yoğunluğunda incelenmiştir. Tallusların; Ca, K, Mg, Na, P, Zn, Mo, Mn, Fe, Cu, Cr, Co, Cd ve As kompozisyonları analiz edilmiştir. Sonuçlarımıza göre, makroelement ve iz element kompozisyonları farklı deney grupları arasında önemli varyasyonlar gösterdi. Türde bulunan makroelementlerin sıralaması şu şekildedir: K > Na > Ca > Mg > P, aynı zamanda iz elementler şu sırayı takip etmektedir: As > Zn > Mn > Cr > Co > Cu > Cd > Mo > Fe. Deneme grupları arasında, makro elementlerin en yüksek değeri K elementi için 1041.3±22.2 mg kg^{-1} olarak belirlenirken, en düşük değer P için 26.607±0.02 mg kg^{-1} olarak belirlendi. İz elementler arasında ise en yüksek değer As için 1339.86±5.27 µg kg^{-1} tespit edilirken, en düşük değer Fe için 1.930±0.04 mg kg^{-1} olarak belirlendi. Bulgular, LED aydınlatma koşullarının *G. barbata* türünün elementel kompozisyonunu önemli ölçüde etkileyebileceğini vurgulamaktadır.

Introduction

Macroalgae, commonly known as seaweeds, are considered as multifaceted marine resources with diverse ecological, nutritional, and industrial significance (Lourenço-Lopes et al., 2020). These photosynthetic organisms are pivotal contributors to global oxygen production and hold immense potential as sustainable sources of essential minerals, bioactive compounds, and functional ingredients for human consumption (Fleurence, 1999). Moreover, macroalgae possess a rich nutritional profile and bioactive compounds with potential antioxidant, anti-inflammatory, and anti-diabetic properties, positioning them as a potential functional food source (Rodrigues et al., 2015).

These sessile organisms adapt to the ever-changing underwater light environment, influencing their physiological responses, pigmentation, and growth patterns (Figueroa et al., 2014). Environmental factors such as solar radiation, water column depth, and dissolved organic matter

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influence light conditions underwater, leading to variations in the composition and growth of macroalgal communities (Lüning, 1991). Light conditions play a pivotal role in shaping mineral accumulation dynamics of macroalgae, a diverse group of marine organisms with substantial promise for addressing nutritional deficiencies and enhancing human health. The nuanced interplay of light spectra, notably the red: far- red and blue: red ratios, is recognized for its role in signaling diurnal and annual photoperiods, thereby influencing the growth cycles of macroalgae (Figueroa et al., 1995)

Indoor algal aquaculture has been transformed by the use of light-emitting diodes (LEDs), surpassing conventional lighting approaches. It is a fundamental driver of photosynthesis, metabolism, and overall growth in conventional fluorescent lamps regarding economic efficiency and technological advancement (Schulze et al., 2014). LEDs offer a host of advantages, including the ability to finely tune light intensity to simulate natural sunlight fluctuations throughout the day, the capacity to generate high light levels with minimal heat emission, and the extended lifespan, typically 20–30 nm at half-peak height in their narrow emission spectrum, enabling precise control for studies in photomorphogenesis and other plant responses to light (Choi et al., 2015; Yeh and Chung, 2009). In their study, Öztaşkent and Ak, (2021) evaluated the effects of LED lights on the pigment, growth, and biochemical composition of *Treptecanta barbata* (formerly known as *Cystoseira barbata*). Red LED light sources were observed to have a significant impact on the specific growth rate of *T. barbata*, leading to a remarkable 61% increase compared to the control group. Notably, it was found that red LED light specifically elevated the protein content in this brown seaweed. Light and salinity effects on the mineral levels of *T. barbata* were also examined by Ak et al., (2022). The elemental values of these brown algae from wild stocks were compared with those of cultured thallus. In the culture experiments, 11 trials with light intensity ranging from 50 to 150 μmol photons m^{-2} s^{-1} and salinity ranging from 24 to 42 ppt were designed using response surface methodology (RSM). According to the results, light intensity and salinity changes were modeled for element accumulation in *T. barbata*. Most of the elements were influenced by salinity rather than light intensity. Okumura et al., (2014) have harnessed these features by developing LED array systems tailored to specific algal growth requirements, demonstrating notable enhancements in growth efficiency and cost-effectiveness.

In the study by Kim et al., (2015), it was observed that the use of mixed-color LED lighting, as opposed to cool white fluorescent lighting, resulted in higher concentrations of chlorophyll and carotenoids during the cultivation of *Gracilaria tikvahiae*. The mineral content within macroalgae has garnered considerable attention due to its potential to provide essential nutrients and trace elements crucial for various physiological processes (Rodrigues et al., 2015; Afonso et al., 2018). Macroalgae can accumulate calcium, magnesium, potassium, iron, and iodine minerals essential for human well-being (Circuncisão et al., 2018).

Understanding the intricate relationship between light conditions and mineral composition within macroalgae is pivotal for comprehending their physiological responses. Among the extensive array of macroalgal species, those belonging to the genus *Gongolaria*, a representative of the brown algae group, play a critical ecological role as "ecosystem engineers" in temperate rocky reefs (Thibaut et al., 2016). Macroalgae, including *Gongolaria* species, have drawn attention as promising candidates for addressing dietary mineral deficiencies and enhancing the nutritional value of human diets. These marine organisms have demonstrated a unique capacity to accumulate and concentrate minerals from their surrounding aquatic environments (Holdt and Kraan, 2011).

While there have been studies on the effects of monochromatic LED lights and salinity on the elemental composition of *Gongolaria barbata* (Stackhouse) Kuntze, 1891, there is currently no research available regarding how different types and intensities of dichromatic LED lights impact the chemical content of this species.

The aim of this study is to investigate the effects of three different dichromatic LED light sources and varying photoperiod durations on the mineral content and trace element fluctuations in *G. barbata* cultivated under controlled conditions. During the experimental phase, red-blue, red-green, green-blue, and fluorescent lights were examined at 16:16, 12:12, and 8:16 Light: Dark (L:D) photoperiods, each with 150 μmol photons $m^{-2}s^{-1}$ intensity of light. The elemental compositions of the thallus samples, including Ca, Mg, P, Na, K, Zn, Mo, Mn, Cu, Fe, Cr, Co, As and Cd were analyzed. These investigations aim to provide valuable insights into optimizing artificial lighting systems for closed macroalgae production environments, facilitating sustainable seaweed cultivation for diverse industrial and ecological applications.

Material and Methods

Macroalgae media and cultivation

In this study, *G. barbata* thallus were obtained from established stock cultures and were cultivated in specialized 50 l Plexiglas tanks. These tanks were filled with UV-sterilized seawater and maintained at temperature of 24 °C. The tanks were illuminated with an irradiance level of 150 μmol photon m^{-2} s^{-1}, which was monitored using an LI-250 light meter from Li-Cor (USA). Throughout the acclimatization period, the cultures were exposed to continuous illumination for 24 hours daily to prevent shading among thallus and ensure consistent light exposure. For the experiments, healthy thalli were carefully selected and placed into glass bottles. The experiments were carried out for five weeks. Three color combinations of LED lights (blue-red, green-red, and blue-green) and three photoperiods (16:8, 12:12, 8:16 (L:D)) were employed for experimentation, while the control group was subjected to the daylight fluorescent light (Table 1). The positioning of the LED lights was arranged symmetrically in the culture chambers.

To conduct the experimental trials, 3 l cylindrical glass bottles, each with a diameter of 15 cm, were employed. The stock density for these trials was established at 5 g l^{-1}, maintaining consistency across the experiment and facilitating accurate observations and measurements. The bottles were aerated by introducing air bubbles, creating a conducive environment for the thalli. The choice of growth medium was crucial for the experimental setup, and the Conway medium was employed consistently throughout the experiments (Tompkins et al., 1995). The pH values were diligently measured and recorded throughout the experiments. These measurements were conducted utilizing a pH meter manufactured by Hanna Instruments (model HI8314). Other culture parameters of culture like salinity and temperature maintained according to the specifications detailed by (Ak et al., 2022). Specifically, the salinity level was maintained at a constant 36‰, while the temperature was regulated to remain within the range of 24 ± 1 °C.

Element analysis

For element analysis, the samples were subjected to examination using the Varian Liberty AX Sequential ICP-AES instrument, employing the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) technique. This analysis encompassed a wide range of elements, including silver (Ag), potassium (K), arsenic (As), aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), calcium (Ca), phosphorus (P), and zinc (Zn), as outlined by the Nordisk Metodikkomité for Næringsmidler (2007). To ensure accuracy and prevent secondary contamination, procedures recommended by the Marine Strategy Framework's 8th and 9th task groups were rigorously followed (Law et al., 2010). All analyses were performed in triplicate, and each set of measurements was carried out in three replicates to account for variability and ensure robustness. The measured values were reported as parts per million (ppm) and per billion (ppb), quantitatively representing the elemental concentrations within the samples.

Data evaluation

To evaluate variances in elemental compositions among the different trials, a one-way analysis of variance (ANOVA) was conducted.

Table 1. Experiment group design

Photoperiod (L:D)	LED Condition	Code
16:8	Fluorescent	16:8 F
	RB (Red LED %50 + Blue LED %50)	16:8 RB
	RG (Red LED %50 + Green LED %50)	16:8 RG
	GB (Green LED %50 + Blue LED %50)	16:8 GB
12:12	Fluorescent	12:12 F
	RB (Red LED %50 + Blue LED %50)	12:12 RB
	RG (Red LED %50 + Green LED %50)	12:12 RG
	GB (Green LED %50 + Blue LED %50)	12:12 GB
8:16	Fluorescent	8:16 F
	RB (Red LED %50 + Blue LED %50)	8:16 RB
	RG (Red LED %50 + Green LED %50)	8:16 RG
	GB (Green LED %50 + Blue LED %50)	8:16 GB

Results and Discussion

In our study, we aimed to investigate how photoperiods (16:8, 12:12, and 8:16 L:D) and dichromatic light colors (F, RB, RG, and BG) impact the cultivation of *G. barbata*. The macro and trace elements of the *G. barbata* samples, cultivated under various LED photoperiod conditions, are given in Table 2. Among the experiment groups, the highest value of the macro elements was recorded as 1041.3±22.2 mg kg^{-1} dry weight (dw) for the element K, and the lowest value was determined as 26.61±0.02 mg kg^{-1} dw for the P element. For trace elements, the highest value was determined for the As 1339.86±5.27 µg kg^{-1} dw, and the lowest was determined at 1.93±0.04 mg kg^{-1} dw for the Fe. According to our results, the highest Ca value was recorded in the 16:8 photoperiod control group, at 186.45±0.37 mg kg^{-1}. Generally, the lowest values were recorded in the 12:12 photoperiod, with the lowest being 87.17±19.00 mg kg^{-1} dw in the RG group. The highest Mg value was recorded in the 16:8 F, with values in the 8:16 groups being the lowest. The macro elements identified in the species were ranked in the following order from highest to lowest concentration: K > Na > Ca > Mg > P. For the trace elements found in the species, they were arranged in the following order from highest to lowest concentration: As > Zn > Mn > Cr > Co > Cu > Cd > Mo > Fe.

Macro elements

Our research findings indicated that, based on elemental data, the highest Na values were recorded in the 12:12 photoperiod group with 564.68±10.18 mg kg^{-1}, while the lowest value recorded in the 8:16 photoperiod, 311.23±0.99 mg kg^{-1} (Table 2). In the study by Aşıkkutlu and Okudan (2021), the sodium (Na) content was determined to be 6.46 mg g^{-1} in *Cystoseira foeniculacea* and 8.36 mg g^{-1} in *Gongolaria montagnei*. Meanwhile, Kravtsova et al. (2014) reported the highest recorded Na values for *C. barbata*, ranging from 11.3 mg g^{-1} to 26.60 mg g^{-1}, and the highest average Na values for *C. crinita* species were in the range of 20.00 mg g^{-1} to 25.30 mg g^{-1}. Upon comparing our study's data with previous studies, it is evident that the sodium content values in our study were lower than those reported in the earlier research. The highest value was recorded for K in the 12:12 (L:D) photoperiod control group (1041.3±22.2 mg kg^{-1}), while the lowest was measured at 559.33±1.56 mg kg^{-1} in the 8:16 (L:D) photoperiod RB group. K was found to be 28.97 mg g^{-1} in *C. foeniculacea* species and 22.71 mg g^{-1} in *G. montagnei* species, according to the study Aşıkkutlu and Okudan (2021). Our study's values are lower than those of previous studies.

Our study found Mg values higher at 16:8 F, 139.05±1.48 mg kg^{-1} and lowest at 8:16 (L:D) BG, 91.89±0.72 mg kg^{-1} (Table 2). Manev et al. (2021) found a Mg composition of 6.60 mg kg^{-1}, and Szelag-Sikora et al. (2016) found the highest mean in the *Cystoseira barbata* species as 6.26 mg g^{-1} and the lowest value of 4.70 mg g^{-1}. Also, Aşıkkutlu and Okudan (2021) found Mg as 0.63 mg g^{-1} in *C. foeniculacea* and 0.62 mg g^{-1} in *G. montagnei*. According to previous studies our results are higher than those reported in the study of Manev et al. (2021) and lower than those reported by Aşıkkutlu and Okudan (2021) and Szelag-Sikora et al. (2016).

For Ca, our highest value for *G. barbata* was 186.45±04 mg kg^{-1}, 16:8 F (L:D), and the lowest was 105,06±0,25 mg kg^{-1} at 12:12 BG (L: D) (Table 2). According to Aşıkkutlu and Okudan, (2021), Ca was determined as 7.84 mg g^{-1} in *C. foeniculacea* and 11.23 mg g^{-1} in *G. montagnei*. Manev et al., (2013) found the Ca composition of *C. barbata* from Cape Rusalka near the Black Sea at 41.60 mg kg^{-1}. Szelag-Sikora et al. (2016) found the highest mean value for Ca in the *C. barbata* as 24.83 mg g^{-1}, and the lowest was 9.03 mg g^{-1}. We observed that our study's values were lower when compared to previous studies.

Our study detected a P range between 78.77±0.25 mg kg^{-1} (8:16 RB (L:D)) to 28.60±0.02 mg kg^{-1} (12:12 RG (L:D) (Table 2)). In previous studies, P values were recorded as 316.84 mg kg^{-1} in *C. foeniculacea* and 370.35 mg kg^{-1} in *G. montagnei* (Aşıkkutlu and Okudan, 2021). Szelag-Sikora et al. (2016) found ranges between 1.24 mg g^{-1} to 0.47 mg g^{-1} in *C. barbata*. According to previous studies our results were lower than Aşıkkutlu and Okudan (2021) and Szelag-Sikora et al. (2016).

Na/K ratio was detected as the highest 0.68±0.01 mg kg^{-1} in our study. Low Na/K ratios characterize macroalgae. This ratio is considerably lower than those found in various foods, such as olives (43.6), cheddar cheese (8.7) and sausages (4.9). Previous research data indicate that green algae have Na/K ratios ranging from 0.9 to 1 mg kg^{-1}, red algae from 0.1 to 1.8 mg kg^{-1}, and brown algae from 0.3 to 1.5 mg kg^{-1}. The World Health Organization (WHO) recommends Na/K ratios close to unity, highlighting the importance of evaluating the consumption of foods with this ratio or lower for healthy cardiovascular purposes. However, it is worth noting that many developing and developed countries currently have an average daily Na intake of 3.95 mg/day, nearly double the recommended daily intake, which could lead to a general increase in Na intake without considering alternative dietary sources. Using seaweeds like *Cystoseira* in processed foods instead of NaCl (table salt) could be an excellent strategy to reduce overall sodium consumption while increasing potassium intake and enhancing the intake of other essential elements not typically found in the diet.

Trace elements

In Table 3, previous studies for trace elements are given. Macroalgae can accumulate and concentrate elements from their surrounding aquatic environments (Holdt and Kraan, 2011). Seaweeds can serve as a source of trace elements essential for maintaining health, including Fe, Mn, Cu, Zn, and Co. These organisms can accumulate elements necessary for sustaining human health, including Ca, Mg, P, and Fe. Furthermore, macroalgae possess a rich nutrient profile and harbor potential bioactive compounds with antioxidant, anti-inflammatory, and anti-diabetic properties, positioning them as a potential functional food source (Rodrigues et al., 2015; Afonso et al., 2019).

Looking at trace element data, for Zn, the highest value was recorded in the 16:8 F group with 908.06±1.0 µg kg^{-1} while the lowest value was found in the 8:16 RB group at 300.25±0.78 µg kg^{-1}. Different researchers have documented considerable fluctuations in Zn concentrations, even when studying identical macroalgae species. For instance, Akçalı and Küçükksezgin (2011) found Zn content in *Cystoseira sp.* 75.5 ± 12.3 µg g^{-1} collected from Çanakkale/City and 36.9 ± 2.04 µg g^{-1} Çanakkale/Dardanos. Aşıkkutlu and Okudan (2021) found 3.32 mg kg^{-1} in *C. foeniculacea* and 6.87 mg kg^{-1} in *G. montagnei*.

With respect to Fe, the highest value was measured in the 12:12 F group as 5.96±0.03 mg kg^{-1}, while the lowest value was recorded in the 8:16 BG group as 1,93±0,04 mg kg^{-1}. The mean iron concentrations of *Cystoseira sp*. were reported as 302.3 ± 16.6 mg kg^{-1} in the Çanakkale/city center region and 186.6 ± 19.4 mg kg^{-1} in the Çanakkale/Dardanos region (Akçalı and Küçükksezgin, 2011). In the study conducted by Arıcı et al. (2019), the average Fe concentration in *Cystoseira crinita* was reported as 322.5 mg kg^{-1}. In the research conducted by Aşıkkutlu and Okudan (2021), the Fe content was determined as 0.29 mg g^{-1} in *Cystoseira foeniculacea* and 0.67 mg g^{-1} in *Gongolaria montagnei*.

Table 2. Elemental Composition of *G. Barbara* cultivated under different LEDs and photoperiods (DW)

		Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	P (mg kg^{-1})	Na/K (mg kg^{-1})	Fe (mg kg^{-1})	Mo (µg kg $^{-1}$)
16:8	F	186,45±0,37^{a}	809,55±1,08^{c}	139,05±1,48^{a}	297,57±0,46^{d}	31,91±0,15^{d}	0,37±0,01^{d}	3,21±0,017^{c}	5,54±0,22^{a}
	RB	128,15±2,57^{b}	845,36±13,6^{b}	116,10±0,05^{c}	387,20±5,65^{b}	35,35±0,01^{b}	0,46±0,011^{a}	3,33±0,01^{b}	5,55±1,04^{a}
	RG	113,25±0,61^{c}	738,65±3,60^{d}	126,12±0,39^{b}	330,71±2,46^{c}	33,11±0,01^{c}	0,45±0,01^{b}	4,54±0,02^{a}	6,09±0,6^{a}
	BG	105,06±0,25^{d}	1030,10±5,9^{a}	129,10±2,16^{b}	423,71±3,12^{a}	36,71±0,08^{a}	0,41±0,01^{c}	2,56±0,02^{d}	6,56±0,62^{a}
12:12	F	101,47±1,92^{b}	934,44±15,6^{b}	124,35±0,19^{b}	475,41±7,98^{b}	32,04±0,08^{b}	0,51±0,01^{d}	3,89±0,002^{c}	7,96±0,08^{a}
	RB	115,76±1,59^{a}	1041,3±22,2^{a}	134,25±1,77^{a}	564,68±10,2^{a}	39,49±0,12^{a}	0,54±0,01^{b}	5,96±0,03^{a}	7,92±0,43^{a}
	RG	87,17±1,96^{c}	859,3±18,8^{c}	123,353±1,2^{b}	446,4±10,06^{c}	28,61±0,02^{c}	0,52±0,01^{c}	2,65±0,02^{d}	6,01±0,31^{b}
	BG	116,97±2,47^{a}	791,6±13,92^{d}	110,03±0,01^{c}	541,31±9,32^{a}	28,82±0,07^{c}	0,684±0,01^{a}	5,48±0,013^{b}	8,37±0,45^{a}
8:16	F	106,18±0,58^{b}	614,43±8,11^{c}	98,47±0,56^{a}	336,04±3,67^{b}	46,95±0,55^{d}	0,55±0,01^{b}	2,99±0,01^{a}	6,40±1,135^{a}
	RB	110,36±0,24^{b}	559,33±1,57^{d}	94,53±0,97^{b}	311,23±0,99^{c}	78,77±0,25^{a}	0,56±0,02^{a}	2,29±0,013^{b}	6,16±1,07^{a}
	RG	120,03±3,23^{a}	640,839±0,9^{b}	94,963±0,6^{b}	313,36±1,08^{c}	72,05±0,07^{b}	0,49±0,01^{d}	2,15±0,01^{c}	6,04±0,56^{a}
	BG	107,57±2,21^{b}	687,01±3,90^{a}	91,89±0,72^{c}	351,08±1,81^{a}	64,45±0,29^{c}	0,51±0,01^{c}	1,93±0,04^{d}	6,98±0,45^{a}

*Different lowercase letters show the significant differences between the groups according to the ANOVA results ($p<0.05$)

Table 2. Continued

		Mn (µg kg $^{-1}$)	Zn (µg kg $^{-1}$)	Cu (µg kg $^{-1}$)	Cr (µg kg $^{-1}$)	Co (µg kg $^{-1}$)	Cd (µg kg $^{-1}$)	As (µg kg $^{-1}$)
16:8	F	140,15±0,78^{b}	908,06±1,00^{a}	62,1±33,2^{b}	49,80±0,47^{a}	8,48±0,09^{b}	8,89±0,34^{a}	861,86±5,61^{c}
	RB	116,03±1,36^{d}	503,31±1,51^{d}	52,1±28,8^{cd}	51,69±0,86^{a}	9,57±0,46^{a}	4,01±0,09^{b}	828,43±2,47^{d}
	RG	147,51±1,19^{a}	734,81±1,98^{c}	52,7±28,8^{c}	49,91±1,12^{a}	9,96±0,14^{a}	4,229±0,325^{b}	900,6±12,71^{b}
	BG	120,30±0,83^{c}	766,99±0,75^{b}	74,1±30,5^{a}	43,94±1,07^{b}	8,38±0,66^{b}	4,160±0,21^{b}	1329,86±5,3^{a}
12:12	F	180,68±0,43^{c}	551,54±3,14^{b}	53,8±26,6^{a}	65,38±0,00^{cA}	86,579±0,28^{b}	3,183±0,19^{b}	1199,52±3,4^{b}
	RB	227,04±1,68^{a}	657,29±4,20^{a}	61,1±33,0^{a}	114,85±1,91^{a}	88,14±0,67^{a}	4,2980±0,02^{a}	1339,19±2,4^{a}
	RG	120,79±0,05^{d}	547,82±2,89^{b}	54,0±29,0^{a}	48,56±0,72^{d}	70,61±0,36^{d}	4,76±0,05^{a}	842,25±1,12^{d}
	BG	223,81±0,69^{b}	528,98±3,11^{c}	52,2±28,8^{a}	96,453±0,47^{b}	81,56±0,68^{c}	2,68±0,35^{b}	1161,1±7,04^{c}
8:16	F	159,04±0,83^{a}	370,75±2,08^{b}	68,5±43,6^{a}	55,83±0,49^{a}	10,48±0,31^{d}	5,12±0,42^{a}	459,33±5,81^{b}
	RB	119,02±1,53^{b}	300,25±0,78^{d}	55,0±33,8^{a}	51,56±0,38^{b}	11,96±0,07^{c}	2,71±0,37^{bc}	366,62±6,08^{d}
	RG	120,91±2,54^{b}	511,38±9,05^{a}	73,9±44,3^{a}	45,417±0,6^{d}	86,28±0,57^{a}	3,07±0,06^{b}	482,76±0,48^{a}
	BG	107,74±0,10^{c}	337,64±2,13^{c}	62,7±37,2^{a}	48,55±0,58^{c}	22,47±0,54^{b}	2,25±0,14^{c}	2,22±0,011^{d}

* Different lowercase letters show the significant differences between the groups according to the ANOVA results ($p<0.05$)

Table 3. Previous studies on trace elements in *Cystoseira* and *Gongolaria* species (mg kg^{-1})

Region/ Site	Species	As mg kg^{-1}	Cd* µg kg^{-1}	Cu mg kg^{-1}	Cr mg kg^{-1}	Zn mg kg^{-1}	Co mg kg^{-1}	Fe mg kg^{-1}	References
Çanakkale	*Cystoseira sp.*	-	89.9± 9.18	6.73± 1.72	2.13± 0.71	75.5± 12.3	6.24± 1.04	302.3± 16.6	Akçalı and Küçükksezgin, (2011)
Dardanos	*Cystoseira sp.*	-	278.3±49.2	3.13± 0.36	4.76± 0.80	36.9± 2.04	2.31± 0.39	186.6± 19.4	Akçalı and Küçükksezgin, (2011)
Sinop	*C.crinita*	0.87	-			47.89	19.09	322.5	Arıcı et al., (2019)
Black Sea	*C. barbata*	-	-	5-37	-	65		327±18	Arici and Bat, (2016)
Greece	*C. barbata*	-	60- 2600	0.7- 8.8	-	8.8- 58.1	0.02- 2.5	-	Sawidis et al., (2001)
Bulgaria	*C.crinita*	0.85	-	-	0.04	1.63	0.02	6.1	Kravtsova et al., (2014)
Crimea	*C. crinita*	28.1	-	-	-	18.6	19.09	1460	Kravtsova et al., (2014)
Antalya	*C. foeniculacea*	-	800± 300	1.66± 0.46	1.00±0.01	3.32± 0.49	1.00± 0.01	289.88±48.96	Aşıkkutlu and Okudan, (2021)
Antalya	*G.montagnei*	-	-	6.22± 0.63	1.64± 0.47	6.87± 0.88	0.98±0.01	667.72±162.21	Aşıkkutlu and Okudan, (2021)

The highest value for Cd was observed in the 16:8 F group as 8.89±0.34 µg kg^{-1}, with the lowest value being determined in the 8:16 RB group as 2.25±0.14 µg kg^{-1}. Reported Cd concentrations in various species of seaweeds are given in Table 3. In the study by Aşıkkutlu and Okudan (2021), it was found that Cd levels were 0.80 mg kg^{-1} in *Cystoseira foeniculacea*. In *Cystoseira crinita,* the mean Cd concentration was 0.23 mg kg^{-1} (Arıcı et al., 2019). Akcalı and Küçüksezgin (2011) conducted a study on *Cystoseira sp*. and reported the mean Cd concentrations as 0.09 mg kg^{-1} in Çanakkale/city center and 0.05 mg kg^{-1} in Çanakkale/Dardanos. Our results on Cd were lower than those reported in previous studies.

For Mn, the highest value was measured at 227.04±1.68 µg kg^{-1}, and the lowest was 107.74±0.11 µg kg^{-1}. Aşıkkutlu and Okudan (2021), reported a Mn level of 0.79 mg kg^{-1} in *G. montagnei*, while it was below the measurement range in *C. foeniculace*. Ak et al., (2020) found that the mean Mn concentration in *C. barbata* was 0.18 mg kg^{-1}. Our results showed lower Mn levels than those reported in the existing literature.

In the present study, the highest value for Cu was measured as 74.1±30.5 µg kg^{-1} at 16:8 BG and the lowest as 52.1±28.8 µg kg^{-1} at 16:8 RG. Aşıkkutlu and Okudan (2021), observed that Cu levels were 1.66 mg kg^{-1} in *C. foeniculacea* and 6.22 mg kg^{-1} in *G. montagnei*. A mean Cu concentration of 10.20 mg kg^{-1} in *C. barbata* and 4.27 mg kg^{-1} in *Cystoseira crinite* were reported by Arıcı et al., (2019). In the study by Akçalı and Küçüksezgin (2011) on *Cystoseira sp*., the mean Cu values were determined as 6.73 mg kg^{-1} in the Çanakkale/city center and 3.13 mg kg^{-1} in the Çanakkale/Dardanos. Our results were considerably lower than those reported in earlier studies.

In this study, the highest Co value was 88.143±0.67 µg kg^{-1} at 12:12 RB, and the lowest was measured as 8,38±0,66 µg kg^{-1} at 16:8 BG. Co is required for the synthesis of vitamin B12, and is essential for propionate metabolism and cytosolic transmethylation of homocysteine. In an earlier study, Co was reported as 1.00 mg kg^{-1} in *C. foeniculacea* and 0.98 mg kg^{-1} in *G. montagnei* (Aşıkkutlu and Okudan, 2021). Arıcı et al. (2019) measured the mean value for Co in *C. barbata*as 0.50 mg kg^{-1} and in *C. crinita* as 0.57 mg kg^{-1}. Our findings were similar to those reported in other studies.

For Cr, the highest value was measured as 114.85±1.91 µg kg^{-1} at 12:12 RB group and the lowest at 16:8 BG as 43.94±1.06 µg kg^{-1}. The mean Cr values for *Cystoseira sp.* were determined as 2.13 mg kg^{-1} in the Çanakkale/city center region and 4.76 mg kg^{-1} in the Çanakkale/Dardanos region (Akçalı and Küçükksezgin, 2011). In a previous study, Cr was found as 1.00 mg kg^{-1} in *Cystoseira foeniculacea* and 1.64 mg kg^{-1} in *Gongolaria montagnei* (Aşıkkutlu and Okudan, 2021). Overall, our results were lower than those reported in the literature.

In the present syudy, the highest value for As was 1339.19±2.35 µg kg^{-1} and the lowest value was 2.22±0.01 µg kg^{-1}. Arıcı et al. (2019) reported 0.87 mg $kg^{-1,}$ and Kravtsova et al. (2015) reported 28.1 mg kg^{-1}.

Table 4. International regulations on trace element permissible limits in macroalgae (µg g^{-1})

Organization/Authority	As	Cd	Zn	Cu	Fe	References
Ceva, 2014	3	0.5				CEVA, (2014)
Australian and New Zealand Food Standard Authority		1	14	10	8	ANZFA, (2005)
Eu Commission(EC No: 488/2014		3				EC (2008, 2017)
Codex Alimentarius Commission (FAO/WHO)		0.2				FAO/WHO, (1995)
Institute of Medicine USA	10					Institute of Medicine (US), (2001)
Max values in the present study	1.33	0.008	0.9	0.07	5.95	

Daily intake of seaweeds

Even though macroalgae are gaining increased attention as a potential food source, their limited consumption in Europe has led to relatively limited regulations and a lack of consistent EU-level guidelines for macroalgae-based foods. Nevertheless, there are established regulatory limits for the toxic metals and trace elements in macroalgae, which are outlined in Table 4. These results can be cross-referenced with the maximum residual limits (MRLs) set by different authorities. According to EC regulations from 2014 and the Codex Alimentarius Commission (FAO/WHO) (as shown in Table 4), the MRL for Cadmium (Cd) is set at 3 µg g^{-1} and 0.2 µg $g^{-1,}$ respectively, for dried seaweed. Our study indicated that all the experiment groups of *G. barbata* had lower Cd levels than indicated by EC regulation and (FAO/WHO). With respect to Cu, Zn, Fe, and Cd, the Australian and New Zealand Food Standard Authority (ANZFA) regulations specify MRLs of 10 µg g^{-1},14 µg g^{-1}, 2 µg g^{-1}, and 1 µg g^{-1}, respectively (ANZFA, 2005). All the experimental groups in the study had Cu, Zn, Fe, and Cd levels below these MRLs. It is important to note that elevated levels of heavy metals in seagrasses could potentially impact their reproduction, physiology, growth rate, and metabolism as documented in previous studies (Farias et al., 2017; Roberts et al., 2008). Therefore, additional research is necessary to gain a comprehensive understanding of the impact of heavy metals on seagrasses and their ecosystems. Research should focus on to the mechanisms of metal accumulation and the potential for biomagnification within the food chain. Today, macroalgae are recognized as a rich source of diverse bioactive compounds with considerable market potential, highlighted by Holdt and Kraan (2011). One of the fastest-growing segments in the European macroalgae market is the "superfood" category, and macroalgae-based dietary supplements are becoming increasingly common in groceries. However, it is worth noting that the European market is still predominantly supplied by imported macroalgae products (FAO, 2019).

Conclusion

This study investigated the impact of various types of dichromatic LED lights and different photoperiod durations on the macro and trace element compositions of *G. barbata*. The findings highlight that LED lighting conditions can significantly influence the elemental composition of this seaweed species.

Specifically, different LED lighting periods and colors indicated pronounced macro and trace element variations. This variability suggests that *G. barbata* may have distinct nutritional requirements and potential applications based on specific lighting conditions. Furthermore, the ability of macroalgae to accumulate essential elements underscores their potential as a functional food source. This research emphasized the importance of understanding how dichromatic LED lighting conditions could affect the elemental composition of organisms like *G. barbata*, and their potential for sustainable production and diverse industrial applications.

In conclusion, this study contributes to understanding the seaweed's potential for future food and industrial applications by demonstrating how different LED lighting conditions impact the elemental composition of *G. barbata*.

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Conflict of Interest

There is no conflict of interest between the authors.

Author Contributions

MY, and IA contributed the concepts and design of experiments. MY and IA. have performed the experiments MY was carried out data analysis. IA has responsibility for the integrity of the work and the correspondence.

Ethics Approval

Ethics committee approval is not required for this study

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RESEARCH ARTICLE

Spatial and Temporal Variability of the Surface Temperature in the Black Sea Between 2000-2022

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Abstract: This study presents a comprehensive assessment of the spatio-temporal variability of Sea Surface Temperature (SST) in the Black Sea at monthly to interannual scales, with a focus on understanding its connection to major large-scale atmospheric forcing during the period 2000-2022. Monthly variations of SST in the Black Sea reveal distinct seasonal patterns. The study evaluates the potential impacts of large-scale atmospheric patterns on interannual SST variations using climate indices such as the North Atlantic Oscillation (NAO), East Atlantic/West Russia (EA-WR) and El Niño-Southern Oscillation (ENSO) during the winter months. The results indicated that these large-scale atmospheric oscillations played a substantial role in influencing SST anomalies, with the NAO and EA-WR indices particularly affecting the Black Sea's SST anomalies. The NAO index exhibited negative values during warm winters and positive values during cold winters, with extreme cold and warm winters corresponding to specific years, as observed in 2003, 2006, 2012, 2017 (cold) and 2018, 2020, 2021 (warm). Notably, the relationship between NAO and SST anomalies was not as dominant during 2000-2022. This difference might be explained by the combined influence of NAO and ENSO, which is beyond the scope of this study. The EA-WR pattern was identified as another significant large-scale atmospheric dynamic affecting the Black Sea's SST. Although it explains the cold SST anomalies in certain years, it cannot account for extreme warm SST years. While the influence of ENSO remains somewhat inconclusive for the extreme warm period, the SST pattern between 2016-2022 aligns closely with El Niño events, particularly in 2018 and 2021 when positive SOI index values coincide with warm SST years in the Black Sea.

Anahtar kelimeler:

Karadeniz
Deniz yüzey sıcaklığı (DYS) değişkenliği
DYS anomalisi
İklim
Isınma eğilimi
İklim endeksi

Karadeniz'de 2000-2022 Yılları Arasında Yüzey Sıcaklığının Mekansal ve Zamansal Değişimi

Öz: Bu çalışma, 2000-2022 yılları arasında Karadeniz'deki Deniz Yüzeyi Sıcaklığının (DYS) uzamsal-zamansal değişkenliğinin aylık ve yıllar arası ölçeklerde kapsamlı bir değerlendirmesini sunmakta olup, uzun dönemli DYS değişimlerinin büyük ölçekli atmosferik sistemlerin bağlantısının anlaşılmasına amacındadır. Karadeniz'deki DYS'nin aylık değişimleri farklı mevsimsel dağılım biçimleri göstermektedir. Çalışma, kış aylarında Kuzey Atlantik Salınımı (NAO), Doğu Atlantik/Batı Rusya (EA-WR) ve El Niño-Güney Salınımı (ENSO) gibi iklim endekslerini kullanarak büyük ölçekli atmosferik modellerin yıllar arası DYS değişimleri üzerindeki potansiyel etkilerini değerlendiriyor. Sonuçlar, incelenen büyük ölçekli atmosferik salınımların SST anomalilerini etkilemede önemli bir rol oynadığını, özellikle NAO ve EA-WR endekslerinin Karadeniz'in SST anormalliklerini etkilediğini göstermektedir. NAO endeksi, 2003, 2006, 2012, 2017 (soğuk) ve 2018, 2020, 2021 (sıcak) yıllarında gözlemlendiği, sıcak kışlarda negatif değerler ve soğuk kışlarda pozitif değerler sergilemiştir. NAO ve DYS anomalileri arasındaki ilişki 2000-2022 döneminde o kadar baskın değildir; bu farklılık, muhtemelen bu çalışmanın kapsamı dışında tutulan NAO ve ENSO'nun birleşik etkisinden kaynaklanmıştır. EA-WR paterni, Karadeniz'in DYS'nı etkileyen bir diğer önemli büyük ölçekli atmosferik dinamik olarak tanımlanmıştır. Belirli yıllardaki soğuk DYS anomalilerini açıklasa da aşırı sıcak DYS yıllarını açıklayamaz. ENSO'nun etkisi DYS ekstrem ısınma dönemlerini açıklamak için bir şekilde sonuçsuz kalsa da 2016-2022 arasındaki DYS değişimleri, özellikle pozitif SOI endeks değerlerinin Karadeniz'deki sıcak SST yıllarıyla çakıştığı 2018 ve 2021'deki El Niño olaylarıyla yakından uyumludur.

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Introduction

The river runoff predominately from the northwestern Black Sea and the Mediterranean inflow through the Bosporus Strait are the major fluxes to the Black Sea conserving extremely stable two-layer stratification in the Black Sea (Oğuz et al., 1993a; Özsoy & Ünlüata, 1997; Sur et al., 1994). The upper layer is confined to approximately 150m which leads the Black Sea surface layer sensitive to atmospheric conditions. The physical characteristics change further by the circulation dynamics which are mainly the Rim Current following the continental slope surrounding the cyclonic gyres inside and anticyclones on the peripheries (Korotaev, 2003a; Oğuz et al., 1993a). One of the most important features in the Black Sea is the accumulation of cold waters at an intermediate depth above the main halocline, the so-called Cold Intermediate Layer (CIL), maintaining the sinking of surface waters, making colder and denser by loss of heat towards the atmosphere in the winter (Akpınar et al., 2017; Akpınar et al., 2022; Belokopytov, 2011; Capet et al., 2012, 2020; Gregg, 2005; Miladinova et al., 2018; Oğuz & Beşiktepe, 1999; Stanev & Chtirkova, 2021).

Sea Surface Temperature (SST) is one of the essential parameters for oceanography to evaluate physical dynamics and biogeochemical processes and ecological conditions in the upper ocean as well as reflecting the climate signal on marine ecology (Capet et al., 2012; Ginzburg et al., 2004, 2007, 2021; Oğuz et al., 2003, 2006; Stanev et al., 2019). The winter SST variations give insight about the trend of the Cold Intermediate Layer (CIL) formed in the winter as observations of the mean CIL temperature follows the same trend with the winter SST (Akpınar et al., 2017; Oğuz et al., 2006). Therefore, SST is an indicator of the intensity of convective mixing and hence the CIL formation. Black Sea SST has significant variability at seasonal to inter-annual time scales as being subject to varying atmospheric conditions together with the combined effect of meso-scale to large-scale dynamics (Artamonov et al., 2020; Capet et al., 2012; Efimov & Komarovskaya, 2018; Ginzburg et al., 2004, 2007; Kazmin & Zatsepin, 2007; Shapiro, Aleynik, et al., 2010). In the long term time scales, many studies have shown a warming trend of SST began in 1900's in the Black Sea and leading changes in its physical and biochemical properties (Capet et al., 2012, 2016, 2020; Çokacar et al., 2004; Ginzburg et al., 2004; Kazmin & Zatsepin, 2007; Kubryakova et al., 2018; Mikaelyan et al., 2011; Oğuz et al., 2003; Shapiro, Aleynik, et al., 2010; von Schuckmann et al., 2016). Black Sea is considered one of the hotspots globally (Bulgin et al., 2020). The intermediate layers warming trend is found to be even stronger than the surface layers (Miladinova et al., 2017). As Stanev et al., (2019) pointed out this tendency could result in disappearance of a distinctive layer in the Black Sea.

Large-scale atmospheric systems determine the variations in the ocean-atmosphere climatic system and have an important role on regional climatic changes. The North Atlantic Oscillation (NAO), the East Atlantic-West Russia (EA-WR) atmospheric system and El Niño-Southern Oscillation (ENSO) are the systems that are mostly studied for the Black Sea. The positive phase of the NAO shows colder winters, and the negative case corresponds to the milder and more wet winters in the Black Sea. It was shown that during 1980-1995, SST under cold winter conditions was in accord with the positive face of NAO index, whereas 1960-1980 period corresponded to negative phase of NAO index with warm SST. While the north Atlantic Oscillation (NAO) significantly influences the Mediterranean weather and the Black Sea hydrodynamics (Hurrell & Deser, 2009), the east Atlantic-west Russia (EA-WR) pattern, has been found to be more closely associated with the Black Sea SST (Oğuz et al., 2006). It is also shown that the combination of the NAO and EA-WR teleconnection patterns is effective in explaining variability link to climate change in the Black Sea region (Oğuz et al., 2006). In addition, Ginzburg et al., (2004) observed that the El Niño Southern Oscillation (ENSO) contributes to the occurrence of extreme (i.e., minima and maxima) sea surface temperature (SST) values in the Black Sea.

The decade between 2000 - 2009 is called the "global warming hiatus" during which the global mean SSTA changed less than those in the previous or subsequent decades (Fyfe et al., 2016; Trenberth & Fasullo, 2013). Therefore, our study focused on the period between 2000 - 2022. This study aims to improve our limited knowledge on the spatial and temporal variability of SST and the general pattern of the long-term SST variability in the Black Sea during 2000–2022, associated with the major large-scale atmospheric forcing. Including the analysis of trends in SST with the latest data, this study complements earlier studies based on the analyses of spatial and temporal changes of SST and their links to large scale atmospheric systems.

Material and Methods

Data

The Copernicus Marine Environment Monitoring Service (CMEMS) SST analyses regional SST product is used in this study. Reprocessed Black Sea SST dataset (REP BS SST, https:// resources. marine. copernicus.eu / product- detail / SST_ BS _ S ST_ L4_REP_ OBSERVATIONS _ 010_ 022 / INFORMATION, is accessed on 2 June 2023. The collated Level- 3C (merged single-sensor, L3C) climate data record provided by the ESA Climate Change Initiative (CCI) and the Copernicus Climate Change Service (C3S) initiatives, but also include in input an adjusted version of the AVHRR Pathfinder dataset is built from reprocessed BS SST products (Merchant et al., 2019; Pisano et al., 2016). Basic statistics were computed with the daily SST data, including monthly temporal means. Monthly means were estimated from daily data spanning January 2000 and December 2022. The spatial distribution of SST analyses to evaluate seasonal changes built on 12 months of a year and winter months associated with the standard deviations were constructed from data in between the years 2000 and 2022.

The most relevant measure of large-scale air pressure oscillations to Black Sea are North Atlantic Oscillation (NAO), the East Atlantic – Western Russia (EA-WR) and El Niño–Southern Oscillation (ENSO). The index data sets were obtained from https://psl.noaa.gov/. The NAO index is based on the surface sea-level pressure difference between the Subtropical (Azores) High and the Subpolar Low. The EA-WR index associated with four positive phase is associated with four centers: Europe-northern China, and central North Atlantic-north of the Caspian Sea. Southern Oscillation (SOI) index is based on differences in sea level pressure (SLP) between Tahiti and Darwin, Australia. i.e. between western and eastern tropical Pacific, oscillating with El Niño and El Nina events. The winter means are calculated by averaging December, January, February and March following the approach in earlier studies (Ginzburg et al., 2007; Oğuz et al., 2006).

Results

Monthly variabilities of SST

The monthly means computed using 23 years SST data (2000-2022) shows a clear seasonal cycle with the highest temperatures in July and August (Figure 1). In general, temperature spatial contrasts are larger in the coldest months with 6-7 °C differences between the northwestern and southeastern areas. The spatial temperature differences in the summer are reduced to 3-4 °C when the SST can exceed 25 °C. The winter conditions typically persist for January, February and March, after which the circulation structure evolves to its subsequent spring phase during April, May and June. The period between December to March is characterized by west to east SST gradient with lower temperatures in the western basin and warmer in the eastern (Figure 1). This meridional gradient in the SST is related to the Rim Current. The coldest waters in the northwestern shelf spread along the western and southern coasts to the eastern part along the Rim Current periphery while the relatively warm water from the east follows the periphery area to the west in accordance with cyclonic circulation (Korotaev, 2003b; Korotaev et al., 2006; Oğuz et al., 1993b; Özsoy & Ünlüata, 1997). The zonal gradient in the central cyclonic area can be explained by the exchanges between the Northwestern Shelf and the cyclonic interior part mainly mediated by the Sevastopol eddy (Capet et al., 2012; Oğuz & Beşiktepe, 1999; Shapiro, Stanichny, et al., 2010). With the onset of spring, the thermocline starts in the upper water column. The southwestern and northeastern parts of the basin SST are colder than the rest of the basin (Figure 1). The eastern part becomes the warmest area in the basin. In fact, it is related with the formation of the Batumi anticyclone in this area. Batumi eddy, which was absent in the winter months, begins to appear in the southeastern corner of the basin and persists during the spring period. The Batumi anticyclone is the greatest and most persistent coastal eddy which forms in early March and lives until the end of October. In summer, the lowest SST distribution is again observed in the northwestern part of the sea. These cold-water observations are related with the wind driven coastal upwelling frequently observed in the area in summer (Ginzburg et al., 2002; Tolmazin, 1985). Then the anticyclonic eddies propagate this cold water along the continental slope (Ginzburg et al., 2002; Oğuz & Beşiktepe, 1999). May, June July can be considered as a transitional period because of the shift of SST gradients from zonal to meridional. Finally in August, basin SST gradients shift to meridional gradient in which the colder temperatures are observed in the north and warmer in the south. The temperature in the inner cyclonic part and the NWS are the lowest SST areas compared to the rest of the area.

In the autumn, the temperature minimum off the southeast of Crimea stands out (Figure 1). These relatively cold waters can be related to the divergency. On the other hand, warmer waters in the northern border of this cold formation are related with the Rim Current and the coastal anticyclones propagating with the Rim Current (Ginzburg et al., 2004).

The 23-years temporal mean of winter SST shows a significant meridional temperature difference (Figure 2 top). While the NWS has the lowest temperatures, the highest temperatures are observed in the southeast of the basin. In the entire basin, the mean winter temperature ranges between 4 °C and 11 °C. The variation of SST are highest on the northwestern shelf (Figure 2, bottom) which is related to changes in river fluxes; wind forcing and propagation of anticyclonic eddies within the area (Ginzburg et al., 2002; Shapiro, Stanichny, et al., 2010). In the southern Rim Current periphery and southeast of the Black Sea, the standard deviations of SST are also high (Figure 2 bottom) due to the local dynamical features branches of the Rim Current and anticyclones (Akpınar et al., 2022; Ginzburg et al., 2007; Korotaev, 2003a; Oğuz et al., 1993a).

Interannual variabilities of SST link to large scale atmospheric variabilities

The seasonal cycle spatial means of monthly SST is given in Figure 3a. Overall, 2010 was the hottest summer with 27.93 °C and 2006 winter is the coolest winter with 6.23 °C basin wide mean SST's. The winter SSTA indicated that 2003, 2006, 2012 and 2017 were colder in the winter about ~-1°C STA (Figure 3b). In addition, 2021 winter was the warmest (0.9°C) and warm sea surface temperature anomalies were observed in 2018 and 2020. The 2020 winter SSTA was slightly lower than that in 2021. The entire mean winter SSTA data exhibited a linear warming trend with 0.68 °C per decade. The rise in SST is higher (0.78 °C per decade) if all seasons are considered for the calculations. This is consistent with the global warming trend observations (Bulgin et al., 2020; Merchant et al., 2019). There is considerable decadal variation in sea surface temperatures. SSTA variations of the last decade showed mainly positive variations during 2010-2021 while in the preceding decade it was mainly negative. This 10-

year cycles of cold and warm periods are explained by large scale atmospheric dynamics influencing the Black Sea in the study (Oğuz et al., 2006). In order to show the response of the Black Sea SST to NAO, EA-WR, ENSO, we first evaluated similarities between the extreme winter SST values. We used means of the winter months as the indices to compare with winter SSTA. The sign of NAO index was negative for the warm winters and positive in the cold winters. Only 3 winters (2010, 2012, 2021) out of 7 years matched with the extreme cold (2003, 2006, 2012, 2017) and hot (2018, 2020, 2021) winter years. The correlation between the negative phase of NAO and SSTA was determined to be -0.5. The EA-WR pattern was concerned as the next important large scale atmospheric dynamics affecting the Black Sea (Capet et al., 2012; Oğuz et al., 2006). Positive winter EA-WR index corresponds to cold and dry air masses from the north to Black Sea, at its negative values, warm and moist air masses arrive to basin from the south. The combinations of winter EA-WR indices with the winter NAO was found in the studies of Capet et al., (2012) and Oğuz et al., (2006). The study identified that different combinations of the positive and negative winter NAO and EA-WR index values defined either cold or warm winter in the Black Sea. For example, at EA-WR > 0 and NAO < 0, the anomalously cold winters were observed. EA-WR < 0 and NAO < 0 corresponded to warm winters (Capet et al., 2012). Since the evaluation of the combined effect requires the assessment of the change of positions of high and low atmospheric pressure centers, it is not considered in this study. The cold SSTA years 2003, 2006, and 2017 match with positive EA-WR (Figure 3d). On the other hand, extreme warm SSTA years cannot be explained by EA-WR index. Nonetheless, the negative phase of EA-WR demonstrates the highest correlation (-0.63) with SSTA among the indices.

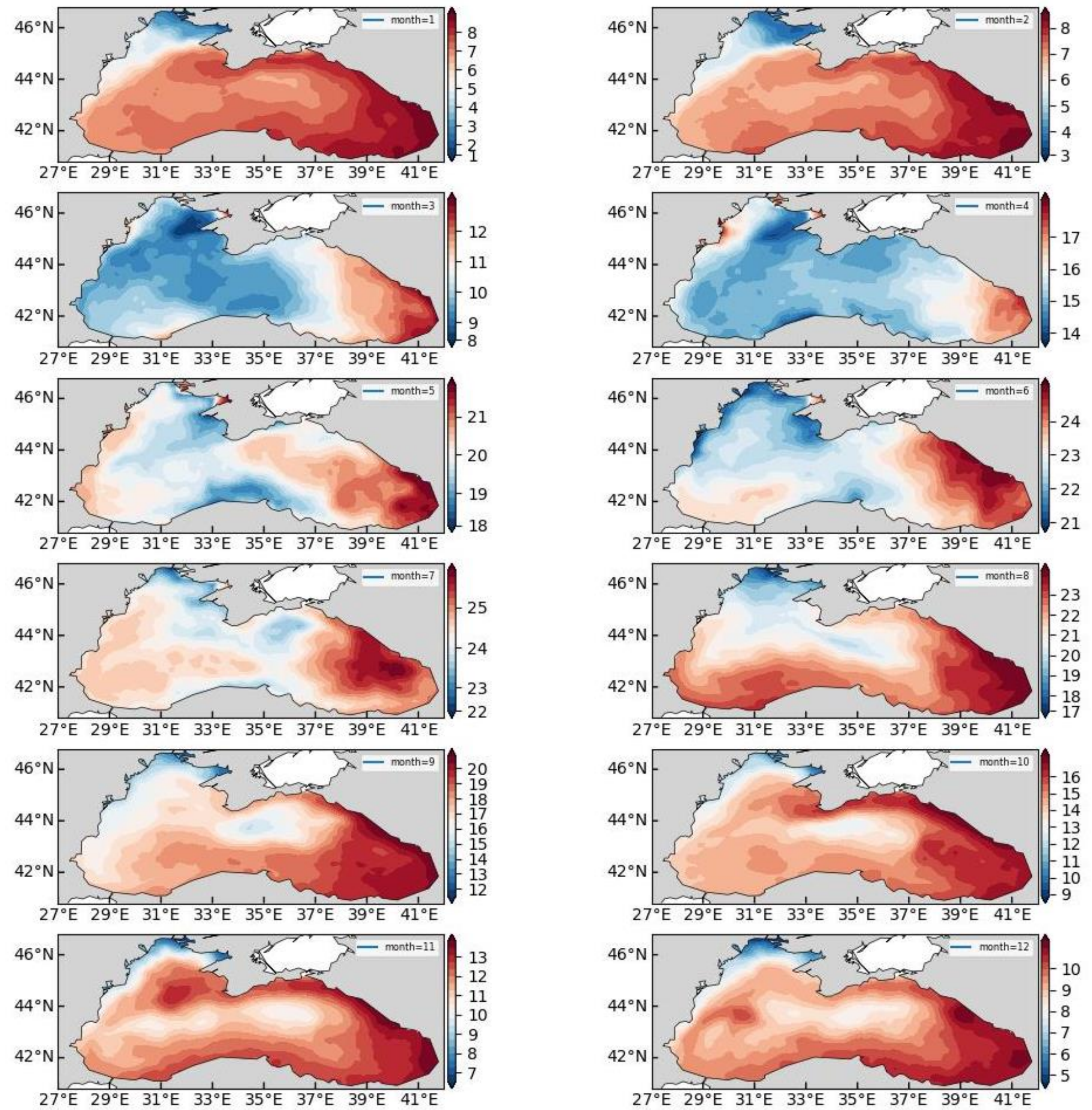

Figure 1. Monthly mean SST calculated from Copernicus CMEMS gridded BS-SST product data during 2000-2022

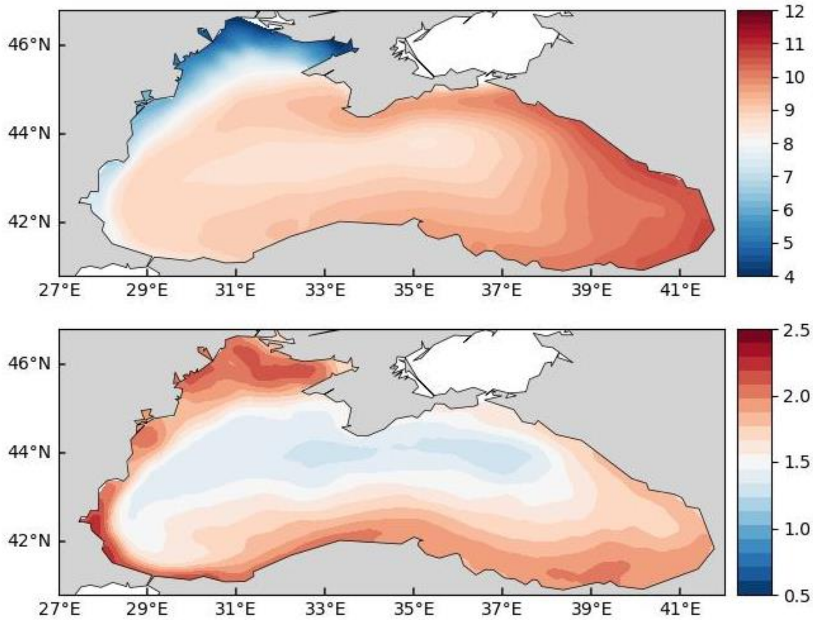

Figure 2. (a)Winter mean and (b) standard deviation of SST calculated from Copernicus CMEMS gridded BS-SST product data during 2000-2022.

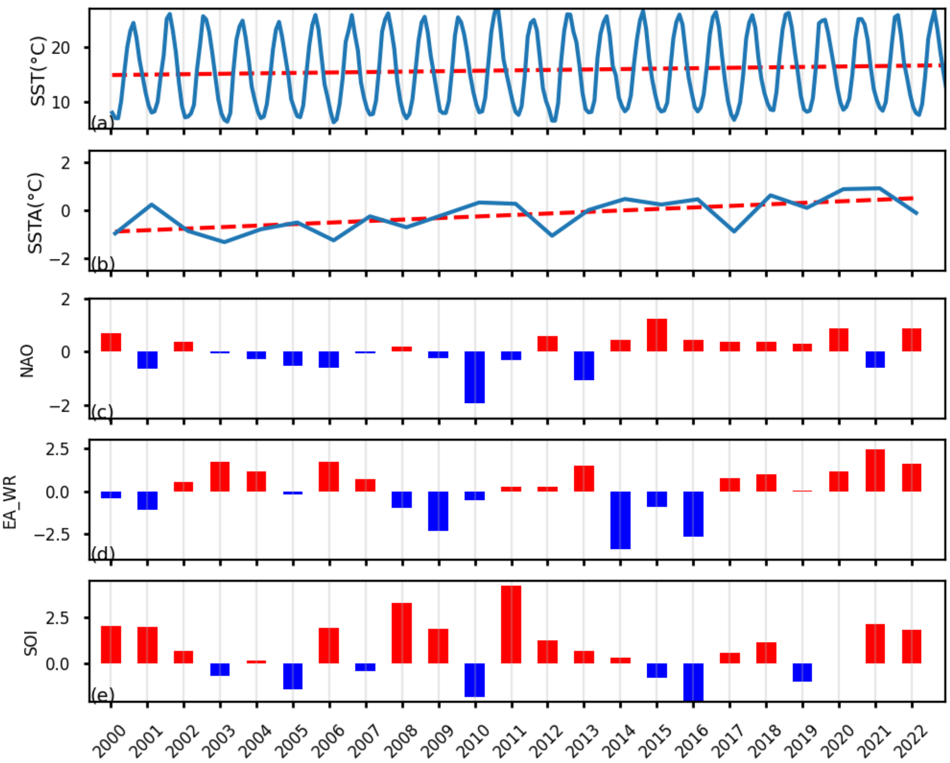

Figure 3. Inter-annual variations of a) the winter (January-March) mean sea surface temperature b) winter mean SSTA calculated from Copernicus CMEMS gridded BS-SST data. Red dash line represents the trend line c) NAO d) EA-WR e) SOI indices.

ENSO is low-frequency oscillation in comparison with the NAO. In the literature, although a consistency was found between the extreme anomalies of SST and the El Niño phases (Ginzburg et al., 2004), no clear relationship was found in the study of Oğuz et al. (2006). The character of the changes in the SSTA during 2016-2022 is very close to the El Niño events. As follows from Figure 3e in the period 2000–2022, El Niño events (positive SOI index) in 2018 and 2021 agreed with the warm SST years in the Black Sea.

Discussion

This study assesses the spatial and temporal variability of SST in monthly to interannual scales and analyses the connection between the general pattern of the long-term SST variability in the Black Sea and the major large-scale atmospheric forcing during 2000–2022, including the analysis of trends of SST in this period.

In the winter, meridional gradient in the SST field was observed with the warming gradient from western to east of the basin. In the spring, by the onset of seasonal warming, the Batumi anticyclone is generated in southeast of the basin. Moreover, SST is colder in the southwestern and northern part of the basin compared to the rest of the basin. The Batumi anticyclone persists in the area between early March and end of October. In the summer, the lowest SST distribution is again observed in the northwestern part of the sea. May, June July can be considered as a transitional period that SST gradients shift from zonal to meridional. Meridional gradients form in SST where the colder temperatures in the north and warmer in the south are observed in the summer. The temperature in the inner cyclonic part and the NWS are the lowest SST areas compared to the rest of the area. In the autumn, the temperature minimum off the southeast of Crimea stands out. Winter SST distributions 23 years seasonal mean of winter SST shows a significant variation in the southern Rim Current periphery and southeast of the Black Sea due to the local dynamical features of the Rim Current and accompanied by small to mesoscale scale anticyclonic formations.

The potential impacts of large-scale atmospheric patterns on the interannual variations on the SST are assessed by the climate indices NAO, EA-WR and ENSO obtained for the winter months. Different time scales of influence from large-scale teleconnection patterns on air temperature (AT) are distinguished: the East Atlantic/West Russia (EA-WR) pattern influences short-term (1-5 years) variations in the sea surface temperature (SST), whereas the long-term trends of the North Atlantic Oscillation (NAO) drive prolonged (8-10 years) SST trends (Capet et al., 2012). The SST anomaly follows both the long-term influence of the NAO index and the rapid oscillation governed by the EA-WR index. Simultaneous cold and warm cycles, each with an approximate duration of 5 to 10 years, are noted in SSTA time series. The sign of NAO index is negative for the warm winters and positive in the cold winters. The SSTA analysis depicts extreme cold winters for the years 2003, 2006, 2012, and 2017 and hot winters for 2018, 2020, and 2021. The correlation between NAO and SSTA found to be exist for negative phase of the NAO. In contrast, the general consistency found between the interannual SST variations and the NAO in the Black Sea (Capet et al., 2012; Oğuz et al., 2006). This relation is not so dominant during 2000-2022. This difference may be explained by analyzing the combined NAO and ENSO during the years 2000-2022. However, it requires the analysis of the change in positions of high and low atmospheric pressure centers, which is out of the scope of this study. The EA-WR pattern is concerned as the next important large scale atmospheric dynamics affecting the Black Sea (Capet et al., 2012; Oğuz et al., 2006). The cold SSTA years 2003, 2006, and 2017 match with positive EA-WR however, extreme warm SSTA years cannot be explained by EA_WR index. Among the indices, the negative phase of EA-WR demonstrates the highest correlation with SSTA. Even though the influence of the ENSO index could not be deduced clearly from the analysis in this study, the changes the SSTA pattern during 2016-2022 is very close to the El Niño events. El Niño events (positive SOI index) occurred in 2018 and 2021 agreed with the warm SST years in the Black Sea. The dataset, spanning 23 years, is relatively short for accurately estimating SST trends. However, the analyses of the influence of NAO, EA_WR and ENSO on SSTA shows statistically significant correlations for negative phases of the EA-WR pattern and NAO index between the years 2000-2022. This study complements earlier studies based on the analyses of spatial and temporal changes of SST and their interaction to large scale atmospheric systems. This study improves our limited knowledge during the period of 2000-2022 and compares the recent results with the literature.

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Conflict of Interest

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

Ethics Approval

Ethics committee approval is not required for this study.

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RESEARCH ARTICLE

Extraction and Characterization of Acid Soluble Collagen From Golden Grey Mullet (*Chelon auratus*) Scale

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Key words:

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Collagen characterization

Abstract: In the present study, the potential of waste fish scales for obtaining a valuable bioactive material was evaluated. Acid soluble collagen from golden grey mullet (*Chelon auratus*) scales was isolated and characterized successfully. Proximate composition, denaturation temperature, Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE), amino acid composition, Scanning electron microscopy and Energy dispersive X-ray spectroscopy (SEM-EDS), Fourier transform infrared spectrophotometer (FTIR), Thermogravimetric analysis (TGA), antioxidant and antimicrobial activity analyses were performed for the extraction of collagen and extracted collagen was compared with commercial collagen. As a result, an alternative and useful source for mammalian collagen for the industrial applications like food and cosmetics was extracted from the fish scale waste material, which may help mitigate the management of natural wastes or environmental problems.

Anahtar kelimeler:

Biyoaktif madde
Asitte çözünür kollajen
Balık pulu
Altınbaş kefal
Kollajen karakterizasyonu

Altınbaş Kefal (*Chelon auratus*) Pullarından Asitte Çözünür Kollajen Ekstraksiyonu ve Karakterizasyonu

Öz: Bu çalışmada değerli bir biyoaktif materyal elde etmek amacıyla atık balık pullarının potensiyali incelenmiştir. Altınbaş kefal (*Chelon auratus*) pullarından asitte çözünür kollajen izole edilerek karakterize edilmiştir. Ekstrakte edilen kollajenin besin kompozisyonu, denatürasyon sıcaklığı, Sodyum dodesil sülfat jel elektroforezi (SDS-PAGE), amino asit bileşimi, Taramalı elektron mikroskobu ve Enerji dağılımlı X-ışınları spektrometre (SEM-EDS), Fourier dönüşümü kızılötesi spektrofotometre (FTIR), Termogravimetrik analiz (TGA), antioksidan ve antimikrobiyal aktivite analizleri yapılarak ticari kollajenle karşılaştırılmıştır. Sonuç olarak, balık pulu atıklarından, doğal atıkların veya çevre sorunlarının yönetimine katkı sağlayacak, memeli kollajenine alternatif, gıda ve kozmetik gibi endüstriyel alanlarda kullanılabilecek bir kaynak elde edilmiştir.

Introduction

Bioactive substances of fish such as lipids, proteins, vitamins, minerals, and also other fish by-products are considered important functional sources due to their high nutraceutic and cosmeceutic and therapeutic potential (Atef and Ojagh, 2017; Ashraf *et al.,* 2020). These resources have a wide variety of applications i.e in the field of nutrition as a dietary supplement, in pharmacy as antitumor and anti-viral, in agriculture due to their herbicide, insecticidal, and fungicidal activities, and in cosmetics as sunscreen and anti-aging (Zayed, 2018). Today, various fish wastes such as skin, muscle, skeleton, bone and internal organs are used to isolate many bioactive materials (Ashraf *et al.*, 2020). The annual total production of aquatic products exceeded one hundred million tons in the world, and approximately 10 % of this is discarded due to spoilage (Yu and Gu, 2015). These wastes cause danger for the environment because of high biological and chemical oxygen demand, associated pathogens and organic substances, etc. (Knidri *et al.,* 2018). Recently, in numerous scientific studies, the potential of the versatile by-products of fish and crustaceans processing, by converting into high-value bio-compounds such as collagen and gelatin, bioactive peptides, enzymes and specific proteins, chitin, chitosan and pigments, were reported to provide economic and environmental benefits (Hamdi *et al.,* 2017).

Fish scales constitute about 5 % of fish waste and are considered as an important industrial waste (Fehng, 2016). Fish scales are not generally recycled and therefore serious environmental problems are caused by their storage, incineration or disposal to water bodies. Hence, it is very

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important to evaluate these wastes which are a good sources of proteins, minerals, bioactive peptides, fish oil, enzymes and amino acids (Bhuimbar *et al.*, 2019).

Collagen is the most abundant structural protein in the extracellular matrix of the various connective tissues in the body (i.e., skin, bones, ligaments, tendons, and cartilage) (Jafari *et al.*, 2020). It plays an important role in maintaining the structural and biological integrity of the extracellular matrix and provides physiological functions and mechanical strength to tissues. Collagen has multifunctional properties related to their groups of protein side chains such as having high nutritional value, gelling, emulsifying, foaming and film-forming properties, high water absorption capacity, biocompatibility, bio-degradability, low toxicity, non-antigenicity, high stability, easy processing, native ability to combine with other materials and large-scale extraction (Shalaby *et al.*, 2020; Subhan *et al.*, 2021; Tang *et al.*, 2022). All these properties make them functional ingredients in various food systems, and also it is used in cosmetics, pharmaceutical and biomedical industry in the forms of injectable solutions, thin substrates, porous sponges, nanofibrous matrices, and micro- and nano-spheres (Shalaby *et al.*, 2020; Subhan *et al.*, 2021, Tang *et al.*, 2022; Rajabimashhadi *et al.*, 2023). Marine collagen obtained from fish parts such as skin, bone, cartilage and scales, as well as from marine vertebrates and invertebrates, are more bioavailable and a good alternative to bovine or porcine collagen (Jafari *et al.*, 2020).

Golden grey mullet has high economic value that is frequently fished in Dalyan,Köyceğiz (Türkiye). According to Dalyan Fisheries Cooperative, 408 tonnes of golden grey mullet were caught in 2018. Therefore, golden grey mullet is an important source of fish scale in this region. Although Turkey has important aquaculture resources, utilization of these resources for valuable bioactive components is very limited. In the present study, and in accordance with the concept of sustainability, a valuable bioactive substance, collagen, was isolated from golden grey mullet fish scales.

Material and Methods

Golden grey mullet (*Chelon auratus*) scales were used as study material. Commercial marine fish skin collagen (MM Ingredients Ltd./UK) in the powder form was used to compare the isolated collagen as control. The golden grey mullet scales were obtained from DALKO Fisheries Cooperative (Köyceğiz/Muğla/Turkey). Scales were transferred to Muğla Sıtkı Koçman University Fisheries Faculty Quality Control Laboratories under cold storage. Scales were washed with tap water then dried for 3 days at room temperature (approximately 24 °C).

Acid soluble collagen (ASC) extraction

Acid soluble collagen (ASC) was extracted from golden grey mullet scales according to the method of Ali et al. (2017) with slight modifications. Non-collagenous protein was removed from fish scales using 0.1 M NaOH for 6 h (1:10 w/v). The solution was changed every 3 hours (2 buffer changes). After 6 hours, the pH was decreased by washing with distilled water. Demineralization of the filtered scales was achieved with 0.5 M $EDTA_2$-Na solution (pH:7.4) (1:10 w/v) using a magnetic stirrer for 48-hour. At this stage, the solution was changed every 12 hours and the removal of non-collagen proteins and demineralization phase was completed. The scales were stored at –80 °C for further procedures. After pretreatment, scales were extracted with 0.5 M acetic acid for 48 hours. After 48 hours, the scales were filtered with a double layer of cloth and the treatment continued with the liquid part. The extract was precipitated with 2.5 M NaCl + 0.05 M Tris (hydroxymethyl) aminomethane at pH 7.0 (1:1 v/v) and centrifuged at +4 °C 14,000 g for 1 h using a high speed refrigerated centrifuge machine (HANIL SUPRA 22K). Precipitated gels were dissolved in 0.5 M acetic acid (w/v, 1:9) and dialyzed. Dialysis was carried out with 0.1 M acetic acid (24 hours/2 buffer changes) and than with distilled water (5 hours). Purified collagens were then dried with freeze-dryer for 72 hours at –50 °C. Entire process of extraction was performed at 4 °C.

Proximate composition analysis

The protein, lipid and ash contents of the ASC from fish scale and commercial collagen samples were determined according to the method of AOAC (2006a), AOAC (2006b) and AOAC (2002), respectively. Moisture content was measured with an automatic moisture analyzer (SARTORIUS MA 35). A conversion factor of 5.95 was used for calculating protein content of fish scale (Chuaychan *et al.*, 2015) and 6.38 for collagen (Chen *et al.*, 2018).

Denaturation temperature

The denaturation temperature was determined by the method of Nagai and Suzuki (2000) with slight modification by measuring its viscosity. Viscosity of 1.5 mg/ml of collagen solution in 0.1 M acetic acid was measured. The thermal denaturation curve was obtained from 10 to 60 ºC by measuring at temperature intervals of 2 ºC. The denaturation temperature, *Td*, was determined as the temperature at which change in viscosity was half completed and calculated by the following equation of Wang *et al.* (2008):

Fractional viscosity = (Measured viscosity – Minimum viscosity) / (Maximum viscosity – Minimum viscosity)

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

SDS-PAGE of collagen extracted from grey mullet fish scale was performed according to the method of Laemmli (1970) with 8% separating gel and 4 % stacking gel. 10.6 µg of protein was loaded in each well. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 and then destained in a solution of 40 % (v/v) methanol and 7 % (v/v) acetic acid to visualize band formation. A wide range molecular-weight marker was used to estimate the molecular weight of collagen samples. Molecular weight was calculated by densitometric analysis in Image J (1.52a) program (Günlü *et al.*, 2014).

Amino acid analysis

Amino acid analysis of fish scale and commercial collagen was performed according to modified method of Erkan et al. (2010) using a Shimadzu Prominence LC-20A (Kyoto, Japan) high performance liquid chromatograph (HPLC) system by comparison of their retention time with those of authentic standard (Pierce, Amino Acid Standard Hydrolyzate, product no: 20078 20088 20089 1800180 NCI0180, Rockford, IL 61105 USA).

Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS)

Scanning electron microscopy analysis of fish scale and commercial collagen was examined with JSM 7600F Field Emission Scanning Electron Microscope (JEOL, Japan) at an accelerating voltage of 15 kV. A piece of powder was placed on a specimen stub with double-sided adhesive carbon tape. Elemental analysis of collagen samples was also carried out by energy dispersive X-ray spectroscopy (EDS) (Oxford Instruments, UK) combined with SEM.

FTIR spectrum analysis

FTIR spectrums of fish scale collagen and commercial collagen was monitored by FTIR (Thermo Scientific Nicolet iS10-ATR, USA) at a resolution of 4 cm^{-1} in potassium bromide (KBr) pellets and the spectrum were recorded in the wavelength range from 4,000 to 400 nm^{-1}.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis of the fish scale and commercial collagen was executed by using a TGA instrument (Perkin Elmer TGA 4000, Perkin Elmer, Waltham, MA). Samples were heated from 30 to 600 °C at a rate of 10 °C/min under a nitrogen flow rate of 20 ml/min.

Antioxidant and antimicrobial activity

The antioxidant activity (AA %) of fish scale collagen and commercial collagen were carried out by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity according to method of Brand-Williams *et al.* (1995). The scavenging activity percentage (AA %) was calculated according to Mensor *et al.* (2001).

Antimicrobial activity of the fish scale and commercial collagen was carried out using agar well diffusion method (NCCLS, 1993). For this purpose, a Gram positive strain, *Staphylococcus aureus* ATCC (American Type Culture Collection) 25923, a Gram negative strain, *Escherichia coli* ATCC 25922 and a yeast *Candida albicans* (ATCC 10231) provided from Ankara Refik Saydam Hıfzısıhha Institute (Ankara, Turkey), were used. After the convenient incubation period for each microorganism, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms.

Statistical analysis

All experiments were carried out in triplicate and for each parameter, results were reported as mean and standard deviation. Means were compared by the analysis of variance (ANOVA) in SPSS (Version 21, SPSS Inc., Chicago, IL, USA) software. Tukey's multiple range test ($p < 0.05$) was used to detect differences among mean values of all test intervals.

Results and Discussion

Proximate composition

The proximate composition of fish scale, fish scale collagen and commercial collagen was shown in Table 1. The protein, lipid, ash and moisture contents of golden grey mullet fish scales were 39.99 ± 1.54 %, 0.08 ± 0.01 %; 40.07 ± 0.20 % and 21.42 ± 0.09 %, respectively. As a result of the ash analysis after demineralization of the collagen production, the ratio of ash obtained was 0.04 ± 0.02 %. In this context, it has been observed that the removal of minerals was carried out successfully.

Protein, lipid, ash and moisture values were similar to those reported for *Sciaenops ocellatus* scales; 41.1 ± 0.1 %; 0.4 ± 0.1 %; 42.4 ± 0.1 % and 16.1 ± 0.1 % respectively (Chen *et al.*, 2016). These values were 24.32 %; 0.68 %; 47.31 % and 26.72 %; respectively, for the scales of seabass (*Lates calcarifer*) (Chuaychan *et al.*, 2015). Scale composition reported for mullet was 31.12 % protein, 0.20 % lipid, 30.01 % ash and 37.87 % moisture (Cao *et al.*, 2017). Thuy *et al.* (2014) found protein content of mullet scale as 50.4 ± 0.4 % and ash content as 47.9 ± 0.6 %, while Wang *et al.* (2008) found the amount of protein in *Sebastes mentella* scale as 56.9 %; ash amount as 39.4 %. The nutrient contents of golden grey mullet scale examined in the present study are similar to those reported by other researchers. With respect to lipid content, our findings were low as expected.

The values of protein, lipid, ash and moisture of fish scale collagen isolated in the present study were found as 94.52 ± 2.02 %, 0.67 ± 0.11 %, 0.98 ± 0.01 % and 0.17 ± 0.01 %, respectively. Also the protein, lipid, ash and moisture values of the commercial fish skin collagen were determined as 98.89 ± 0.85 %, 0.03 ± 0.01 %, 1.49 ± 0.02 % and 3.09 ± 0.93 %, respectively. Collagen, a connective tissue protein, is expected to have a high content of protein and a fairly low content of lipid, moisture and ash. It was determined that the difference between protein, lipid, ash and moisture values in fish scale collagen and commercial collagen was statistically significant ($p < 0.05$) (Table 1). Nurilmala *et al.* (2019) detected 82.95 % protein, 0.96 % lipid, 3.64 % ash and 12.07 % moisture for skin collagen from yellow fin tuna (*Thunnus albacares*).

Table 1. Proximate composition of fish scale, fish scale collagen and commercial collagen (dw %)

%	Fish scale	Fish scale collagen	Commercial collagen
Protein	39.99 ± 1.54	94.52 ± 2.02[b]	98.89 ± 0.85[a]
Lipid	0.08 ± 0.01	0.67 ± 0.11[a]	0.03 ± 0.01[b]
Moisture	21.42 ± 0.09	0.17 ± 0.01[b]	3.09 ± 0.93[a]
Ash	40.07 ± 0.20	1.49 ± 0.02[a]	0.98 ± 0.01[b]

*Data are expressed as the mean ± SD. Lower case letters in the same line show statistical difference between fish scale collagen and commercial collagen.

Denaturation temperature

Denaturation temperature of fish scale collagen and commercial collagen was determined as 30 ± 0.02 ºC and 28 ± 0.01 ºC, respectively ($p < 0.05$) (Figure 1).

Pati *et al.* (2010) found the denaturation temperature of collagen from scales of *Labeo rohita* and *Catla catla* as 35 °C. They stated that the denaturation temperature is closely related to the temperatures in the natural habitat of fish. El-Rashidy *et al.* (2015) determined the denaturation temperature of the scale collagen from Nile Tilapia (*Oreochromis niloticas*) as 32.09 °C and concluded that scale collagen was close to mammalian collagen. Sionkowska *et al.* (2015) found the denaturation temperature of *Brama australis* skin collagen as 24 °C in their study. The use of collagen from different species in different applications depends on the thermal stability of the obtained species, which depends on the environmental conditions and body temperature. Similarity between the denaturation temperature of the fish collagen and the mammalian collagen is an important advantage in terms of its use in biomedical applications (Pati *et al.*, 2010). Therefore, the denaturation temperature of the collagen obtained from the mullet scale in this study, offers advantages for its use in food and cosmetics industries.

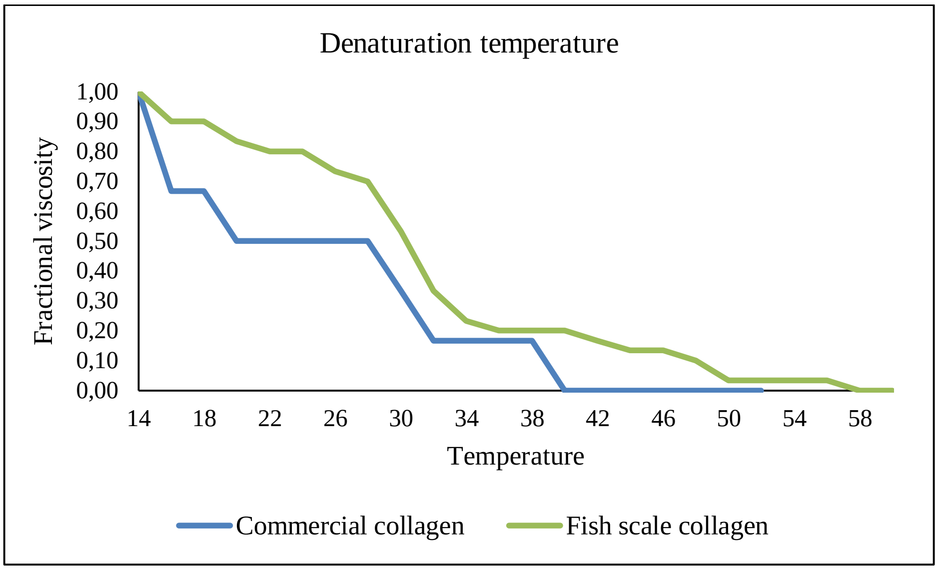

Figure 1. Denaturation temperature of fish scale collagen and commercial collagen

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

Figure 2 shows the electrophoretic pattern and densitometric analysis results of the fish scale collagen. ASC from fish scale was composed of two different α chains, α1 and α2; density wise α1 was far intense than α2 chain, with high molecular weight components β (dimmer) and γ (trimmer). According to subunit composition of SDS-PAGE, ASC was mainly composed of type I collagen. Molecular weights of α1 and α2 chains were found with densitometric analysis as 126 and 116 kDa, β and ɣ chains were 197 and 210 kDa, respectively. Similar results were obtained by Pal *et al.* (2016); the molecular mass of collagen from Mrigal carp (*Cirrhinus cirrhosus*) scale was about 120 kDa for α1 and 110 kDa for α2.

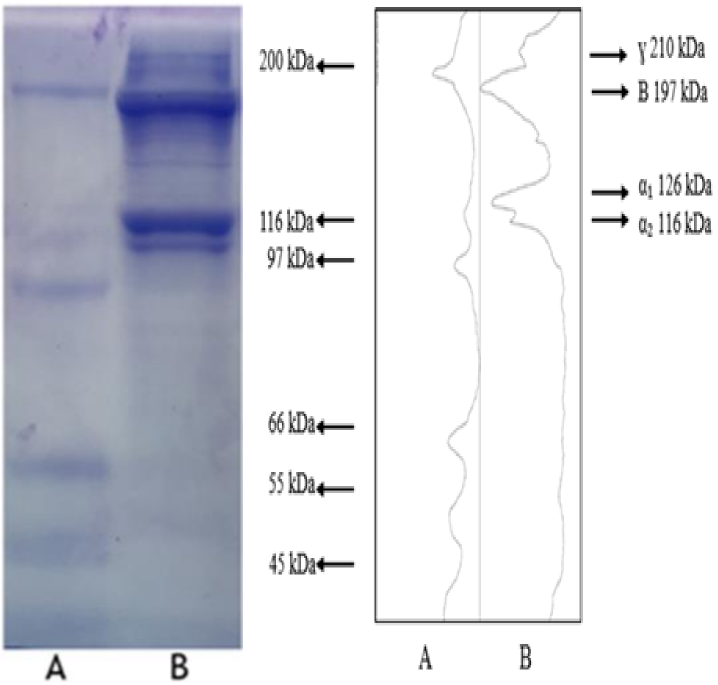

Figure 2. SDS-PAGE pattern and densitometric analysis of protein marker (A) and collagen from fish scale (B)

Chinh *et al.* (2019a) reported that they obtained type I collagen from the scales of tropical freshwater carp, molecular weights of α1 and α2 chains were detected as 139 kDa and 129 kDa, respectively, and the β-chain was also observed. Chen *et al.* (2016) extracted acid-soluble collagen from tilapia (*Oreochromis niloticus*) scales and skin and reported that the electrophoretic positions of the α chains in the scale collagen (α1-132 kDa; α2-121 kDa) was in the higher position compared to skin collagen (α1-130 kDa; α2-120 kDa). In the present study, slightly lower molecular weight was observed compared with those of ASC. Tan and Chang (2018) observed the molecular weights of α1 and α2 chains in the collagen they extracted from the skin of canal catfish (*Ictalurus punctatus*) as 123 kDa and 113 kDa, respectively. The molecular weights of β and γ chains, was found to be 226 kDa and 338 kDa, respectively. They found the molecular weights of β and γ chains in higher positions compared with our study. Different collagen extraction methods applied in fish species can reveal small differences in the structure of collagen. In this context, depending on the species and collagen extraction method, molecular weights may also differ.

Amino acid

The amino acid composition of fish scale and commercial fish skin collagen is presented in Table 2. The extracted collagen was rich in arginine, threonine, glycine and proline. The highest amino acid was arginine + threonine with 39.86 ± 0.93 g/100 g. The peaks of arginine and threonine amino acids were calculated together since they cannot be distinguished clearly. Glycine (13.75 ± 0.34 g/100 g), proline (8.05 ± 0.31 g/100 g) and arginine had higher levels compared to other amino acids. Alanine level was found to be 1.64 ± 0.03 g/100 g. In the commercial fish skin collagen, the highest amino acid was arginine with 13.39 ± 0.09 g/100 g, followed by glycine with 12.56 ± 0.42 g/100 g, and proline with 6.67 ± 0.00 g/100 g. The total amount of essential amino acids of commercial collagen was found to be lower than that of the fish scale collagen ($p < 0.05$). Both collagens had low concentrations of tyrosine, histidine and methionine.

Pal and Suresh (2017) found glycine as the highest amino acids of acid-soluble collagen extracted from two cyprinids species (*Catla catla* and *Labeo rohita*) with 22.43 and 22.63 g/100 g, respectively. Glycine was followed by proline with 15.40 and 15.30 g/100 g, alanine with 12.81 and 12.88 g/100 g, while arginine was found to be 8.52 and 7.24 g/100 g. Similar findings have been reported in our study. The amount of arginine was also lower in the study of Pal and Suresh (2017). In their study, in which collagen was extracted from the tropical frehwater carp scales, threonine was the most dominant amino acid Chinh *et al.* (2019a) with 39.79 % followed by proline with 11.37 %, glutamic acid with 12.84 % and arginine with 9.92 %. Glycine level was found as 3.27 %, alanine as 5.09 %, and hydroxyproline was not detected. Reported glycine content was lower than that determined in the present study. In the amino acid sequence of the collagen, there is one glycine in every three amino acids which explains the higher abundance of glycine.

Table 2. Amino acid composition of the fish scale collagen and commercial collagen (g/100 g)

Amino acids	Fish scale collagen	Commercial collagen
Lysine	2.32 ± 0.11[a]	2.10 ± 0.01[a]
Methionine	1.73 ± 0.05[a]	1.37 ± 0.05[b]
Arginine + Threonine*	39.86 ± 0.93[a]	17.02 ± 0.09[b]
Isoleucine	0.99 ± 0.01[a]	0.92 ± 0.02[a]
Leucine	1.78 ± 0.03[a]	1.44 ± 0.02[b]
Phenylalanine	1.34 ± 0.02[a]	1.04 ± 0.01[b]
Valine	1.77 ± 0.03[a]	1.53 ± 0.00[a]
Histidine	0.55 ± 0.01[a]	0.60 ± 0.00[a]
Serine	1.08 ± 0.03[a]	0.89 ± 0.01[a]
Cysteine	7.00 ± 0.01[a]	2.37 ± 0.02[b]
Tyrosine	0.89 ± 0.02[a]	0.33 ± 0.01[b]
Alanine	1.64 ± 0.03[a]	1.61 ± 0.00[a]
Aspartic acid	2.6 ± 0.02[a]	2.47 ± 0.03[a]
Glutamic acid	3.89 ± 0.13[a]	3.24 ± 0.04[a]
Glycine	13.75 ± 0.34[a]	12.56 ± 0.42[a]
Proline	8.05 ± 0.31[a]	6.67 ± 0.00[b]
ΣAA	91.11[a]	57.02[b]

Data are expressed as the mean ± SD. Lower case letters in the same line show statistical difference between fish scale collagen and commercial collagen.
*The peaks of arginine and threonine were calculated together since they cannot be distinguished clearly.

Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS)

As shown in SEM images of fish scale and commercial collagen (Figure 3), fibrous structures and a porous, cottony structure were observed in scale collagen. A similar morphology was observed in the commercial collagen and porous structures. More fibrillar structures were found in the scale collagen compared to commercial collagen. In addition, nodular structures were observed in the commercial collagen. Microstructure may be affected by the method of collagen purification and the solvent used. Collagen can form fibrillar structures spontaneously under suitable conditions such as pH, temperature and ionic strength. The pore size of collagen increases as a factor of the amount of water used during collagen production.

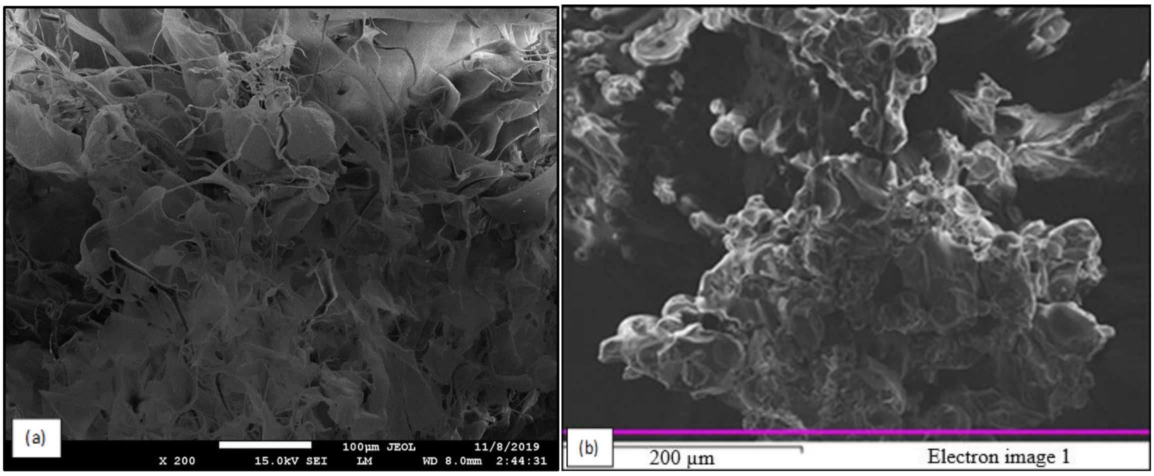

Figure 3. SEM image of fish scale collagen (a) commercial collagen (b)

Shalaby *et al.* (2020) characterized scale collagen from tilapia for wound healing purposes. The SEM image of tilapia collagen is similar to the collagen analyzed in the present study. According to Shalaby *et al.* (2020), the microstructure of lyophilized tilapia collagen shows homogeneous, multi-layered, orderly and highly fibrillated structures which is beneficial to the cell adhesion and proliferation.

Pal and Suresh (2017) examined the physico-chemical properties and fibril structure of ASC and PSC collagen from carp (*Catla catla* and *Labeo rohita*) scales. According to SEM images, porous, three-dimensional collagen fibrils was observed. Nodular structures were seen in PSC. In *Catla catla* scale collagen more porous structures were observed in PSC compared to ASC, but also more branched structures were seen in ASC. ASC from *Labeo rohita* were more porous and fibril structures were less complex than PSC. According to Pal and Suresh (2017) uniform fibril structure is a desired property for collagen.

EDS spectrum of scale and commercial collagen are shown in Figure 4. For scale collagen percent mass weight of C, N and O was found as 46.09 %, 22.05 % and 28.48 %; percent atomic weights as 52.53 %, 21.55 % and 24.37 %, respectively. In the commercial collagen the percent mass weights of the C, N, O elements were determined as; 43.79 %, 23.52 % and 32.69 % and atomic weights as 49.48 %, 22.79 % and 27.73 %, respectively. In scale collagen, unlike commercial collagen, Na and Cl elements were observed corresponding to mass weights of 1.14 % and 2.25 %, atomic weights of 0.68 % and 0.87 %, respectively. It is likely that NaCl, which was used to precipitate collagen proteins dissolved in acetic acid, could not be completely removed during the collagen production process.

Element	Weight%	Atomic%	Element	Weight%	Atomic%
C K	46.09	52.53	C K	43.79	49.48
N K	22.05	21.55	N K	23.52	22.79
O K	28.48	24.37	O K	32.69	27.73
Na K	1.14	0.68	Totals	100.00	
Cl K	2.25	0.87			
Totals	100.00				

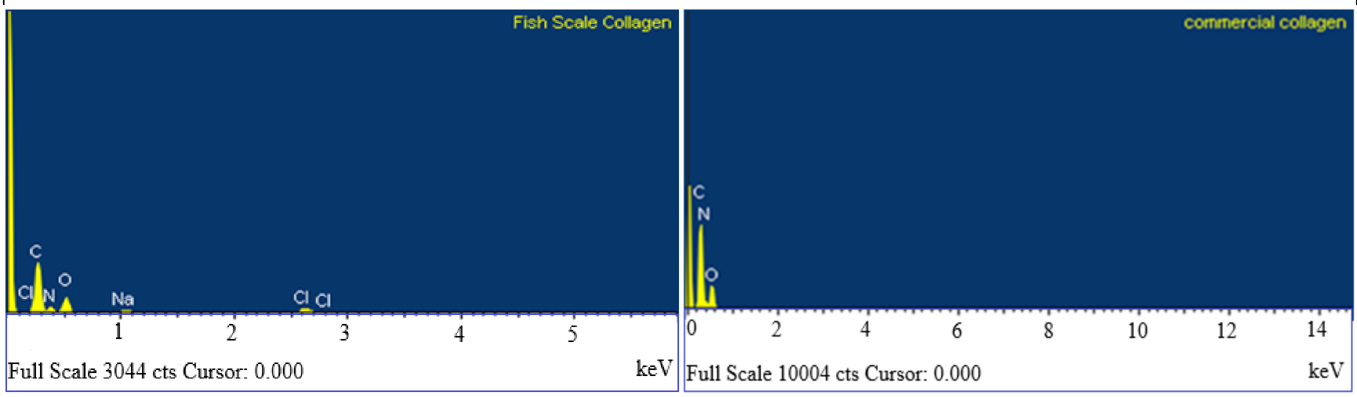

Figure 4. EDS spectrum of fish scale and commercial collagen

Chinh *et al.* (2019b) found the mass weight of Na, Cl, O, C and N in hydroxy apatite and collagen from tilapia fish scale as 33.68 %, 51.06 %, 2.23 %, 11.38 % and 1.65 %, atomic weights as 35.65 %, 35.04 %, 3.40 %, 23.05 %, and 2.86 % according to the EDS analysis. They reported that the higher contents of Na and Cl is due to the NaCl solution which is used for precipitation of the collagen. In future studies, the amount of NaCl solution should be decreased to obtain pure collagen.

FTIR spectrum

Secondary structure of fish scale collagen and commercial collagen was detected by FTIR (Figure 5). FTIR spectrum of fish scale collagen showed main absorption bands of amide A (3262.97 cm^{-1}), amide B (2873.86 cm^{-1}), amide I (1639.38 cm^{-1}), amide II (1532.85 cm^{-1}) and amide III (1234.13 cm^{-1}). In commercial collagen amide A, B, I, II and III bands were observed at 3274.42, 2879.21, 1639.55, 1522.36 and 1237.20 cm^{-1}, respectively. The resulted spectrum was similar for both fish scale and commercial collagen.

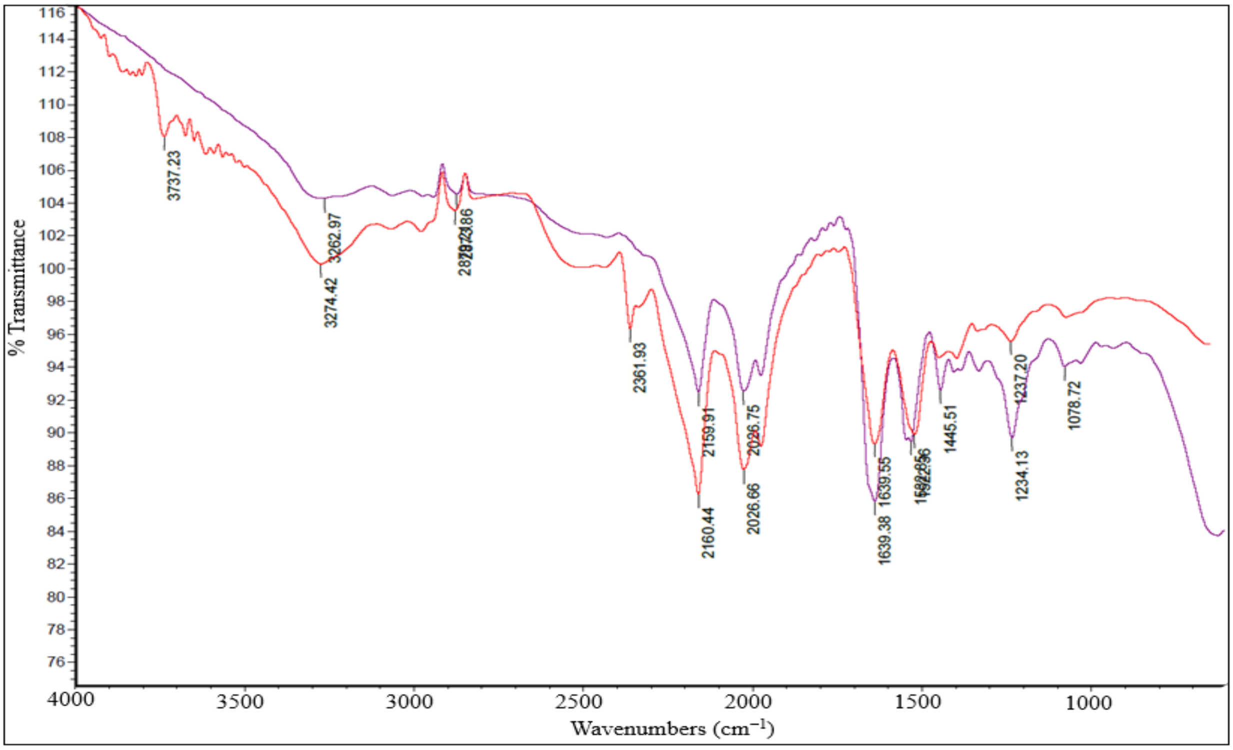

Figure 5. FTIR spectrum of fish scale collagen (purple) and commercial collagen (red)

Amide A consists of N–H stretching vibration. Amide B band consociated with CH_2 asymmetrical stretching (Bhagwat and Dandge, 2016). The range of amide A, I, II, and III is known to have a direct relation to the polypeptide chain (Abinaya and Gayathri, 2019). The amide I wave number ranges from 1600 to 1700 cm^{-1}, which is related to the carbonyl (C=O) stretching vibration of the peptide chain and is the most important factor for determining the secondary structure of proteins. Similarly, the ASC absorption of the regional band was observed at 1639.38 cm^{-1} in the present study. Amide II is related to N–H bond and C-N expansion, whereas amide III is related to CN stretching and N–H bending and participates in the triple helix structure of collagen (Woo *et al.*, 2008). Chen *et al.* (2016) found the main absorption bands in the collagen of red drum (*Sciaenops ocellatus*) scale at 3328, 3080, 1658, 1548 and 1240 cm^{-1}, respectively. Chinh *et al.* (2019b) determined the characteristic peaks at 3451.95, 1635.26, 1400.15, 1065.54 and 462.32 cm^{-1} wavelengths as a result of FTIR measurements made on tilapia fish scale collagen. In this present study, the main bands of the collagen in FTIR spectrum was observed in the range of values specified in the literature, and are quite similar to purified collagen from other fish species.

Thermo gravimetric analysis (TGA)

According to the results of mass changes examined with thermogravimetric analysis, there was no significant weight loss up to 100 °C for both collagens. The first weight loss in scale and commercial collagen occurred at 308 ºC and 286 ºC, respectively. The initial decrease in this temperature is thought to be due to the breakdown of organic compounds. The major lost also occurred in scale and commercial collagen at 350 ºC and 410 ºC. After 600 ºC there was no significant change in weight (Figure 6).

According to TGA results of collagen obtained from the puffer fish (*Lagocephalus inermis*) skin, no significant weight loss was observed before 100 °C (Iswariya *et al*,. 2018). The major weight losses occurred at 315 °C and 520 °C. Pati *et al.* (2010) reported that according to the TGA results, there were no weight losses before 100 ºC in the type I collagen from *Labeo rohita* and *Catla catla* fish scales. The main weight and organic compound losses occurred at 286 ºC and 411 ºC, respectively. Similar to our findings, in both studies, no significant lost or change was observed after 600 °C.

Antioxidant activity

In the present study the DPPH radical scavenging activity of fish scale collagen and commercial collagen was determined as 81.90 ± 1.27 % and 80.54 ± 3.34 %, respectively ($p > 0.05$). Pal and Suresh (2017) found the the DPPH radical scavenging activity of the collagen from *Catla catla* and *Labeo rohita* in the range of 9–24 % for ASC. Collagens can inactivate reactive oxygen species, reduce hydroperoxides, enzymatically eliminates specific oxidants and scavenge free radicals, which may contribute to their antioxidant activities. (Pal and Suresh, 2017). Nakchum and Kim (2016) determined the DPPH radical scavenging activity of squid skin collagen as 57.38 %. Slimane and Sadok (2018) produced films with collagen extracted from the common shark (*Mustelus mustelus*) skin and chitosan, and reported that DPPH radical scavenging activity of films with a pure collagen and mixture of collagen:chitosan (1:1) was 30.88 ± 0.03 % and 23.91 ± 1.15 %, respectively. These results are considerably lower than the DPPH radical scavenging activity obtained in our study. It was reported that the antioxidant properties can change depending on the peptide sequence and molecular weight (Bu *et al.*, 2017). In our study, it was determined that the DPPH radical scavenging activity of collagen was quite high and similar to that of the commercial collagen. These results further highlight the importance of golden grey mullet scale collagen.

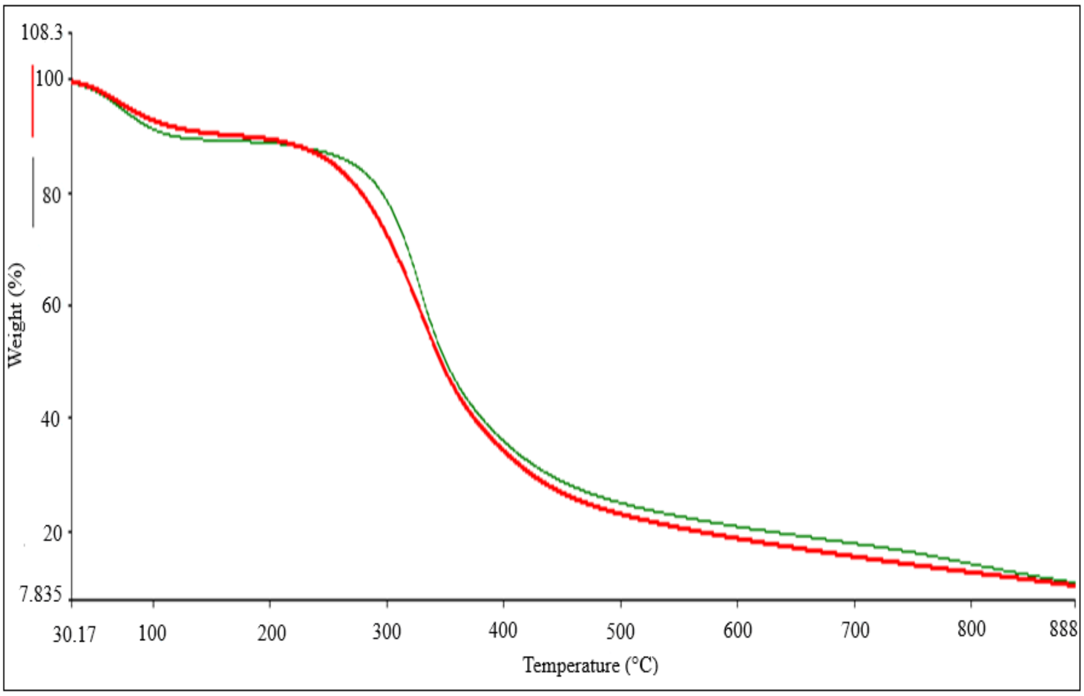

Figure 6. TGA results of the scale collagen (green) and commercial collagen (red)

Antimicrobial activity

The antimicrobial activity of the scale and commercial collagen was evaluated against two common pathogenic bacteria; a Gram negative strain *E. coli* and a Gram positive strain *S. aureus* and a yeast *C. albicans*. There was no inhibition zone against *E. coli*, *S. aureus* and *C. albicans* in both scale and commercial collagen. Venkatesan *et al.* (2017) reported that peptides from *Setipinna taty* and *Scomber scombrus*, produced by enzymatic hydrolysis using different enzymes (pepsin, papain, trypsin) inhibited *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria innocua*. It is thought that due to the small structure of the peptides formed by the hydrolysis of collagen, penetration into microorganisms occurs more easily. Shalaby *et al.* (2020) produced and characterized collagen from fish scales for wound healing purposes, and reported that the mean inhibition circle diameters on *Escherichia coli* and *Staphylococcus aureus* were 0.08 mm and 0.5 mm, respectively, having a very low antibacterial effect againts these bacteria.

Conclusion

Collagen was extracted by using acetic acid from the scale of golden grey mullet. Based on proximate and amino acid composition, denaturation temperature, SDS-PAGE, SEM-EDS, FTIR, TGA, antioxidant and antimicrobial activity, ASC was characterized and compared with commercial fish skin collagen. Extracted collagen was quite similar to commercial collagen, yielding even higher amounts of total amino acids and higher antioxidant properties. ASC from scale of golden grey mullet can be a promising alternative source to mammalian collagen from the fish processing industry. The present study will contribute to the reduction of the environmental pollution as well as having economic and scientific benefits by using unevaluated fish scale wastes for collagen extraction. Isolated collagen is a promising renewable biological source that can be used in various sectors such as food as a functional ingredient and cosmetics, that will contribute to human health.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Cansu Metin Hacisa and Taçnur Baygar planned and designed the research. Cansu Metin Hacisa carried out laboratory analysis. All authors contributed to the writing of the final manuscript.

Ethics Approval

Ethics committee approval is not required for this study.

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- D. Çayan and E. Unur conceived the ideas of the study and writing manuscript; D. Çayan, M. Nisari, D. Patat and E. Dağlı performed data collection and analysis; H. Akalın performed gene expression stages.

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