JMSF

Çanakkale Onsekiz Mart University

Journal of Marine Sciences and Fisheries

E-ISSN : 2651-5326 **Cilt** | Volume : **8** **Sayı** | Issue : **1** **Yıl** | Year : **2025**

Çanakkale Onsekiz Mart University
Journal of Marine Sciences and Fisheries JMSF

E-ISSN: 2651-5326 | **Cilt**/Volume: **8** | **Sayı**/Issue: **1** | **Ay**/Month: **Temmuz** /July | **Yıl**/Year: **2025**

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İNDEKSLER / INDEXES

TR Dizin
DOAJ
Biological Abstract
BIOSIS Previews
Zoological Record
Crossref
Google Scholar

Çanakkale Onsekiz Mart University
Journal of Marine Sciences and Fisheries
JMSF

E-ISSN: 2651-5326 | Cilt/Volume: 8 | Sayı/Issue: 1 | Ay/Month: Temmuz /July | Yıl/Year: 2025

TABLE OF CONTENTS / İÇİNDEKİLER

Çanakkale Onsekiz Mart University
Journal of Marine Sciences and Fisheries

E-ISSN: 2651-5326 | **Cilt**/Volume: **8** | **Sayı**/Issue: **1** | **Ay**/Month: **Temmuz** /July | **Yıl**/Year: **2025**

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

SHORT COMMUNICATION / KISA BİLDİRİ

COMU J Mar Sci Fish 2025 8(1): 1-13
https://doi.org/10.46384/jmsf.1552135
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Determination of the Compensatory Growth Response, Mineral and Antioxidant Enzyme Activities of European Sea Bass Fingerlings (*Dicentrarchus labrax*) in Fasting and Re-feeding

Seval Dernekbaşı[1*], Dilara Kaya Öztürk[1], Keriman Yürüten Özdemir[2], İsmihan Karayücel[1]

[1]Sinop Üniversitesi, Su Ürünleri Fakültesi, Sinop, Türkiye https://orcid.org/0000-0001-5735-2486
[1]Sinop Üniversitesi, Su Ürünleri Fakültesi, Sinop, Türkiye https://orcid.org/0000-0003-2505-231X
[2]Kastamonu Üniversitesi, Kastamonu, Türkiye https://orcid.org/0000-0002-5561-5702
[1]Sinop Üniversitesi, Su Ürünleri Fakültesi, Sinop, Türkiye https://orcid.org/0000-0003-2520-7545

Received: 18.09.2024 /Accepted: 06.02.2025 / Published online: 14.07.2025

Keywords:

European sea bass
Dicentrarchus labrax
Antioxidant enzymes
Mineral matters
Fasting
Re-feeding
Compansatory growth

Abstract: Understanding compensatory growth in European sea bass (*Dicentrarchus labrax*) is also crucial for optimizing aquaculture practices as it can lead to more efficient feeding strategies and improved fish health. To this end, this study evaluated the effects of fasting and re-feeding on growth performance, mineral and antioxidant enzyme activities of European sea bass fingerlings. The experiment involved five treatments, each with three replicates. The control treatment was fed continuously, while treatments A1, A2, A3, and A4 were fasted for one, two, three, and four weeks, respectively. Following the fasting period, all treatments were fed to satiation twice daily for four weeks. At the end of the trial, the control treatment showed the best growth during the fasting period, while the growth of the other treatments decreased with the duration of fasting ($p<0.05$). Consequently, the highest specific growth rate (SGR), feed conversion ratio (FCR) and hepatosomatic index (HSI) were observed in the control treatment ($p<0.05$). During the re-feeding period, potassium (K) and calcium (Ca) levels significantly decreased compared to the fasting period. The ratios of Ca, K, sodium (Na), and iron (Fe) were higher during the fasting period than during the re-feeding period. A4 treatment, which lasted for four weeks, had the highest mineral content during the fasting period compared to the control treatment in both periods. In the re-feeding period, the control treatment had the lowest levels of Ca, Fe, phosphorus (P), manganese (Mn), and zinc (Zn) ($p<0.05$). Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and lipid peroxidation (LPO), were highest in treatments A2, C, and A1 during the fasting period ($p<0.05$). These values significantly decreased by the end of the re-feeding period ($p<0.05$). Therefore, this study concludes that re-feeding after fasting resulted in partial compensatory growth in the European sea bass fingerlings examined.

Anahtar kelimeler:

Levrek
Dicentrarchus labrax
Antioksidan enzimler
Mineral madde
Açlık
Yeniden besleme
Telafi büyümesi

Avrupa Levreği Yavrularının (*Dicentrarchus labrax*) Açlık ve Yeniden Beslenmede Telafi Edici Büyüme Tepkisi ile Mineral ve Antioksidan Enzim Aktivitelerinin Belirlenmesi

Öz: Beslenme eksikliğinden sonra yeniden besleme döneminden sonra oluşan telafi büyümesi, balık çiftçiliğinin genel verimliliğini ve ekonomik uygulanabilirliğini önemli ölçüde etkileyebilir. Avrupa levreğinde (*Dicentrarchus labrax*) telafi edici büyümeyi anlamak, daha verimli besleme stratejilerine ve iyileştirilmiş balık sağlığına yol açabileceğinden su ürünleri yetiştiriciliği uygulamalarını optimize etmek için de önemlidir. Bu amaçla, bu çalışmada açlığın ve yeniden beslemenin levrek yavrularının büyüme performansı, mineral içeriği ve antioksidan enzim aktiviteleri üzerindeki etkileri değerlendirilmiştir. Deney, her biri üç tekrarlı beş uygulamayı içermektedir. Kontrol grubu (C) sürekli beslenirken, A1, A2, A3 ve A4 grupları bir, iki, üç ve dört hafta boyunca aç bırakılmıştır. Açlık döneminin ardından, tüm deneme grupları dört hafta boyunca günde iki kez doyuncaya kadar beslenmiştir. Denemenin sonunda, kontrol grubu açlık döneminde en iyi büyümeyi gösterirken, diğer grupların büyümesi açlık süresiyle azalma göstermiştir ($p<0,05$). Sonuç olarak, en yüksek spesifik büyüme oranı (SGR), yem dönüşüm oranı (FCR) ve hepatosomatik indeks (HSI) kontrol grubunda gözlenmiştir ($p<0,05$). Yeniden besleme döneminde potasyum (K) ve kalsiyum (Ca) düzeyleri açlık dönemine kıyasla önemli derecede azaldı. Ca, K, sodyum (Na) ve demir (Fe) oranları açlık döneminde yeniden besleme dönemine göre daha yüksek tespit edilmiştir. Dört hafta süren A4 grubu, her iki dönemde de kontrol uygulamasına kıyasla açlık döneminde en yüksek mineral içeriğine sahip bulunmuştur. Yeniden besleme döneminde kontrol uygulaması en düşük Ca, Fe, fosfor (P), manganez (Mn) ve çinko (Zn) düzeylerine sahip bulunmuştur ($p<0,05$). Süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx) ve lipid peroksidasyonu (LPO) dahil antioksidan enzim aktiviteleri, açlık döneminde A2, C ve A1 gruplarında en yüksek tespit edilmiştir ($p<0,05$). Bu değerler yeniden besleme döneminin sonunda önemli ölçüde azalmıştır ($p<0,05$). Bu nedenle, bu çalışma açlıktan sonra yeniden beslemenin incelenen levrek yavrularında kısmi telafi büyümesinin gözlendiği sonucuna varmaktadır.

*Corresponding author: sdernekbasi@sinop.edu.tr

How to cite this article: Dernekbaşı, S., Kaya Öztürk, D., Yürüten Özdemir, K., & Karayücel, İ. (2025). Determination of the compensatory growth response, mineral and antioxidant enzyme activities of European sea bass fingerlings (*Dicentrarchus labrax*) in fasting and re-feeding. COMU J. Mar. Sci. Fish, 8 (1): 1-13. doi:10.46384/jmsf.1552135

Introduction

In a certain period of their lives, all living organisims may encounter hunger that can lead to death due to seasonal reasons such as shortage of food resources or sudden temperature changes (McCue, 2010). The frequency and duration of these hunger periods in nature may differ seasonally (Ali et al., 2003; McCue, 2010). The term hunger is expressed as the ability of an animal to give up feeding opportunity after digestion through some hormonal or metabolic activities (Doucett et al., 1999). After this period, the biological term given to the rapid increase in the growth performance of living organisms with the start of feeding again is compensatory growth (Ali et al., 2003). Compensatory growth is also defined as growth in which an organism catches the weight of those whose growth has never decreased, following a period of limited growth or negative growth, usually resulting from low feed consumption (Hornick et al., 2000).

The effect of fasting period applied for certain periods in breeding conditions on nutrition, feed intake, feed conversion rate and growth has been studied in many fish species. While there is a rapid growth after re-feeding in some fish species exposed to fasting, it was determined by some researchers that the feed utilization rate did not change (Hayward et al., 2000). This mechanism has brought along a good feed conversion efficiency as well as rapid growth in some studies (Ali and Jauncey, 2004). This event is not only a theoretical issue but also commercially applicable, increasing feed conversion efficiency with growth (Wang et al., 2000). Previous studies have investigated various fish species, such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), and Nile tilapia (*Oreochromis niloticus*), to understand the effects of fasting and re-feeding. In rainbow trout, fasting followed by re-feeding has been shown to enhance growth rates and improve feed conversion efficiency (Quinton and Blake, 1990). In Atlantic salmon, compensatory growth was observed with a significant increase in feed intake and growth rate upon re-feeding (Johansen et al., 2001). Similarly, Nile tilapia exhibited improved growth performance and feed utilization efficiency after a period of fasting and re-feeding (Ali et al., 2003). When comparing these findings to sea bass, it is observed that while the mechanisms of compensatory growth, such as increased feed intake and metabolic adjustments, are similar, the specific responses in growth performance and feed utilization may vary due to species-specific differences in metabolism and dietary requirements.

The effects of nutritional factors on the physiological and health of fish have been comprehensively studied, but feeding frequency may also affect fish's antioxidant ability and immunity (Garcia and Villarroel, 2009; Sheikhzadeh et al., 2012). Ding et al. (2017) reported that malnutrition not only causes oxidative damage but also results in immunosuppression. The effects of oxidative stress resulting from the disruption of prooxidant-antioxidant balance in living organisms due to any stress factor can be eliminated with the help of antioxidant substances that strengthen the antioxidant defense system. Changes in the activities of SOD, CAT and GPx, which are the main enzymatic antioxidants, can be used as an indicator of oxidative stress (Ekambaram et al. 2014). These enzymes provide defense against the damage caused by reactive oxygen species (ROS) in living systems and cells are well protected by these antioxidant enzymes (Altan et al., 2005).

Many stressors in the animal world are universal because of the similarity of the basic needs of animals. Social interaction requirements such as non-optimal environmental conditions (temperature, oxygen, etc.), malnutrition, sunlight, openness to predators and the need to determine the area are defined as universal stressors. Fish and other aquatic organisms are exposed to more stress factors than land animals. This is due to the fact that homeostasis mechanisms are highly dependent on the environmental conditions in which they operate (Harper and Wolf, 2009). Looking at today's conditions, it can be said that the environment in which fish live is under threat of change (due to reasons such as climate change). Therefore, just as there is always a risk of fish fasting under natural conditions, there is also a risk for aquaculture. Aquaculture is not only carried out offshore but also in coastal areas, where hatcheries and earthen ponds are commonly established. Inaccessibility to aquaculture systems due to weather conditions, lack of food, malnutrition, technical problems, and other factors can lead to periods of fasting for fish in both settings. Therefore researchers have started to focus on this topic recently. Also, there is no research regarding the effect of fasting and re-feeding on the mineral matter in sea bass.

Sea bass is a fish with high economic value and can easily adapt to climatic conditions. It is carnivorous and feeds on shrimp, crabs, worms, and fish in nature, therefore it has a wide range of feeding habits. Sea bass, which is highly able to gain live weight, is also preferred by consumers for its meat quality, an important criterion in farming. Its rich vitamin, mineral, and trace element content holds special importance in healthy nutrition. These fish have a high probability of starving in nature and farming. Therefore, in the present study, the changes in growth performance, mineral content, antioxidant enzyme activities, and lipid peroxidation activities of sea bass after fasting and re-feeding period were examined, and the contributions of these changes to compensatory growth were investigated. It also hypothesized how physiological and biochemical changes affect compensatory growth in sea bass and how re-feeding after a period of starvation would lead to significant improvements in growth and health markers. This research is novel in its comprehensive approach and is expected to contribute valuable insights into optimizing feeding strategies for sustainable aquaculture practices, enhancing both the economic viability and health of cultured sea bass.

Material and Methods

Animals, experimental conditions and water parameters

The experiment was carried out in Sinop University Fisheries and Aquaculture Faculty Research and Application Center. Sea bass (300 fish) provided from a commercial sea bass farm (Kızılırmak Su Ürünleri Inc., Samsun/Turkey) were used. The transport of fish was carried out with water-filled and ventilated transport tanks. The fish brought to the research center were stocked in 3-ton tanks, and they were acclimatized to the experimental conditions by taking them for a 2-week feeding period with commercial sea bass feed. After acclimatization, fish (weight ~32.09±0.07 g) were fasted for a day, weighted and randomly distributed into 120-L rectangle glass aquaria in a recirculation seawater system.

Fish were maintained under a stress-free conditions during the experimental period. The water quality parameters of the recirculated system were measured daily; temperature (21.11 ± 0.05°C), dissolved oxygen (DO) (7.08±0.01 mg/L), pH (8.14±0.05) and salinity (19.65±0.06‰) did not change significantly between treatments during the experiment. A recirculated system consisting of 15 tanks and five sump filter systems was established. Mechanical, chemical and biological filtration were provided thanks to the continuous water circulation and filter materials in the sump filter system. Aeration was realized by two air stones placed in the sump filter system.

Experimental design and feeding

The experiment was designed with three replications using a total of 270 sea bass fingerlings in 15 square tanks with 18 fish in each tank for eight weeks. The experiment was divided into two phases: fasting (weeks 0-4) and re-feeding (weeks 5-8). All treatments were fed commercial sea bass pellets, with their approximate composition, mineral content, and amino acid composition detailed in Table 1. Throughout the experiment, the control treatment (C) was fed commercial diets twice daily until satiation. The other four treatments underwent varying fasting periods: fish in treatments A1 (one week fasting), A2 (two weeks fasting), A3 (three weeks fasting) and A4 (four weeks fasting) were starved for one, two, three, and four weeks, respectively.

From weeks 5 to 8, fish in the control treatment (C) and the fasting treatments A1, A2, A3 and A4 were fed twice a day until satiation. To remove unnecessary food and feces, the tanks were regularly siphoned. Approximately 10% of the total water volume was replaced daily throughout the experiment. The fish were weighed every 15 days to monitor growth performance. They were kept under a natural light regime, and tank aeration was provided by an air pump.

Sampling

Sampling was done in the 4th and 8th weeks of the study. During these weeks, a total of 6 fish from each group (2 fish from each tank) were sampled. First, the length and weight measurements of the sampled fish were measured. During these weighing and measuring processes, in order to prevent the fish from being damaged by mechanical effects and from getting stressed and to make accurate measurements, the fish were stunned in benzocaine solution (50 mg/lt). The weighed fish were taken to experimental tanks that were cleaned and water was added after being separated in a separate tank that was continuously ventilated and contained clean water. The weighing process was carried out in the early morning hours when the fish were hungry and since the fish were not fed on the weighing days, these days were not included in the experimental period. Then, the abdomen was dissected, the gastrointestinal organs and liver were removed. The liver was weighed to determine the hepatosomatic index (HSI) and placed in small storage bags. For enzyme analysis, the livers were transported to the laboratory in a cold chain and stored at -20°C in sucrose until analysis. The fish meat, after being cleaned of bones and skins, was weighed, homogenized, and stored at -80°C for biochemical composition, amino acid, and mineral matter analysis.

Amino acid analysis of fish feed

Agilent Technologies/6460 Triple Quad LC-MS/MS Liquid Chromatography Mass/Mass Spectrometer was performed for amino acid analysis; 5 separate standard, internal standard, mobile phases, reagents, chromotographic separation and mass detection parameters are developed using the Jasem LC-MS/MS amino acid analysis kit (Pt Berca Niaga Medica/Indonesia) with updated sample preparation including acid hydrolysis procedure (Bilgin et al., 2018).

Mineral matter analysis of fish fillets

Element analysis was determined in the laboratories of Sinop University Scientific and Technological Researches Center (SUBITAM). Analyses (Se, P, Zn, Ca, Na, K, Mg, Mn, Co and Fe) were performed by wet digesting of the samples in HNO_3/H_2O_2. 0.5 g of the sample from the homogenous sample was placed in the 90 ml heat and pressure-resistant dried or lyophilized teflon containers of the microwave device. The sample was treated with 7 ml of 65% HNO_3 suprapur purity and 1 ml of 30% H_2O_2 suprapur purity before being broken up in the necessary microwave program. Up to 50 mL of ultrapure water was filled into the containers and read on the ICP-MS (inductively coupled plasma/optical emission spectrometer).

Analytical procedures of antioxidant enzymes

At the end of fasting and re-feeding periods, fish liver samples were taken to determine the activity of antioxidant enzymes such as SOD, CAT, GPx and LPO. All samples were stored at −80 °C until analysis. After thawing, liver samples were washed with sterile physiological saline, dried with filter paper, and homogenized in a Potter-Elvehjem homogenizer with approximately 1 g liver sample and inserted in a homogenization medium (0.25 M sucrose, 0.5 mM EDTA and 10 mM Tris-HCl; pH 7.4) for

4°C centrifugation, 20000 g for 45 minutes (Hisar et al., 2009). The supernatant was isolated after centrifugation and used for enzyme activity assays. In the analysis, SOD was determined using the SIGMA 19160-1KT-F SOD Assay Kit; catalase activity was determined using the Cayman 707002 Catalase Assay Kit; glutathione peroxidase activity was determined using the Cayman 703102 Glutathione Peroxidase Assay Kit; and lipid peroxidation was determined using the Cayman 10009055 TBARS Assay Kit. All these methodologies have been carried out following the guidance of the manufacturer.

Statistical analysis

Results were presented as mean±SE. The data collected at the conclusion of the study were analyzed using one-way analysis of variance (ANOVA), and Tukey's multiple comparisons procedure was used to determine the significance level of differences within and between groups (IBM SPSS 21). The relationships between fillet and diet fatty acids were evaluated by regression analysis. The tables and figures were created using the MS Office 2010 program. Prior to statistical analysis, arcsine square root transformations of percentage data were performed to ensure homogeneity of variances. When $p<0.05$, differences were deemed significant.

Results

Amino acid and mineral matter contents of fish feeds

The commercial sea bass feed used in the study contained 46% crude protein and 19% crude lipid. It was rich in minerals, including Calcium (Ca, 22897.07 mg/kg), Potassium (K, 8346.08 mg/kg), and Phosphorus (P, 15207.15 mg/kg). The feed also included essential amino acids such as histidine (1.47%), isoleucine (1.42%), leucine (3.73%), lysine (3.31%), methionine (0.90%), phenylalanine (2.15%), taurine (2.09%), and valine (1.90%) (Table 1). The essential amino acid profiles of the experimental diets meet the requirements for the sea bass (NRC, 1993). All the essential amino acids for growth were found in the diets. The amino acid composition of the experimental diet is shown in Table 1.

Table 1. Biochemical composition, mineral matter contents and amino acid composition of commercial sea bass pellets used in the study

Biochemical composition (% wet weight)	NRC (1993)	Mineral matters (mg/kg)	NRC (1993)	Essential Amino Acids (g/100g)	NRC (1993)	Non-essential Amino Acids (g/100g)	NRC (1993)
Crude protein 46	45-55	Calcium (Ca) 22897.07	12000-20000	Histidine 1.47	0.8-1.2	Alanine 2.90	3.0-4.5
Crude lipid 19	10-20	Iron (Fe) 312.94	50-100	Isoleucine 1.42	1.2-1.8	Aspartic acid 4.23	4.5-6.5
Ash 10	<12	Potassium (K) 8346.08	9000-12000	Leucine 3.73	1.8-2.5	Glutamic acid 6.43	6.5-10.0
Fiber 1.7	-	Magnesium (Mg) 1781.65	500-1000	Lysine 3.31	2.5-3.5	Glycine 2.93	4.0-6.0
		Sodium (Na) 3123.82	2000-4000	Methionine 0.90	0.9-1.2	Serine 2.52	2.0-3.5
		Phosphorus (P) 15207.15	7000-12000	Phenylalanine 2.15	1.2-1.8	Tyrosine 1.24	0.9-1.3
		Manganese (Mn) 55.71	10-30	Threonine 2.09	1.2-1.8		
		Zinc (Zn) 150.96	30-50	Valine 1.90	1.4-2.0		
		Cobalt (Co) 2.13	-	Arginine 2.69	1.8-2.5		
		Selenium (Se) 1.35	0.15-0.3				
		Ca/P 1.51	1.33-1.67				

Growth performance

The best growth during the fasting period was in the control group, growth in other groups decreased according to the degree of fasting (Figure 1). As a result of the first weighing of the fish with the same average initial weights on the 15th day, a slight decrease was determined in all groups including the control. The control group showed a rapid recovery and growth rate dollowing a decline after day 15th. A1 and A2 groups also showed a recovery after the decline on day 15th, but they did not grow as fast as the control group. Groups A3 and A4 also showed growth after the decline on day 15th, but the recovery was not as strong as the other groups. At the end of the study, treatments were ranked as control>A1>A2>A3>A4 with respect to observed growth rates.

Although fish weights increased rapidly after the re-feeding period, the best growth was determined in the control group as there was no full compensatory growth. Accordingly, the highest SGR, FCR and HSI were also in the control group (Table 2). HSI was quite low in groups A2 (fasting for two week), A3 (fasting for three week) and A4 (fasting for four week) during the fasting period. HSI values increased when re-feeding was started ($p<0.05$).

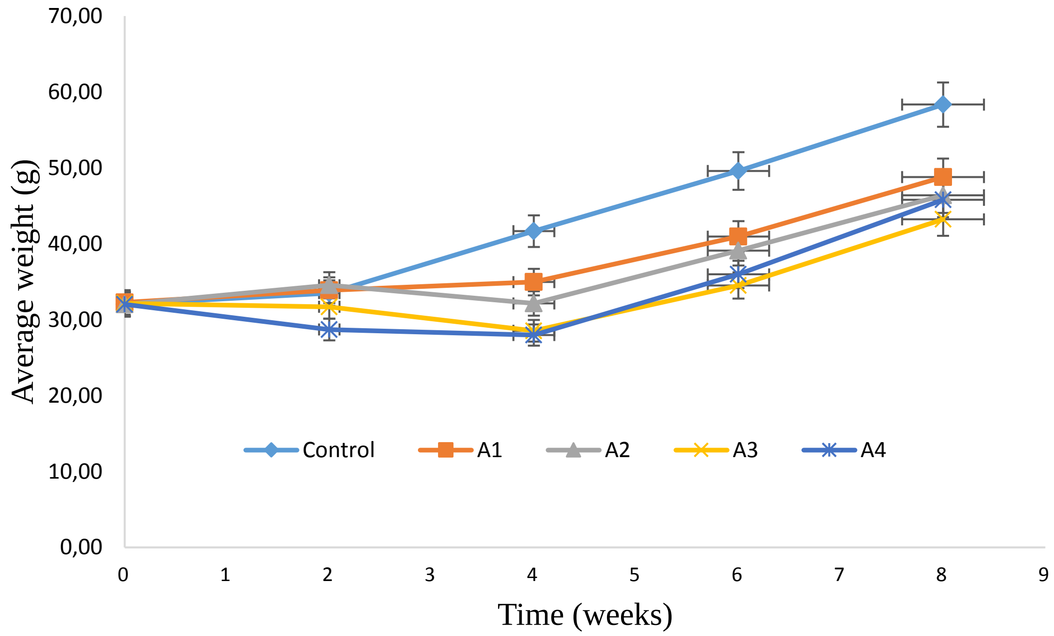

Figure 1. Changes in the average weight of fish during the study period

Table 2. Growth and feed utilization of sea bass fingerlings fed with fasting (week 0 to 4) and re-feeding (week 5 to 8) for 8 weeks.

	Treatments				
	Control	**A1**	**A2**	**A3**	**A4**
Initial weight (g)	32.03±0.02[a]	32.28±0.19[a]	32.01±0.38[a]	32.13±0.18[a]	32.01±0.14[a]
Weight (0-4 weeks; g)	41.66±1.05[a]	34.95±0.82[b]	32.14±0.78[c]	29.07±0.11[d]	27.98±0.26[e]
Lenght (0-4 weeks; cm)	15.62±0.22[a]	15.38±0.22[a]	15.12±0.17[a]	15.15±0.23[a]	14.98±0.22[a]
Final weight (5-8 weeks; g)	58.33±2.96[a]	48.79±2.85[b]	46.38±1.11[c]	43.21±1.34[d]	45.79±2.02[c]
Final length (cm)	17.69±0.05[a]	16.73±0.39[b]	16.49±0.06[b]	16.13±0.13[b]	16.39±0.23[b]
Weight gain (g)	26.30±2.97[a]	16.51±2.90[b]	14.37±1.26[c]	11.08±1.62[d]	13.78±2.14[c]
SGR (g day^{-1})	1.07±0.13[a]	0.74±0.15[b]	0.66±0.07[c]	0.53±0.04[d]	0.64±0.11[c]
Daily feed intake	24.08±0.77[a]	18.40±0.75[b]	14.94±0.19[c]	14.98±0.19[c]	11.90±0.62[d]
FCR	1.37±0.12[d]	2.03±0.30[b]	2.12±0.30[b]	1.84±0.48[c]	2.82±0.74[a]
HSI (0-4 weeks; %)	1.75±0.26[a]	1.17±0.06[b]	0.80±0.06[c]	0.70±0.59[d]	0.82±0.07[c]
HSI (5-8 weeks; %)	1.72±0.21[c]	1.65±0.12[d]	1.82±0.15[b]	1.87±1.71[a]	1.76±0.19[c]

Data are reported as mean ± standard errors of three replicates (3). Means with different superscript letter in a row are significantly different ($p<0.05$). SGR:Specific Growth Rate,,FCR: Feed Conversion Ratio, HSI: HepatosomaticIndex

Mineral matter contents of fish fillets

Comparisons of fillet mineral matter contents (mg/kg) during the fasting period (week 0 to 4) and re-feeding period (week 5 to 8) of sea bass fingerlings are shown in Table 3. In the fasting period, calcium, potassium, sodium and iron ratios were more dominant than the re-feeding period. The A4 group (fasting for four week) had the highest mineral content during the fasting period compared to the control group of both periods. In the re-feeding period, the lowest calcium, iron, phosphorus, manganese and zinc were in the control group, the other groups had slightly higher values.

Table 3. Comparisons of fillet mineral matter contents (mg/kg) during fasting period (week 0–4) and re-feeding period (week 5–8) of sea bass fingerlings

	Treatments				
	Control	**A1**	**A2**	**A3**	**A4**
Fasting period (mg/kg; week 0-4)					
Calcium	1391.88±5.79^{c}	823.01±2.96^{e}	1104.22±4.27^{d}	1465.66±1.37^{b}	1860.47±4.51^{a}
Iron	7.65±0.54^{c}	8.19±1.07^{b}	6.65±0.64^{d}	10.20±0.94^{a}	4.83±0.52^{e}
Potassium	3044.18±4.36^{c}	2612.03±3.09^{e}	2874.93±3.98^{d}	3150.80±2.22^{b}	5403.33±3.28^{a}
Magnesium	322.26±4.61^{b}	310.35±3.54^{c}	319.87±5.11^{c}	337.96±2.91^{b}	357.60±2.96^{a}
Sodium	400.33±4.60^{c}	401.02±3.66^{c}	443.70±3.70^{b}	455.29±2.44^{b}	527.40±2.82^{a}
Phosphorus	2453.91±4.54^{d}	2831.07±3.19^{c}	2869.16±4.09^{c}	3238.18±2.99^{b}	3509.70±2.89^{a}
Manganese	0.70±0.11^{a}	0.53±0.07^{c}	0.57±0.06^{bc}	0.60±0.07^{b}	0.61±0.11^{b}
Zinc	6.76±0.64^{b}	7.09±0.97^{b}	6.95±0.71^{b}	6.81±0.78^{b}	7.56±0.99^{a}
Selenium	0.25±0.02^{a}	0.25±0.02^{a}	0.25±0.01^{a}	0.26±0.00^{a}	0.26±0.02^{a}
Re-feeding period (mg/kg; week 5-8)					
Calcium	201.35±1.17^{e}	304.47±0.78^{d}	511.88±2.81^{b}	398.42±1.96^{c}	841.53±3.22^{a}
Iron	5.69±0.07^{e}	7.97±0.05^{d}	32.39±0.18^{a}	15.88±0.11^{b}	14.16±0.04^{c}
Potassium	1739.88±1.75^{a}	1798.09±2.05^{a}	1669.62±1.82^{b}	1683.15±2.31^{b}	1695.59±1.61^{b}
Magnesium	289.31±1.76^{b}	307.48±1.05^{a}	306.86±1.61^{a}	281.94±1.24^{b}	304.38±1.46^{a}
Sodium	545.76±2.39^{a}	377.54±1.15^{b}	334.89±1.39^{c}	356.40±1.52^{bc}	352.98±1.67^{bc}
Phosphorus	2349.48±1.81^{c}	2581.99±1.50^{b}	2481.07±1.92^{c}	2481.57±1.99^{c}	2863.49±2.12^{a}
Manganese	0.28±0.01^{d}	0.36±0.01^{c}	0.89±0.01^{a}	0.76±0.01^{b}	0.73±0.01^{b}
Zinc	5.29±0.10^{b}	5.39±0.06^{b}	6.39±0.09^{a}	5.55±0.09^{b}	5.93±0.07^{a}
Selenium	0.22±0.01^{a}	0.22±0.01^{a}	0.24±0.01^{a}	0.22±0.01^{a}	0.20±0.01^{a}

Data are mean ± SE. Means with different superscript letter in a column are significantly different ($p<0.05$)

Antioxidant enzyme activities of fish

In this study, antioxidant enzyme activities (SOD, CAT, GPx, LPO) were evaluated to determine the effects of experimental treatments (Figure 2). In all groups, SOD, CAT, GPx and LPO activities, which were high during the fasting period (especially in the A2 group), decreased during the re-feeding period except for A4 group.

SOD activities determined on the fasting and re-feeding periods in Table 4. The highest SOD activity was recorded in A2 group (76.23±1.97 U/mg protein), whereas, the lowest was in A4 group fish (30.16±1.84 U/mg protein) on the fasting period. On the re-feeding period, the highest SOD value was in A2 and A4 groups. On the other hand, control group fish had lowest SOD activity ($p<0.05$). Lipid peroxidation (LPO) was examined in liver tissues of fish. LPO results determined on the fasting and re-feeding periods are presented in Table 4. On the fasting period, the highest LPO value was in A1 group fish. On the other hand, control, A2 and A3 groups fish had the lowest muscle LPO (p<0.05). During the re-feeding period, A3 group had the highest LPO value and it decreased significantly in fish of all the experimental groups and control (p<0.05). Highest GPx activity was observed in A1 and A3, on the fasting period (p<0.05). There were no significant differences in GPx values between control and experimental groups on the re-feeding period (p>0.05). In terms of CAT, the highest value was in the control group on the fasting period (Table 4). The lowest value was estimated in A2 on the same period. On the re-feeding period, fish of all experimental groups were significantly higher than that of the control (p<0.05).

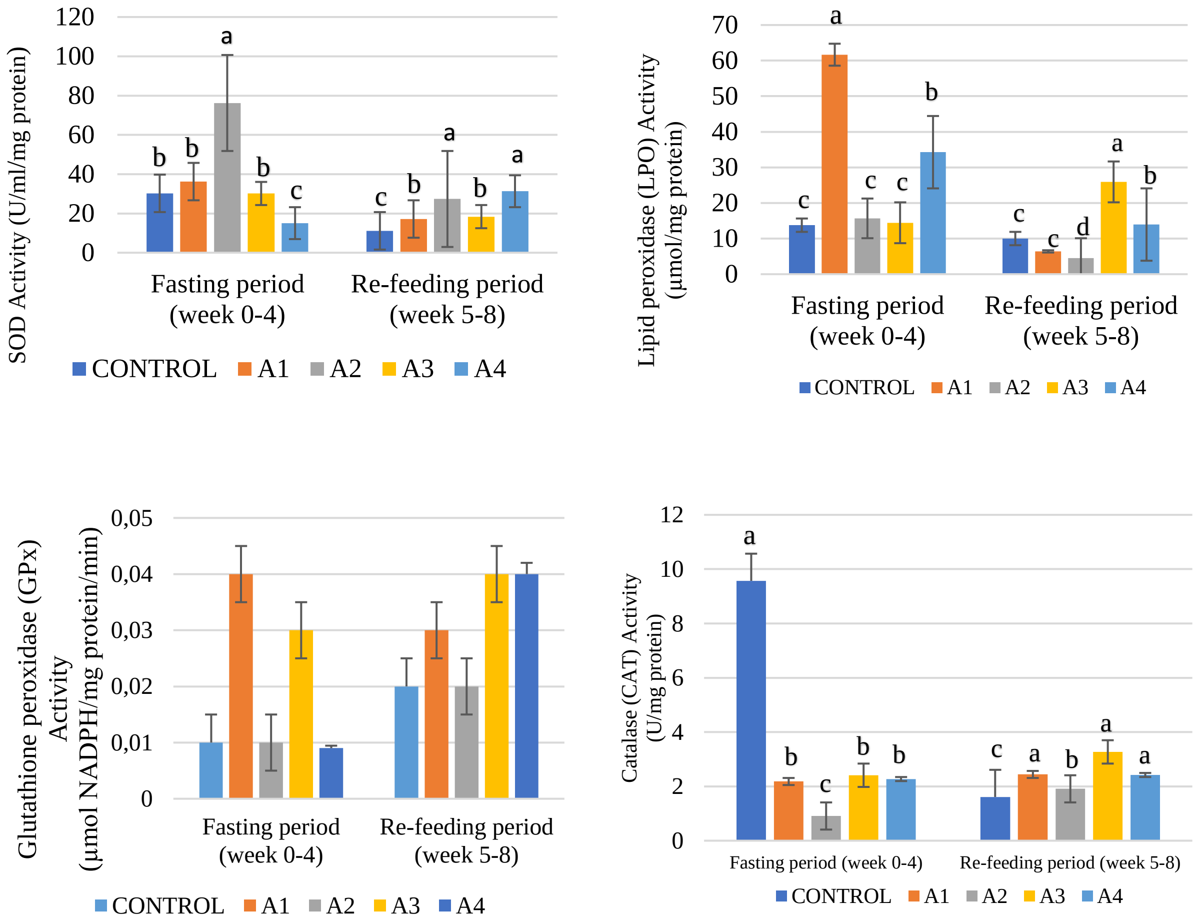

Figure 2. Changes in Superoxide Dismutase (SOD), Catalase (CAT), Lipid Peroxidase (LPO) and Glutathione Peroxidase (GPx) activity of sea bass fingerlings fed with fasting (week 0 to 4) and re-feeding (week 5 to 8) for 8 weeks.

Table 4. Antioxidant enzyme activities of sea bass fingerlings fed with fasting (week 0 to 4) and re-feeding (week 5 to 8) for 8 weeks.

Groups	Antioxidant Enzyme Acitvities			
	SOD	LPO	GPx	CAT
Fasting period (week 0-4)				
Control	30.21±1.96[b]	13.78±1.23[c]	0.01±0.01[a]	9.57±1.10[a]
A1	36.21±1.67[b]	61.67±1.43[a]	0.04±0.02[a]	2.18±1.63[b]
A2	76.23±1.97[a]	15.68±1.09[c]	0.01±0.01[a]	0.91±0.10[c]
A3	30.16±1.84[b]	14.45±0.67[c]	0.03±0.01[a]	2.41±0.18[b]
A4	15.05±1.52[c]	34.28±0.22[b]	0.01±0.01[a]	2.27±1.81[b]
Re-feeding period (week 5-8)				
Control	11.14±1.91[c]	10.02±0.28[c]	0.02±0.01[a]	1.61±0.27[c]
A1	17.16±1.80[b]	6.42±0.49[c]	0.03±0.01[a]	2.44±1.81[a]
A2	27.36±1.93[a]	4.53±0.05[d]	0.02±0.01[a]	1.91±0.79[b]
A3	18.37±1.56[b]	25.94±1.17[a]	0.04±0.03[a]	3.27±1.24[a]
A4	31.30±1.09[c]	13.95±1.18[b]	0.04±0.01[a]	2.42±0.58[b]

Data are mean ± SE. Means with different superscript letter in a row are significantly different ($p<0.05$)

Discussion and Conclusion

Fish can absorb minerals not only through food digestion but also by ingesting seawater and through exchanges between body tissues such as skin and gill membranes. Ca is readily absorbed from seawater, while freshwater contains low levels of calcium. However, most feedstuffs, especially those derived from animal proteins, are rich in Ca, indicating that calcium deficiency in fish caused by nutrient deficiencies is very unlikely. Conversely, both seawater and freshwater have minimal P content, making P levels in feeds and feed ingredients nutritionally very important (Anonymous, 2024a,b). It is well known that maintaining a favourable Ca and P balance is vital for fish, as it is for many vertebrates. The recommended ratio in fish feed is 1:1 or 1:1.5 (Martinéz-Valverde et al., 2000).Burrow et al. (2020) emphasized the physiological importance of Ca and P and stated that these minerals are essential for growth and energy production. In their study, Ca/P ratio was found to be 1.51 mg/kg. It was also reported that phosphorus supplementation to the diets of Atlantic salmon (*Salmo salar*) reared in seawater increased growth, improved feed utilisation and supported bone mineralisation (Akyurt, 1994).

Factors affecting the growth of fish in the feedback process after the fasting period are the amount of consumed feed, the quality of feed, and the water temperature (Ali et al., 2003; Eroldoğan et al., 2006; Pérez-Jiménez et al., 2007; Türkmen et al., 2012). Also, during periods of nutrient deprivation, the activation of body energy reserves to sustain life processes in fish, leads to a loss of body weight (Gisbert et al., 2011). Although partial compensatory growth (Morales et al., 2004; Mozanzadeh et al., 2017) and full compensatory growth (Pascual et al., 2003; Taravat et al., 2019), were reported, overcompensation growth is rare (Yılmaz, 2012). Adaklı and Taşbozan (2015) reported that short-term fasting and multiple cycles showed partial compensatory growth in sea bass. A partial compensatory growth was determined in the current study. Some similar results were obtained in studies on feeding patterns with different fish species (Eroldoğan et al., 2006; Mozanzadeh et al., 2017). Several studies have shown that compensatory fish growth is likely to be related to the length and impact of dietary restrictions imposed before re-feeding (Ali et al., 2003; Mozanzadeh et al., 2017). Applying different fasting and re-feeding protocols in sea bream juveniles (*Sparus aurata*) showed partial growth after feeding during the cycle period and even until satiation for 3 more weeks. Some degree of compensation was also achieved during the re-feeding period, but the best growth rate and SGR were reported in the control group (Eroldoğan et al., 2006).

Compensatory growth is a complex phenomenon influenced by various mechanisms, including metabolic adjustments and increased feed intake efficiency. After a period of starvation, fish often exhibit a hyperphagic response upon re-feeding, consuming more food than during normal feeding periods. This increased feed intake can lead to accelerated growth rates as the fish efficiently utilize the available nutrients to catch up on the lost growth

(Ali et al., 2003; Johansen et al., 2001). Additionally, metabolic adjustments, such as enhanced protein synthesis and reduced energy expenditure during the re-feeding period, play a crucial role in facilitating compensatory growth (Won and Borski, 2013). These mechanisms enable fish to not only regain lost weight but also improve their overall physiological condition, contributing to better growth performance and health outcomes. Graynoth and Taylor (2000) applied restricted feeding for 33 days in two species of eels (*Anguilla australis* and *A. dieffenbachii*) which were then fed to satiation for 10 days and determined that compensatory growth occured at the end of the experiment. Mozanzadeh et al. (2017) reported that with the same nutritional regimen (1-day fasting, 2 days re-feeding) in sobaity seabream (*Sparidentex hasta*) showed full compensatory growth. This suggests that fasting and re-feeding cycles provide a species-specific response. Again, Taravat et al. (2019) reported that after the re-feeding period, groups with food deprivation showed full compensatory growth and from the results sobaity seabream had the ability to maintain optimal growth after periods of food deprivation and re-feeding. Pérez-Jiménez et al. (2007), who subjected sea bass to a 9-day fasting period followed by 12 days of re-feeding with diets containing 42% (42P) and 50% (50P) protein, reported that fish experienced rapid metabolic adjustments to both fasting and re-feeding, and that no significant differences in growth performance, feed intake and feed efficiency were observed between groups. Türkmen et al. (2012) concluded that sea bass responds rapidly to cyclical fasting and re-feeding and that the 25% restricted feeding rate is insufficient to induce a compensatory growth response in sea bass.

The majority of fish species rely on their body's protein and lipid reserves when they are starved, and research on the significance of glycogen as an energy reserve has produced conflicting findings (Hemre et al., 2002; Furné et al., 2012). The main site of glycogen accumulation in fish is the liver. The cases of liver enlargement have been reported as a result of intense glycogen accumulation (Hemre et al., 2002). In the current study, low HSI in the fasting groups (A1, A2, A3, and A4) supported lipid utilization for metabolic energy. Our results are consistent with the observations for several fish species in which rapid regeneration of liver reserves in re-feeding. This demonstrates the importance of the liver during short-term fasting and restricted feeding regimes (Ali and Jauncey, 2004; Pérez-Jiménez et al., 2007). This might be the result of consuming more feed in the early stages of re-feeding in order to replenish muscle glycogen stores quickly and to recover energy (Black and Love, 1986). According to Liu et al. (2022), after seven days of re-feeding, the majority of the starvation-induced alterations in the immune system, gut microbiota, liver transcriptome, and enzyme activity in golden pompano (*Trachinotus ovatus*) progressively returned to normal. Because a species' eating habits influence how it responds metabolically to nutrient scarcity, carnivorous fish—which naturally feed less than omnivorous and herbivorous fish—are more adapted to this environment than other fish species (Furné et al., 2012). Given their carnivorous nature, sea bass juveniles in our study may have chosen to segregate intraperitoneal lipids for various metabolic uses, such as foraging (Eroldoğan et al., 2006).

Fish meat is an important source of minerals despite being high in digestible protein. While K, Na, Cl, Mg, P and Ca contents are higher, Fe, Zn, Cu and I contents are lower (Martinéz-Valverde et al., 2000). These components are also crucial for many aspects of fish metabolism. They give fish bones their strength and toughness. They are primarily involved in the neurological and endocrine systems, as well as in preserving the osmotic equilibrium with the water environment, in bodily fluids. They are parts of blood pigments, enzymes, and other organic substances. They also have a role in metabolic processes related to energy transport (New, 2021). Various studies have shown that the trace mineral content in fish is affected by various factors such as fish size (Yildiz, 2008), sexual maturity (Roy and Lal, 2006; Yildiz, 2008), food sources (Roy and Lal, 2006) and environment (Yamashita et al., 2006). In the present study, there were no significant size variations among all groups and not all fish were sexually mature. All environmental conditions such as salinity, temperature, dissolved oxygen and pH were optimal for these fish species. Only the factors of food deprivation and re-feeding, which were included in the study, were different. Different things happened to each group depending on the circumstances. Fish utilize their endogenous reserve to obtain the energy required to sustain essential functions (brain function, breathing, controlling mineral balance, etc.) and drastically cut energy expenditure from protein conversion in order to survive during periods of unfavorable feeding conditions (Salem et al., 2007). Depending on the species, this response necessitates metabolic adjustments (Wang et al., 2006). Furthermore, fish age or nutritional state are two variables that affect intra-specific modifications unique to these circumstances (Furné et al., 2012). During the fasting period, the highest mineral contents were found in the A4 group (fasting for four week). This suggested that fish tend to store minerals as a response to fasting.

Although similar results were determined among mineral matter compositions in all groups during the re-feeding period, the calcium and phosphorus ratio was significantly lower than the values determined during the fasting period in all groups. Considering that calcium is an important mineral for bone development, it suggested that fish may have used these minerals effectively in bone development. In addition, calcium and phosphorus are physiologically critical minerals that are particularly necessary for growth and energy production (Burrow et al., 2020), suggesting that fish may have stored these minerals during the fasting period. Sodium is a mineral that is part of the carbohydrate and amino acid transport mechanism. Potassium balances the effects of sodium and ensures that fluid levels remain within a certain range. It also helps the proper functioning of body systems, including heart, muscle and bone health. (Akyurt, 1994).

Fish defense mechanisms that either completely eradicate or significantly restrict the spread of infections are involved in the antioxidant enzyme activities of fish (Blazer, 1992). The rise in antioxidant enzyme activity has been viewed by many scientists as a defense mechanism against oxidative stress (Thomas, 2000). According to Florescu et al. (2019), the activities of the enzymes SOD and CAT are thought to be biological indicators of oxidative stress, and an increase in SOD activity could be brought on by an increase in intracellular superoxide radicals (Cheng et al., 2007). Furthermore, oxidative stress resulting from a reduction in oxygen consumption varies among fish species and could be caused by a decrease in activity as a means of preserving body energy during times of food scarcity (Glass, 1968). In our study, activities specific to SOD and CAT increased in the liver of fish that were exposed to the fasting period compared to the re-feeding period. Therefore, excessive ROS production due to fasting, precisely O_2- and H_2O_2, may have triggered an increase in both SOD and CAT activities in the liver. Antonopoulou et al. (2013), contrary to the current study results, reported that there was no significant change in antioxidant enzyme levels in sea bass liver, and that there were significant changes in antioxidant enzyme levels (SOD and CAT) in other tissues they examined (red muscle, intestine and white muscle). Furthermore, in both *Sparus aurata* (Pascual et al., 2003) and *Dentex dentex* (Morales et al., 2004), dietary restriction has been shown to considerably improve antioxidant enzyme activities such as SOD, glutathione reductase, glutathione peroxidase, and CAT. Nonetheless, following a 12-week fast, there has been evidence of elevated CAT activity in the liver of Atlantic cod (Guderley et al., 2003). According to Bayir et al. (2012), antioxidant enzyme activity increased when brown trout (*Salmo trutta*) were deprived of nutrients, and re-feeding did not always result in antioxidant enzyme activity control values. In a similar vein, it was observed that following seven days of undernutrition, the expression of SOD and CAT genes in the liver and gills of *Labeo rohita* fingerlings was considerably elevated, downregulated after three days of re-feeding, and returned to basal levels after eight days of re-feeding (Dar et al., 2019). Growth, biochemical indices, and oxidative stress measures, including as total antioxidant capacity, CAT, and SOD, were all impacted by starving and re-feeding in a study conducted on golden pompano (*Trachinotus ovatus*) (Liu et al., 2022).

When hunger and fasting stress are present, oxidative stress production in fish is typically linked to an increase in LPO levels (Furné et al., 2009). The results, which were consistent with previous research, demonstrated that the fasting phase significantly increased LPO in the liver compared to control fish and that the re-feeding period caused oxidative stress based on LPO levels. In contrast to what we found, Florescu et al. (2019) observed that during the fasting phase and the re-feeding period that followed, the levels of LPO in stellate sturgeon (*Acipenser stellatus*) rose. Sakyi et al. (2020), in line with our research, found that Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) had a decrease in LPO levels during the re-feeding phase.

According to Zeng et al. (2016), one of the key antioxidant enzymes in the fish antioxidant defense system is GPx. In contrast to the control group, Wang et al. (2019) discovered that GPx levels in the tissues (gill, liver, spleen, and kidney) of *Schizothorax wangchiachii* were not significantly impacted by varying food restriction and re-feeding regimens. Although the fish in the current study were stressed out by fasting, the detrimental consequences of this stress were largely offset by re-feeding, so there was no discernible difference in the liver GPx levels between the treatments. In contrast, as re-feeding time rose, antioxidant enzyme levels such as SOD, CAT, and LPO dropped. These findings suggest that oxidative stress in European sea bass may occur from extended fasting. According to research by Furné et al. (2012), oxidative stress indicators were still present in the liver tissue and red blood cells of rainbow trout (*Oncorhynchus mykiss*) and sturgeon (*Acipenser naccarii*) after a protracted fasting period of 72 days and a 60-day re-feeding phase. Variations in species responses, life history phases, culture conditions, duration and degree of food availability, and the particular tissues tested can all be responsible for the observed disparities in the results (Antonopoulou et al., 2013).

The current research results confirm that a fasting and re-feeding regimen can serve as a viable alternative nutritional strategy in aquaculture for European sea bass (*Dicentrarchus labrax*) in low salinity conditions (‰19). The observed partial compensatory growth, along with significant fluctuations in mineral content and antioxidant enzyme activities, suggests that fish can adapt to periods of feed deprivation followed by re-feeding in these specific conditions. However, the impact on fish health and long-term performance indicates that these regimens must be carefully managed. Future studies should focus on optimizing fasting and re-feeding protocols at low salinity levels to minimize stress, ensure optimal growth, and maintain the nutritional and physiological well-being of the fish. These efforts will contribute to the sustainable and economical production of this valuable species in aquaculture under low salinity conditions.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Author Contributions

Seval Dernekbaşı designed the work. Seval Dernekbaşı, Dilara Kaya Öztürk realised the work. Dilara Kaya Öztürk carried out the laboratory studies. Keriman Yürüten Özdemir performed antioxidant enzymes analyses. Ismihan Karayücel and Seval Dernekbaşı analysed the data and interpreted the results. All authors were involved in the setup and finalisation of the experiment.

Ethical Statement

This experiment was carried out according to the ethical principles of animal experiments established by Sinop University Animal Experiments Control Council and the current legislation approved by the Local Animal Use Ethics Committee (Protocol 12/2019).

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COMU J Mar Sci Fish 2025 8(1): 14-22
https://doi.org/10.46384/jmsf.1591598
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Length-Weight Relationship and Diet Composition of *Pomadasys stridens* (Forsskål, 1775) from İskenderun Bay (Eastern Mediterranean Sea)

Sencer Akalın, Tuncay Murat Sever, Dilek İlhan*, Murat Kaya, Burak Altay

Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org//0000-0001-9839-6485
Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org//0000-0001-5231-4077
Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org//0000-0003-1228-105X
Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org//0000-0002-3242-6079
Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org//0000-0003-0410-896X

Received: 26.11.2024 /Accepted: 27.01.2025 / Published online: 14.07.2025

Keywords:

LWR
Diet
Pomadasys stridens
İskenderun Bay
Eastern Mediterranean Sea

Abstract: A total of 513 the striped piggy, *Pomadasys stridens* were collected from İskenderun Bay, Eastern Mediterranean, by a fishing boat at depths of 18-20 m in July and August 2017. The overall sex ratio (female:male) was 1:1. The total length (weight) of females varied between 7.1 cm and 14.6 cm (4.78 g to 44.97 g), and of males between 7.5 cm and 15.5 cm (6.61 g to 59.15 g). The length–weight relationships were: $W=0.00965 \cdot L^{3.164}$ (r=0983) for females, $W=0.00876 \cdot L^{3.195}$ (r=0.986) for males and $W=0.00834 \cdot L^{3.219}$ (r=0.988) for both sexes. A total of 156 *P. stridens* stomachs were analyzed and 22 taxa were identified. Copepods formed the main prey group for all length classes. Amphipoda, Polychaeta and Decapoda were found to be important prey groups for this species feeding in the area. A total of 9 different copepod species were identified with the highest percentage IRI values determined for *Calanopia elliptica* (Calanoida) and *Euterpina acutifrons* (Harpacticoida).

Anahtar kelimeler:

LWR
Besin kompozisyonu
Pomadasys stridens
İskenderun Körfezi
Doğu Akdeniz.

İskenderun Körfezi'nde (Doğu Akdeniz) *Pomadasys stridens* (Forsskål, 1775)'in Boy-Ağırlık İlişkisi ve Besin Kompozisyonu

Öz: Toplam 513 adet *Pomadasys stridens* bireyi İskenderun Körfezi (Doğu Akdeniz)'nden bir balıkçı teknesi ile Temmuz ve Ağustos 2017'de, 18-20 m derinlikten toplanmıştır. Cinsiyet oranı (dişi:erkek) 1:1 olarak hesaplanmıştır. Dişilerin toplam boyu (ağırlığı) 7,1 cm ile 14,6 cm (4,78 g ile 44,97 g), erkeklerin ise 7,5 cm ile 15,5 cm (6,61 g ile 59,15 g) arasında değişmiştir. Boy-ağırlık ilişkileri: dişiler için $W=0,00965 \cdot L^{3.164}$ (r=0,983), erkekler için $W=0,00876 \cdot L^{3.195}$ (r=0,986) ve tüm bireyler için $W=0,00834 \cdot L^{3.219}$ (r=0,988)'dir. Toplam 156 *P. stridens* midesi incelenmiş ve 22 takson tanımlanmıştır. Copepodlar tüm boy sınıfları için esas av grubunu oluşturmuştur. Amphipoda, Polychaeta ve Decapoda'nın bölgede beslenen tür için diğer önemli av grupları olduğu bulunmuştur. Copepoda sınıfından 9 tür tanımlanmış ve en yüksek IRI değerleri *Calanopia elliptica* (Calanoida) ve *Euterpina acutifrons* (Harpacticoida) için belirlenmiştir.

Introduction

In recent years, especially in the Mediterranean, the vast majority of studies on biodiversity are conducted on Lessepsian species. The striped piggy, *Pomadasys stridens* (Forsskål, 1775), is a lessepsian migratory fish of the family Haemulidae. They live in coastal waters and swims in schools at depths of up to 25 m on sandy bottoms. It is considered as a commercially fundamental food fish in the northern Indian Ocean especially Aqaba Gulf, Persian Gulf and the Bitter Lakes in Egypt (Karimi et al., 2014). *P. stridens*, was first recorded in 1969 by Torchio from the Genoa Bay in Italy (Golani et al., 2002). There are various records on the Aegean and Mediterranean coasts (Bilecenoğlu et al., 2009; Ergüden et al., 2015; Vahabnezhad et al., 2015; Akyol & Ünal, 2016; Akyol & Çoker, 2018; Vahabnezhad et al., 2018; Osman et al., 2019). The aim of the present paper was to describe the length-weight relationship (LWRs) and diet composition of *P. stridens* from İskenderun Bay, a very important fishing area in the north-eastern Mediterranean, lies beside the southern Turkish metropolitan cities of Adana and Hatay. LWR of fishes is very important in fisheries research because it is essential in establishing stock composition and evaluating fish condition.

*Corresponding author: dilek.ilhan@ege.edu.tr

How to cite this article: Akalın, S., Sever, T.M., İlhan, D., Kaya, M., & Altay, B. (2025). Length-weight relationship and diet composition of *Pomadasys stridens* (Forsskål, 1775) from İskenderun Bay (Eastern Mediterranean Sea). COMU J. Mar. Sci. Fish, 8 (1): 14-22 doi:10.46384/jmsf.1591598

Material and Methods

The striped piggy specimens were captured between July to August 2017 in the İskenderun Bay (Figure 1) and a total of 513 *P. stridens* samples were examined. Total length (±1.0 mm) and weight (±0.001 g wet weight) were recorded for each fish. The sex ratio was evaluated by using the chi-square test (χ^2) (Zar, 1999).

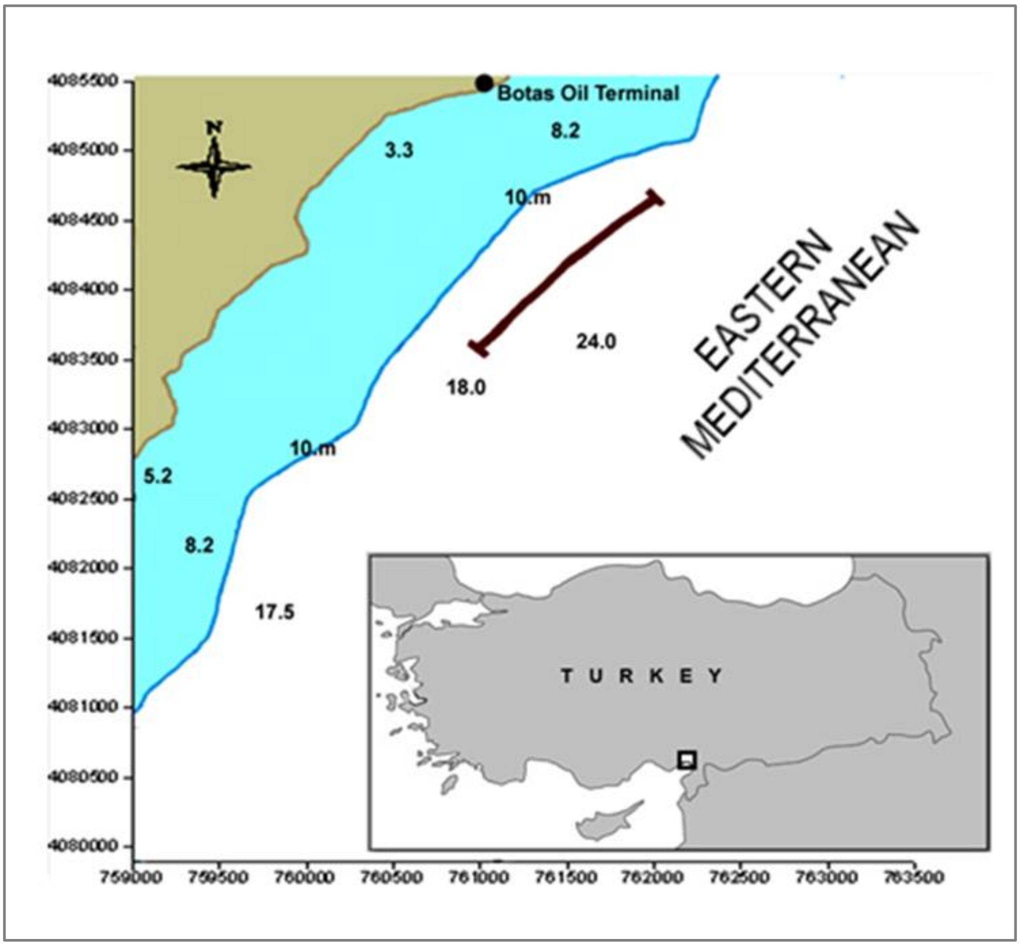

Figure 1. The sampling area of *Pomadasys stridens*

Parameters of the length-weight relationship were obtained by fitting the power function $W=a\cdot L^b$ to length and weight data where; W is the total weight (g), L is the total length (cm) and a (intercept) and b (slope) are regression constant (Sparre and Venema, 1992). The student's t-test was used to test isometric growth (Ricker, 1973).

The fish stomachs were removed immediately following capture and were stored in 4% buffered formalin solution until the contents were analysed. For analysing food composition of length groups, the samples were divided into 5 length groups as; below 8 cm, between 8.1-10.0 cm, between 10.1-12.0 cm, between 12.1-14.0 and bigger than 14.1 cm. The contents of each stomach were placed on a petri dish and identified to the possible lowest taxonomic class using a binocular microscope. All food items were counted and weighed wet to the nearest ±0.0001 g after removing excess liquid. Completely digested food remains were also recorded as unidentified.

Three widely-used measures were utilized to quantify stomach contents and also to reveal the relative importance of various food items in the diet: percentage frequency (%FO), percentage composition by number (%N), and percentage composition by weight (%W). (Bowen, 1996; Tirasin, and Jørgensen, 1999). Additionally, the index of relative importance (IRI) was estimated for each food item (Pinkas et al., 1971).

In order to determine the preference categories of the prey groups IRI (Index of Relative Importance) values suggested by Morato-Gomes (1995) was used. IRI values were reported as percentage as an estimate of relative importance of each prey type in the diet of examined fish and to make comparisons between other studies (Cortés, 1997 and 1998). Main important prey (MIP) was considered when $IRI \geq 30\times(0.15\times\Sigma\%F)$, secondary important prey (SP) was considered when $30\times(0.15\times\Sigma\%F) > IRI > 10\times(0.05\times\Sigma\%F)$ and occasional prey (OP) was considered when $IRI \leq 10\times(0.05\times\Sigma\%F)$.

Percentage Frequency of Occurrence =>
$$\%F_i = \frac{\sum_{j=1}^{j} M_{ij}}{N} \times 100$$

Percentage Composition by Number =>
$$\%N_i = \frac{\sum_{j=1}^{n_j} N_{ij}}{\sum_{i=1}^{n_i}\sum_{j=1}^{n_j} N_{ij}} \times 100$$

Percentage Composition by Weight =>
$$\%W_i = \frac{\sum_{j=1}^{n_j} W_{ij}}{\sum_{i=1}^{n_i}\sum_{j=1}^{n_j} W_{ij}} \times 100$$

Index of Relative Importance =>
$$IRI_i = \%F_i \times (\%W_i + \%N_i)$$

Percentage IRI =>
$$\%IRI_i = \frac{IRI_i}{\sum_{i=1}^{n} IRI_i} \times 100$$

Results

Of the 513 individuals of *P. stridens*, 182 (35.48%) were males, 181 (35.28%) females, and 150 (29.24%) were immature fishes. The female:male ratio was 1:1 and the chi-square analysis indicated that there was no statistically significant differences among sexes ($\chi 2$, $p<0.05$). The total length (weight) of females ranged between 7.1 - 14.6 cm (4.78 g - 44.97 g), and males between 7.5 - 15.5 cm (6.61 g - 59.15 g). The majority of fish belonged to length group of 7.0 cm,accounting for 47% of all samples (Fig 2).

According to Student's t-test, positive allometric growth (P>0.05 was observed for females, males and combined sexes in the research area (Table 1).

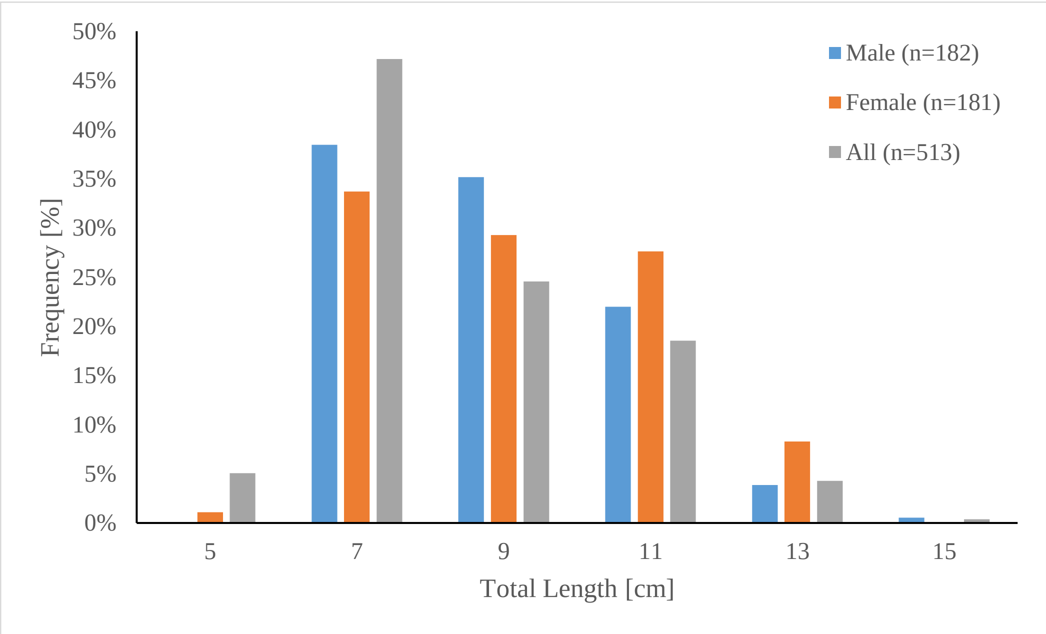

Figure 2. Length frequency distribution of *Pomadasys stridens* from İskenderun Bay, Eastern Mediterranean Sea

Table 1. Parameters of length–weight relation of *Pomadasys stridens* from İskenderun Bay, Eastern Mediterranean Sea

Sex	n	TL (cm)	TW (g)	*a*	*b*	SE*(b)*	R	t-test	Growth Type
♀	181	7.1-14.6	4.78-44.97	0.00965	3.164	0.0433	0.983	3.287[a]	A+
♂	182	7.5-15.6	4.74-52.21	0.00876	3.195	0.0418	0.986	4.665	A+
♀ + ♂	513	5.5-15.6	1.98-58.23	0.00834	3.219	0.0241	0.988	9.087	A+

n, number of specimens studied; TL, total length; TW, total weight; *a*, intercept; *b*, slope; SE*(b)*,standard error of the slope; R, coefficient of determination, GT, growth type; A+, positive allometric; a, t-test, t<t0.05, n>200=1.65

In the present study, l of 156 stomachs analyzed; 14 were empty and 142 contained prey items. The total number of the prey items recovered were 8157 representing. 22 taxa. It was determined that the specimens in all length groups had dominantly full stomachs.

Copepods were identified as the major prey item for all length groups. In addition, Amphipoda were found to be another essential prey item for the length group of 12.1 – 14.0 cm. It was determined that Polychaeta and Decapoda larvae were also important prey items for this species feeding in the area (Table 2).

Our findings indicated that, striped piggy in İskenderun Bay feeds mainly on copepods, followed by decapod crustacean larvae and amphipods. IRI% values was calculated as 92.39% for Copepods, 1.9 % Amphipoda, 2.98 % Decapod crustacean larvae and 2.73 % all other preys.

Table 2. Length groups and total main prey taxa IRI % for *Pomadasys stridens* in İskenderun Bay, Eastern Mediterranean Sea

	Length Groups					
Prey group	**<8.0 cm (n=21)**	**8.1-10.0 cm (n=21)**	**10.1-12.0 cm (n=39)**	**12.1-14.0 cm (n=34)**	**>14.1 cm (n=27)**	**Total (n=142)**
Copepoda	96.40	98.13	93.47	36.10	40.77	92.39
Gastropoda	0.42	0.18	0.33	0.07	0.10	0.27
Ostracoda	0.49	0.41	0.34	0.29	0.66	0.40
Bivalvia	0.01	0.00	0.01	0.00	0.00	0.01
Cirriped nauplius	0.00	0.00	0.01	0.00	0.00	0.01
Mysidacea	0.00	0.00	0.01	0.81	2.57	0.05
Amphipoda	0.03	0.04	2.20	44.49	6.71	1.90
Decapoda larvae	2.52	1.11	1.70	3.44	11.69	2.98
Penaidae	0.00	0.00	0.02	0.00	0.00	0.01
Polychaeta	0.11	0.03	1.79	14.55	36.42	1.89
Chaetognatha	0.00	0.00	0.01	0.00	0.00	0.01
Salpa spp.	0.00	0.00	0.01	0.00	0.00	0.01
Fish	0.00	0.08	0.04	0.26	0.74	0.04

Table 3. Length groups and total main prey copepod species IRI % for *Pomadasys stridens* in İskenderun Bay, Eastern Mediterranean Sea

	Length groups					
	<8.0	**8.1-10.0 cm**	**10.1-12.0 cm**	**12.1-14.0 cm**	**>14.1 cm**	**Total**
Calanoida	2.65	0.03	0.57	0.44	0.45	0.90
Calocalanus pavo	0.01	0.01	0.00	0.00	0.00	0.01
Calocalanus sp.	0.00	0.00	0.01	0.00	0.00	0.01
Calanopia elliptica	22.85	34.33	46.67	11.90	15.52	31.70
Candacia simplex	0.68	0.00	0.00	0.00	0.00	0.08
Candacia sp.	0.01	0.00	0.00	0.00	0.00	0.01
Corycaeus sp.	0.00	0.00	0.00	0.00	0.12	0.01
Oncaea media	0.00	0.01	0.00	0.00	0.00	0.01
Oncaea sp.	0.01	0.00	0.02	0.00	0.00	0.01
Harpacticoida	43.82	51.48	43.88	20.85	20.89	49.09
Euterpina acutifrons	25.35	11.78	1.65	1.17	0.13	8.90
Other Groups	4.61	2.35	7.20	65.64	62.89	9.25

Nine different copepod species were identified (Table 3). Highest IRI values were determined for *Calanopia elliptica* (Calanoida) and *Euterpina acutifrons* (Harpacticoida). *C. elliptica* is an invasive species for the Mediterranean and it is a pelagic species. It is distributed in neritic and epimesopelagic (Razouls et al., 2005-2023). Its distribution in the İskenderun Bay was also reported by zooplanktonic studies (Dönmez, 1998). All the copepod species determined in the present study are characteristic for the depths where *P. stridens* live (Weikert and Trinkaus, 1990; Sever, 1997; Dönmez, 1998).

Table 4. Preferred prey items of *Pomadasys stridens*, according to Morato Index, in İskenderun Bay, Eastern Mediterranean Sea

	Length groups					Total
	< 8.0 cm	**8.0 - 10.0 cm**	**10.1 - 12.0 cm**	**12.1 - 14.0 cm**	**14.1 cm >**	
(MIP)	IRI ≥ 1307.16	IRI ≥ 964.30	IRI ≥ 1384.60	IRI ≥ 1098.54	IRI ≥ 983.34	IRI ≥ 1159.87
	Copepoda	Copepoda	Copepoda	Copepoda Polychaeta Amphipoda	Copepoda Polychaeta	Copepoda
(SP)	IRI > 145.24	IRI > 107.14	IRI > 153.84	IRI > 122.06	IRI > 109.26	IRI > 128.87
	Decapoda larvae	Decapoda larvae	Polychaeta Decapoda larvae	Decapoda larvae	Decapoda larvae Amphipoda Mysidacea	Decapoda larvae Polychaeta
(OP)	IRI ≤ 145.24	IRI ≤ 107.14	IRI ≤ 153.84	IRI ≤ 122.06	IRI ≤ 109.26	IRI ≤ 128.87
	Ostracoda Gastropoda Polychaeta Amphipoda Bivalvia larvae	Ostracoda Gastropoda Amphipoda Polychaeta Other groups	Amphipoda Ostracoda Gastropoda Other groups	Mysidacea Ostracoda Fish Gastropoda	Fish Ostracoda Plants Gastropoda	Amphipoda Ostracoda Gastropoda Other groups

MIS=Morato Index score; MIP=main important prey, SP=secondary prey, OP=occasional prey

According to the Morato Index, copepods were the main prey group for all length groups analyzed. In some length groups (IV-V) Polychaeta and Amphipoda were also observed as the primary prey category. Secondary prey items were Decapod larvae and Polychaeta and the all other prey types were considered as occasional preys like Ostracoda, Gastropoda, and Amphipoda, etc. (Table 4).

Discussion

Lessepsian migrants currently constitute 16.3% of Turkish marine fish fauna, and the invasion of the Mediterranean appears to persist unabated. (Bilecenoğlu, 2024). The correlation between native and invasive fish is crucial for the protection and sustainability of fish stocks. Therefore, these new species' biological characteristics and hosts should be revealed. *P. stridens* is a widespread fish species on the Turkish Eastern Mediterranean coast and has relatively higher abundance and biomass in commercial fish catches, especially those below 50 m (pers. obs. of the first author and communication with the fishermen). The abundance of this exotic species may have negative effects on the native demersal fish species due to competition for food.

Table 5. The number of specimens, female:male ratio and total length range values *Pomadasys stridens* obtained by different authors from different area

Research Area	N	F:M	TL	Author
Arabian Gulf	216	1:0.70	14.0-19.0	Ahmad & Al-Ghais (1997)
Yumurtalık shores, Adana, Eastern Mediterranean	6	-	13.3-15.8	Bilecenoğlu et al. (2009)
Northwest of Persian Gulf, Iran	396	1:0.4	11.1-23.5	Hashemi & Taghavimotlagh (2012)
Karachi Coast, Pakistan	391	1:0.66	5.6-21.0	Safi et al. (2014)
Karachi, Pakistan	192	-	13.8-20.8	Ahmed et al. (2015)
Iskenderun Bay, Eastern Mediterranean	335	-	7.6-17.7	Ergüden et al. (2015)
Khuzestan Coastal Waters (Northwest Persian Gulf)	218	-	7.5-24.0	Hoveizavi et al. (2016)
Persian Gulf and Oman Sea	2	-	18.8-19.0	Jawad et al. (2017)
İskenderun Bay, Eastern Mediterranean	1064	1:0.71	9.8-18.3	Özbek (2017)
Bitter Lakes, Egypt	-	-	7.0-19.9	El-Azim et al. (2017)
İskenderun Bay, Eastern Mediterranean	659	1:1.26	5.0-18.3	Uyan et al. (2018)
Persian Gulf, Iran	276	1:0.4	8.0-20.5	Vahabnezhad et al. (2018)
Marmaris coast, Southern Aegean Sea	4	-	13.6-14.0	Akyol & Çoker (2018)
Gulf of Suez	409	-	6.6-19.0	Osman et al. (2019)
Northern Part of Persian Gulf (Bushehr)	591	1:3.30	11.7-23.0	Karimi et al. (2019)
Gulf of Suez, Red Sea, Egypt	165	-	8.2-16.3	Basuonie et al. (2020)
İskenderun Gulf, North-eastern Mediterranean	1131	1:0.94	7.3-18.9	Avşar et al. (2021)
Mersin Bay, North-eastern Mediterranean	565	1:0.87	5.3-17.6	Tüzün & Gücü (2023)
Syrian coast, Eastern Mediterranean	647	-	7.3-18.9	Nader et al. (2024)
İskenderun Bay, Eastern Mediterranean	363	1:1	7.1-15.5	Present study

For the Mediterranean Sea, *P. stridens* was first recorded in the Gulf of Genoa, Italy by Torchio in 1969 (Golani et al., 2002). After that, it was reported by BenTuvia (1976) from Bardawil Lagoon, Egypt. In recent studies, the reported number of specimens of this species, female:male ratio and total length range values are given in Table 5. In both the species' native distribution areas and in the Mediterranean, females seem to have higher abundance than males. But in some studies, the ratio favored males (Uyan et al., 2018; Karimi et al., 2019). Our findings were 1:1 for the İskenderun Bay, which is almost similar to 1:0.94 reported by Avşar et al., (2021) from the same area. Differences in the sex allocation of *P. stridens* are not clearly explained. They could be coincidental because the size distribution of the male and female specimens overlapped in many studies, reflecting similar growth rates.

Our findings suggest that the striped piggy showed a positive allometric growth in İskenderun Bay. Osman et al. (2019), Ergüden et al. (2015) and Avşar et al. (2021) also reported that this species showed a positive allometric growth in Gulf of Suez and İskenderun Bay. On the other hand, Hashemi and Taghavimotlagh (2012) and El-Azim et al. (2017), stated that isometric growth was observed in the northwest Persian Gulf (Iran) and Bitter lakes (Egypt), respectively. Özbek (2017) noted that females of this species exhibited negative allometric growth, while males and all individuals exhibited isometric growth in İskenderun Bay (Eastern Mediterranean). Differences in b obtained from other locations relate substantially on the form and condition of the species. Additionally, several factors may cause variations in equations of the LWR among seasons and years, such as salinity, temperature, sex, gonadal development (Pauly, 1984; Sparre & Venema, 1992).

Our findings revealed that *P. stridens* feeds on mainly crustaceans (Copepoda, Decapoda, Amphipoda and Mysidacea) and Polychaeta in the İskenderun Bay. Safi et al. (2013) reported that the species feeding on crustaceans, molluscans, teleosts and polychaeta in Pakistan waters of Oman Sea. In addition, they also predicted that the species is an active predator and its feeding habits were related with the abundancy and variety of the prey groups and the environmental factors. Vahabnezhad et al. (2015) indicated that the first preferred food items for the species is crustaceans (Decapoda, Copepoda, Amphipoda, Cumacea and Ostracoda) and molluscs (Gastropoda, Bivalvia). The second preferred prey type was Nematoda and the occasional prey was Echinodermata. El-Azim et al. (2017) stated that *P. strident* is a carnivorous species, fed majorly on crustaceans (62.12%), molluscs (20.31%) small fishes (11.31%), polychaetes (1.26%) in the Bitter lakes, Egypt. Tüzün and Gücü (2023) reported that the species primarily feed on crustaceans (mainly copepods), and additionally on annelids (polychaetes and oligochaetes) in Mersin Bay, Northeastern Mediterranean Sea.

The differences in prey selectivity of this species between different studies could be due to the different composition of the prey items in the areas and discrepancies in length groups of the fishes (Biswas, 1993).

Studies on the feeding of *P. stridens* are lacking both in the Turkish coasts of the Mediterranean Sea and other areas where the species is native or invasive. This study is important as it presents the diet of *P. stridens* for the first time in İskenderun Bay, Eastern Mediterranean. More research is needed to shed light on the reproduction biology and population structure of this species in the area to manage the commercial species at a sustainable level.

Conflict of Interest

The authors affirm that they do not have any conflicts of interest.

Author Contributions

Akalın S.: Conceived, designed and performed analysis, wrote the paper. Sever T. and İlhan D.: Worked laboratory, performs analysis, and wrote the paper. Kaya M.: Collected specimens and wrote the paper. Altay B.: performed analysis, and wrote the paper.

Ethics Approval

No ethics committee approval is required for this study.

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COMU J Mar Sci Fish 2025 8(1): 23-31
https://doi.org/10.46384/jmsf.1607101

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Production and Sustainability in Türkiye's Fisheries Sector: The Role of Economic Variables

Serkan Şengül

Mudanya University, Buırsa, Türkiye

https://orcid.org//0000-0001-9891-9477

Received: 25.12.2024 /Accepted: 24.03.2025 / Published online: 14.07.2025

Keywords:

Capital investment
Energy cost
Fisheries sector
Labor cost
Sustainability
Türkiye

Abstract: This study investigates the impact of basic economic variables on production processes in Türkiye's fisheries sector, emphasizing sustainable development strategies. Using a dataset from the Turkish Statistical Institute covering annual data from 2003 to 2023, the research employs the ARDL model to examine the long-term and short-term effects of capital investments, labor costs, and energy expenses on total production. Results indicate that capital investments significantly enhance productivity, though misallocation can negatively impact efficiency. Labor and energy costs exhibit a negative effect, underlining the importance of cost optimization for sectoral sustainability. Based on these findings, the current study proposes strategic policy recommendations such as; efficient capital allocation, labor cost optimization through training programs, and the adoption of renewable energy sources to reduce operational expenses. These recommendations aim to support sustainable growth in the Turkish fisheries sector, enhance food security, and bolster economic resilience.

Anahtar kelimeler:

Sermaye yatırımları
Enerji maliyeti
Balıkçılık sektörü
İş gücü maliyeti
Sürdürülebilirlik
Türkiye

Türkiye Balıkçılık Sektöründe Üretim ve Sürdürülebilirlik İlişkisi: Ekonomik Değişkenlerin Rolü

Öz: Bu çalışma, sürdürülebilir kalkınma stratejilerine vurgu yaparak, Türkiye'nin balıkçılık sektöründeki üretim süreçleri üzerinde temel ekonomik değişkenlerin etkisini araştırmaktadır. Türkiye İstatistik Kurumu'ndan alınan 2003-2023 yılları arasındaki yıllık verileri kapsayan araştırma, sermaye yatırımlarının, işçilik maliyetlerinin ve enerji giderlerinin toplam üretim üzerindeki uzun vadeli ve kısa vadeli etkilerini incelemek için ARDL modelini kullanmaktadır. Sonuçlar, sermaye yatırımlarının üretkenliği önemli ölçüde artırdığını, ancak yanlış tahsisin verimliliği olumsuz etkileyebileceğini göstermektedir. İşgücü ve enerji maliyetleri olumsuz bir etki göstererek, sektörel sürdürülebilirlik için maliyet optimizasyonunun önemini vurgulamaktadır. Bu bulgulara dayanarak, mevcut çalışma; verimli sermaye tahsisi, eğitim programları aracılığıyla işgücü maliyeti optimizasyonu ve operasyonel giderleri azaltmak için yenilenebilir enerji kaynaklarının benimsenmesi gibi stratejik politika önerileri sunmaktadır. Bu öneriler, Türkiye balıkçılık sektöründe sürdürülebilir büyümeyi desteklemeyi, gıda güvenliğini artırmayı ve ekonomik dayanıklılığı güçlendirmeyi amaçlamaktadır.

Introduction

The fisheries sector plays a fundamental role in the economic development and food security of many countries in the world. Especially in countries like Türkiye, which has a long coastline and abundant seafood resources, fisheries is very important both as an economic resource and andin terms of creating employment opportunity (Bilgin, 2012). In Türkiye, the fisheries sector covers a wide supply chain from production to marketing, as well as fishing and aquaculture activities. However, in recent years, the need to improve the production processes in line with the principles of sustainable development and to increase economic efficiency has become even more important (Topçu, 2012). As stated by the Food and Agriculture Organization (FAO, 2022), fisheries and aquaculture activities contribute to both global nutritional security and livelihoods. However, issues such as overuse of natural resources, overfishing and marine pollution place significant pressure on fish populations (Cochrane, 2001).

In Türkiye, the protection of ecosystems such as the Mediterranean and Black Sea and the dissemination of

*Corresponding author: serkan.sengul@mudanya.edu.tr

How to cite this article: Şengül, S. (2025). Production and sustainability in Türkiye's fisheries sector: the role of economic variables. COMU J. Mar. Sci. Fish, 8 (1): 23-31. doi:10.46384/jmsf.1607101

sustainable fishing practices are of great importance in this context. In the literature, it is frequently emphasized that a balance should be established between the environmental and economic sustainability of the fishing sector (Jacobsen, 2016; Pierce et al., 2012). In studies conducted in line with sustainable development goals in the fishing sector, the protection and efficient use of natural resources are at the forefront. In studies such as Creighton (2015) and Cashion et al. (2017), it has been stated that ensuring environmental sustainability is fundamental to the long-term economic success of the sector. In particular, it has been emphasized that fish stocks are decreasing on a global scale and that current fishing practices are straining environmental boundaries. In addition, studies on environmentally friendly policies implemented in the European Union show that measures taken at the local level can have global effects (Bostock et al., 2016).

The fishing sector in Türkiye is addressed in terms of economic variables such as capital investments, labor costs and energy costs. Capital investments are of critical importance, especially in terms of the renewal and modernization of boats, nets and other technical equipment (Oyakhilomen and Zibah, 2020). Such investments both increase the production capacity in the sector and strengthen international competitiveness (Mobsby et al., 2020). At the same time, optimizing labor costs can positively affect the total production volume of the sector by increasing the efficiency of production processes (Creighton et al., 2015). Energy costs play a decisive role in the economic efficiency of the sector. Energy inputs such as fuel and electricity are one of the main cost elements in both fishing and processing processes (Erkayral and Aksoy, 2022).

In the literature, the balance between energy and capital investments plays a critical role in ensuring the sustainability of production processes (Carneiro et al., 2022). In particular, the development of policies that increase energy efficiency can offer both environmental and economic benefits. In addition, supporting small-scale fisheries and aquaculture practices can promote socio-economic development in rural areas (Muhamad et al., 2024). Small-scale fisheries are considered as a social and economic balancing element in the midst of unbalanced competition with large-scale industrial fisheries (Pierce et al., 2012).

Türkiye's fisheries sector is also vulnerable to the effects of climate change. Blanchard et al. (2012) and Cheung et al. (2009) stated that the effects of climate change on marine ecosystems had not only biological but also economic and social consequences. This situation shows that fisheries policies in coastal countries like Türkiye should be re-evaluated. Innovative policies are required, especially to reduce energy costs and minimize environmental impacts (Mobsby et al., 2020).

The main purpose of this study is to examine the relationships between production and sustainability in the fisheries sector in Türkiye from the perspective of economic variables. The study aims to understand the effects of basic economic factors such as capital investments, labor costs and energy costs on the production processes in the sector and to evaluate the role of these interactions in the context of sustainable development. In addition, it aims to present a holistic analysis of the sector by comparing the dynamics specific to the fisheries sector in Türkiye with the approaches in the global literature.

Material and Methods

The dataset used in this study consists of annual data on the Turkish fisheries sector for the period 2003-2023. All data are obtained from sectoral statistics published by the Turkish Statistical Institute (TurkStat). The research aims to comprehensively analyze the effects of capital investments, energy expenditures and labor costs on production by detailing the main economic variables affecting production processes in the sector.

Capital investment data includes a broad definition of fixed capital expenditures in the sector. In this context, both domestic and foreign investments are considered, and the purchase of various fishing equipment is analyzed. In particular, this includes new or old engines, fishing vessels, nets and technical equipment. These investments are of great importance for adopting technological innovations in the fisheries sector and increasing production capacity. The use of modern equipment contributes to the economic sustainability of fishing activities by increasing productivity in the sector.

Energy expenditure data includes the cost of water, electricity and fuel oil used in the fishing sector. These expenditures stand out as an important cost item in the operational processes of fishing vessels. High energy consumption accounts for a large share of total expenses in the sector, especially due to the operation of motorized vessels and the use of cooling systems. Therefore, energy costs have a significant impact on the efficiency and cost effectiveness of production processes. Optimization of energy consumption is a critical requirement for both economic and environmental sustainability.

In addition to these two key variables, the dataset also includes labor costs. Labor costs include salaries, social security payments and other labor expenditures of employees employed in the sector. The labor-intensive nature of the fisheries sector causes labor costs to occupy an important place in the total cost structure. In this context, the impact of labor costs should be carefully considered in productivity analyses. This data provided by TurkStat allows us to provide a detailed analysis of sectoral costs and provide strategic recommendations for more efficient use of resources.

In the study, long and short-term relationships were analyzed using the ARDL (Autoregressive Distributed Lag) econometric model. This method provides a suitable tool to examine the effects of economic variables in the fisheries sector in detail. The model was designed to measure the causal relationships between economic factors and production processes and the magnitude of these relationships. The data set consists of annual data obtained from reliable national institution, Turkish Statistical

Institute (TUIK) for the period 2003-2023 and statistical tests were applied to determine the correlation between the variables in the analysis.

This dataset provides a comprehensive basis for assessing the impact of capital and energy use and labor costs on production in the fisheries sector. Thus, we aim to contribute to the shaping of policies by providing concrete data on strategies that can be developed to improve the economic sustainability of the fisheries sector. The ARDL model allows both short-run and long-run relationships to be evaluated within the same model and provides efficient results even when the stationarity of the series are different (Pesaran and Shin, 1999). The ARDL method allows stationary series at I(0) and I(1) level to be analyzed together. Thus, the existence of long-run relationships between variables can be tested in a more flexible structure (Pesaran, et al., 2001).

Before constructing the model, unit root tests were applied to determine the stationarity level of the data. The unit root tests used in this study are ADF (Augmented Dickey-Fuller) and Phillips-Perron tests. The ADF test analyzes whether the series are stationary or not and provides a result with improved accuracy by adding additional lag levels according to the autocorrelation structure of the tested series (Dickey and Fuller, 1979). An alternative test, the Phillips-Perron test, provides more robust results than the ADF test, especially in the presence of structural breaks and serial correlation in the series (Phillips and Perron, 1988).

After determining the stationarity levels of the series according to the results of the unit root tests, the long-run and short-run relationships between the variables are analyzed with the ARDL model. Bound test method was used to determine the existence of long-run relationships. Bound test is an approach that tests whether there is a long-run relationship between the dependent and independent variables in the ARDL model and shows the statistical significance of the model (Pesaran et al., 2001). Once the long-run relationships are identified, an error correction model (ECM) is constructed to analyze short-run interactions. The ECM shows how short-run deviations between variables are corrected in the long run and is particularly important in econometric models to analyze the correction of short-term imbalances (Engle and Granger, 1987).

In order to test the appropriateness of the ARDL model, various specification tests were performed. Breusch-Godfrey LM test was applied to examine the presence of autocorrelation in the model. This test detects whether there is serial correlation in the model (Breusch, 1978; Godfrey, 1978). The Breusch-Pagan-Godfrey test was used to test for heteroskedasticity; this test tests the validity of the assumption of constant variance in the error terms of the model (Breusch and Pagan, 1979). The Ramsey RESET test is used to check whether the functional form of the model is correct (Ramsey, 1969). The normality test examines whether the error terms are normally distributed; this test is important for testing the assumptions of the model.

Finally, Cusum and Cusum of Squares plots were analyzed to assess the reliability of the model. These graphs allow to visually assess whether the parameters of the model are stable over time (Brown et al., 1975). If the values in the plots are within the critical limits, the reliability and parameter stability of the model are confirmed. These analyses are an important part of the study to ensure the stability and reliability of the model's prediction results.

Results

The analysis assesses the impacts of energy costs, labor costs and capital investments on total fish production between 2003 and 2023. The results of the data analysis aim to provide important findings on sectoral efficiency and cost-effectiveness.

Table 1. Descriptive statistics of variables (production, capital investment, labor and energy cost)

Variables*	***N***	**Mean**	**Std. Deviation**
Production	21	11.6531	0.33745
Capital Inv.	21	17.4235	0.72443
Labor Cost	21	18.6213	0.58230
Energy Cost	21	18.9934	0.44852

*Since all variables consist of unit data, econometric analysis was performed by taking the natural logarithm of each variable.

Table 1 summarizes mean and SD of log transformed descriptive statistics (production, capital investments, labor costs and energy costs). The production variable has a mean value of 11.65 ± 0.337 and indicated that the production level has a fairly stable structure during the analysis period and shows low fluctuation. The capital investments variable reached a mean value of 17.42 units and exhibited a wider variability compared to the other variables with a standard deviation of 0.724. This situation indicates that capital investments followed a more fluctuating course during the analysis period. The mean of labor costs was determined as 18.62 ±0.582 units indicating higher fluctuation in labor costs compared to the production level. Energy costs have a mean value of 18.99 units and the standard deviation value is calculated as 0.449. The low standard deviation value of energy costs suggests that this variable has a relatively stable structure during the analysis period. In general, the statistical properties of these variables, provide an important basis for understanding the general levels and variability of the economic variables considered in the study. While lower fluctuations are observed in production and energy costs in particular, a wider variation is noted in capital investments and labor costs. This situation can be evaluated as an important finding that should be taken into account in sectoral analyses.

Table 2. ADF unit root test

I(0)			I(1)		
Variables	**t-stat**	**p-value**	**Variables**	**t-stat**	**p-value**
Production	-1.677	0.3992	Production	-4.298	0.0006
Capital Inv.	-2.092	0.6633	Capital Inv.	-5.856	0.0002
Labor Cost	0.636	0.9659	Labor Cost	-4.199	0.0288
Energy Cost	1.437	0.9225	Energy Cost	-1.993	0.0341

Table 3. Philips peron unit root test

I(0)			I(1)		
Variables	**t-stat**	**p-value**	**Variables**	**t-stat**	**p-value**
Production	-1.778	0.4922	Production	-8.238	0.0000
Capital Inv.	-0.834	0.8384	Capital Inv.	-6.021	0.0001
Labor Cost	1.264	0.9003	Labor Cost	-3.217	0.0401
Energy Cost	1.353	0.8920	Energy Cost	-2.642	0.0314

ADF (Augmented Dickey-Fuller) and Phillips-Perron unit root tests were applied to determine the stationarity levels of the variables used in the study. These tests are important to determine whether the time series are stationary and whether it is possible to establish a long-run relationship between the series. In the unit root tests, the stationarity of the variables at level (I(0)) and first difference (I(1)) were analyzed.

According to the ADF test results shown in Table 2, none of the variables tested at level are stationary. This indicates that the variables deviate from the mean over time and do not return to the long-run equilibrium level. However, when the first differences of the variables are taken, it is observed that the variables become stationary. This result indicates that the variables are in the I(1) process, that is, they become stationary when first differences are taken.

The Phillips-Perron test results shown in Table 3 are also consistent with the ADF test. This test developed by Phillips and Perron (1988) has a more robust structure against autocorrelation and heteroscedasticity problems in the series. According to the results of the Phillips-Perron test, variables that are non-stationary at the level become stationary when first differences are taken. This consistency provides strong evidence that the analyzed series can be cointegrated.

In general, both tests indicate that the variables become stationary in their first differences. These results support the appropriateness of the ARDL model to be used in this study. This is because ARDL models have the flexibility to analyze both level stationary (I(0)) and first difference stationary (I(1)) variables. Therefore, the results of unit root tests provide an appropriate basis for analyzing long-run relationships based on the ARDL model.

In this study, ARDL model is used to examine the long-run effects of economic variables affecting production in the Turkish fisheries sector. The chosen model structure analyzes the relationships between the dependent variable total production and capital investments, labor costs and energy costs. The advantage of the ARDL model is that it can take into account short-run dynamics while testing for the existence of long-run relationships between series.

According to the results (Table 4), the long-run effects of capital investments, labor costs and energy costs on total production in the fisheries sector are significant. The positive long-run coefficient of production indicates that productivity can be increased through the use of modern equipment and technologies. This finding emphasizes the importance of capital investments to ensure sustainable production in the sector.

However, the fact that capital investments have a negative coefficient indicates that misallocation of capital may have a negative impact on production. In capital-intensive sectors such as the fisheries sector, making appropriate investments is critical for productivity in the sector.

The negative impact of labor costs on production shows the impact of the labor-intensive structure of the sector on production costs. This finding indicates that labor costs should be managed effectively in order to reduce costs and increase profitability in the sector. Similarly, energy costs have a negative coefficient, indicating the importance of energy efficiency in operational processes in the fisheries sector.

Table 4. General results of ARDL model and long-run relationships (Selected Model: ARDL (2, 2, 0, 2))

Variables	Coefficient	Probability	
Production	0.767851	0.0113**	
Capital Inv.	-0.38257	0.0162**	
Labor Cost	-1.21845	0.0298**	
Energy Cost	-0.50833	0.0064***	
R-squared	0.867345	Akaike info criterion	-1.498745
Adj. R-sq	0.718456	Schwarz info criterian	-1.123657
Log L	19.72346	Hannan – Quinn criter.	-1.632358
		Durbin-Watson stat	2.2073456

* for 10% significance level, ** for 5% significance level, *** for 1% significance level

The overall performance indicators of the model are high, and the explanatory power of the model is satisfactory. These results indicate that capital investments should be directed correctly, and energy costs should be optimized to ensure the economic sustainability of the Turkish fisheries sector. The findings of the study provide a significant contribution to the literature for developing strategies to increase efficiency in the sector.

Table 5. Bound test results

Model	k	m	F	*Signif*	I(0)	I(1)
(2,2,0,2)	3	18	5.2763*	1%	3.42	4.84
				5%.	2.45	3.63
				10%	2.01	3.1

(k): Number of explanatory variables, (m): Maximum number of lags, (*) indicates 1% significance level

According to the bound test results (Table 5), the F-statistic value is calculated as 5.2763 and this value exceeds the boundary values (critical values) at 1% significance level. The bound test is used to test whether there is a long-run relationship between the explanatory variables and the dependent variable in the model. In this test, the F-statistic is compared with the critical values according to certain significance levels.

Since the F-statistic value obtained in this study exceeds both I(0) and I(1) critical thresholds at 1% significance level, it indicates the existence of a long-run relationship in the model. This result indicates that there is a long-run equilibrium relationship between total production in the fisheries sector, which is the dependent variable, and capital investments, labor costs and energy costs.

This finding provides an important clue for developing sustainable production policies in the fisheries sector in line with the objective of the study. The existence of a long-run relationship indicates that variables such as capital investments, labor costs and energy costs can have lasting effects on production processes in the sector and the management of these factors should be taken into account in long-term strategic planning.

Table 6. Short-run error correction model

Variables	Coefficient	t-stat	Probability
CointEq(-1)	-0.2489	5.6378	0.0031
R-squared	0.901569	Akaike info criterion	-1.925130
Adj. R-sq	0.821855	Schwarz info criterian	-1.793851
Log L	18.72457	Hannan – Quinn criter.	-1.96853
		Durbin-Watson stat	2.146852

In this study, the short-run error correction model (ECM) is used to analyze how long-run imbalances are corrected in the short-run. According to Table 6, the error correction term (CointEq(-1)), which is an important component of the ECM, expresses the speed at which the system returns to long-run equilibrium. The coefficient of CointEq(-1), which is called the short-run error correction term, shows how long it takes for the system to return to long-run equilibrium after short-term shocks in the fisheries sector. The value of -0.2489 obtained in this study indicates that the economic imbalances occurring in the short run are corrected at a certain speed and the system returns to the long-run equilibrium level. In natural resource-based sectors such as the fisheries sector, environmental and economic fluctuations are quite frequent. Therefore, the sector's ability to quickly adapt to these shocks is critical for both sustainable production and efficient use of resources.

The fact that the coefficient of CointEq(-1) is significant and positive indicates that the effects of fluctuations in key economic variables such as investments, labor and energy costs in the sector are balanced in a short time. In the fisheries sector, sudden increases or decreases in inputs such as capital investments and energy costs can affect production costs and total output in the short run. However, a strong error correction mechanism ensures that such imbalances in the sector are quickly recovered and the long-run equilibrium level is reached. This reflects the resilience of the fisheries sector and its capacity to adapt to sustainable development goals.

The Turkish fisheries sector is highly sensitive to changes in labor costs due to its labor-intensive structure. This sensitivity makes the impact of labor costs on productivity in the sector even more important. For example, a sudden increase in labor costs may increase production costs in the short run and negatively affect the profitability of the sector. However, the positive value of the coefficient of CointEq(-1) indicates that such cost increases will not destabilize the sector; on the contrary, the sector can adapt to these changes and return to its long-term production targets. This flexibility points to the potential of the fisheries sector to ensure sustainability in production activities.

Energy costs also stand out as an important cost factor in the operational processes of the sector. High energy costs constitute a major cost burden in the sector, especially due to the operations of fishing vessels and the use of cooling systems. However, as the CointEq(-1) coefficient shows, despite the fluctuations in energy costs, the sector has the ability to adapt in the short term. This adjustment mechanism supports the sector's productivity growth and ensures that the negative effects on total output are limited in the long run. Therefore, the strong error correction coefficient suggests that the sector has a high capacity to manage possible shocks in variables such as energy costs and can maintain sustainability in this process.

In conclusion, the significant and positive value of the CointEq(-1) coefficient indicates that the Turkish fisheries sector is resilient to economic and environmental changes and that sustainable production activities in this sector can be carried out in line with sustainable development goals. The fact that the sector has such a flexible structure can be considered as an important finding for decision makers and policy makers.

A series of specification tests, which are shown in Table 7, were applied to test the validity and reliability of the model. These tests are important to assess whether the model ensures autocorrelation, heteroskedasticity, normality and the correct functional form.

Table 7. Specification Tests

Tests	Probability
Serial Correlation LM test (Breusch–Godfrey)	0.1143
Heteroscedasticity test (Breusch–Pagan–Godfrey)	0.8344
Jargue–Bera Normality test	0.4416
Ramsey Reset Test	0.7153

First, the Breusch-Godfrey LM Test for Serial Correlation is applied, and the p-value is 0.1143. This result indicates that the error terms are not serially correlated and there is no autocorrelation problem. The absence of autocorrelation is important in terms of showing that the estimates of the model are independent, and the assumption of inter-series relationship is met (Breusch, 1978; Godfrey, 1978).

Breusch-Pagan-Godfrey test was performed as a heteroskedasticity test and the p-value was found to be 0.8344. This result indicates that the error terms in the model have a constant variance and there is no heteroskedasticity problem. The constant variance of the error terms increases the stability of the model and the reliability of the forecasts. This is especially important for the assumption that the variance between observations obtained in different periods should remain constant.

The Jarque-Bera normality test was applied to check whether the distribution of error terms in the model is normal. The p-value of 0.4416 indicates that the error terms are normally distributed. The normal distribution of the error terms indicates that the model works in accordance with its assumptions and increases the validity of the estimation results. This result indicates that various distributional properties of the model are also confirmed.

Finally, the Ramsey RESET test is used to assess whether the model is in the correct functional form. The p-value of 0.7153 obtained as a result of the test indicates that the model is constructed in a correct functional form. This suggests that there are no missing variables or misspecification in the model and the overall structure of the model is appropriate for the analysis.

The results of these tests indicate that the estimation power and validity of the ARDL model used are high. The absence of autocorrelation and heteroskedasticity in the model, the normal distribution of the error terms and the correct functional form suggest that the model is a reliable tool for analyzing production processes in the Turkish fisheries sector.

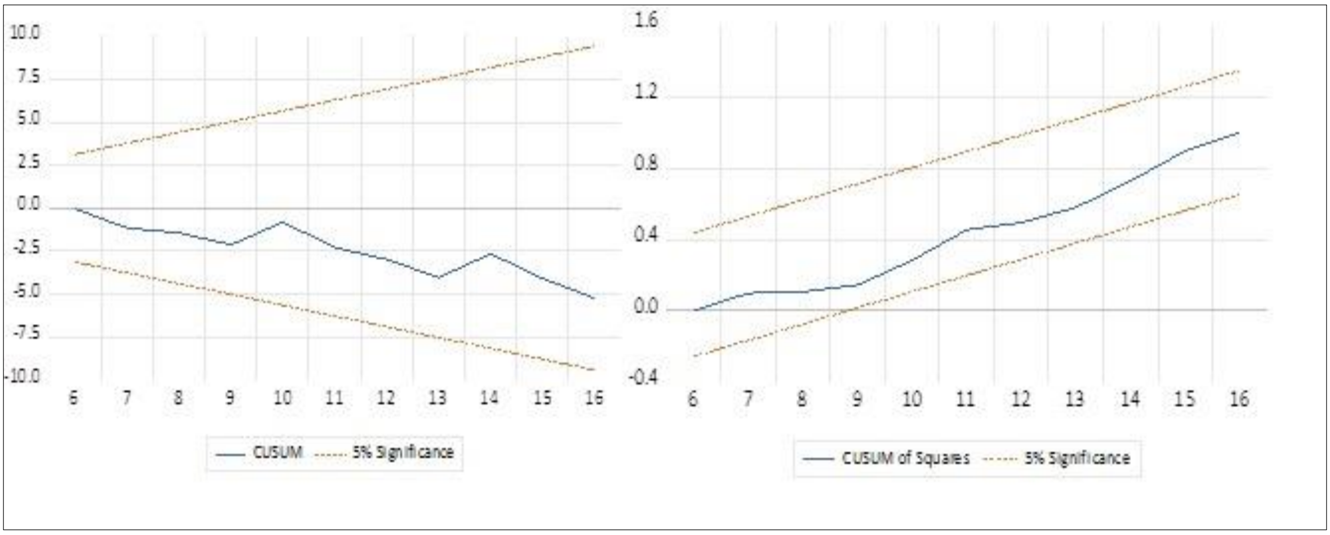

Figure 1. Cusum and cusum square

In figure 1, CUSUM and CUSUM of squares plots are used to assess whether the parameters of the model are stable over time. In these plots, the parameters of the model are considered to be stable as long as the line remains within the 5% significance limits.

The first graph, the CUSUM graph, shows the overall parameter stability of the model. The fact that the blue line is within the 5% significance limits indicates that the parameters in the model are stable over time and the long-run relationships of the variables are preserved in a way that does not affect the predictive power of the model. This supports the reliability of the model in terms of estimating the production processes in the Turkish fisheries sector.

The second graph, the CUSUM of Squares graph, measures the stability of the parameters based on their squares. In this graph, the fact that the blue line remains within the 5% significance limits indicates that the variance of the error terms of the model is constant over time and there is no structural break or sudden change. It shows that the effects of the variables in the model, especially capital investments, labor costs and energy costs, are consistent and stable. This implies that the model produces reliable and valid results.

These analyses show that the predictive performance of the model examining the effects of economic variables on production in the Turkish fisheries sector is high and long-term strategic decisions can be made with confidence based on this model.

Discussion and Conclusion

This study aims to analyze the effects of capital investments, labor costs, and energy costs affecting production processes in the fisheries sector in Türkiye and to provide strategic policy recommendations for sectoral sustainability. The findings of the study have yielded results that are consistent with both national and international literature and have made significant contributions to existing literature.

The findings have shown that capital investments have a significant impact on production in the sector. However, the negative coefficient of capital investments reveals that if the investments are directed incorrectly, they may lead to productivity losses. This result is consistent with the studies conducted by Creighton et al. (2015) and Oyakhilomen and Zibah (2020). Creighton et al. (2015) stated that effective management of capital investments can increase productivity in the fisheries sector. Oyakhilomen and Zibah (2020) emphasize that misdirected investments cause resource waste and long-term production losses. In the context of Türkiye, directing capital investments to the right areas and increasing access to modern technology are of critical importance in terms of ensuring sectoral sustainability. In particular, the use of innovative equipment and environmentally friendly technologies can be encouraged to ensure both economic and environmental sustainability.

The negative impact of labor costs on production shows that the labor-intensive structure in the sector increases total costs and negatively affects productivity. This finding is consistent with the studies conducted by Jacobsen et al. (2016) and Muhamad et al. (2024). While Jacobsen et al. (2016) emphasized the competitiveness-enhancing effects of optimizing labor costs, Muhamad et al. (2024) stated that labor management is a critical element for sustainable production in small-scale enterprises. In the context of Türkiye, encouraging training programs and certification systems for employees to increase labor productivity can optimize labor costs and increase productivity. In addition, focusing on the use of more automation and technology in the sector to balance labor costs can support sustainable development in the sector by controlling costs.

The negative impact of energy costs on production shows that high energy consumption in the sector increases costs and reduces profitability. This finding is consistent with the studies conducted by Mobsby et al. (2020) and Cochrane (2001) are in line with the studies conducted by Mobsby et al. (2020) while emphasizing the positive effects of optimizing energy costs on cost efficiency, Cochrane (2001) detailed the role of energy efficiency increasing policies on sustainability in the fisheries sector. The use of renewable energy sources should be encouraged to increase energy efficiency in the Turkish fisheries sector. In particular, environmentally friendly energy solutions such as solar and wind energy can both reduce costs and minimize environmental impacts. This policy can be considered as a model applicable not only for Türkiye but also for other developing countries with high energy costs.

The policy recommendations include concrete steps for the efficient management of economic variables in the sector. First, effective management of capital investments should be ensured, and public-private sector cooperation should be encouraged to increase access to innovative technologies. This can accelerate technological transformation in the sector and increase efficiency. Secondly, training and certification programs that will increase the competencies of employees in the sector should be expanded in order to control labor costs. Such programs will support sectoral competitiveness by increasing the productivity of the workforce. Thirdly, incentive programs should be developed to increase energy efficiency and to spread the use of sectoral renewable energy. Cost reductions can be achieved in production processes by providing access to low-cost energy solutions for small-scale enterprises.

The results of the study are consistent with the existing findings in the literature, and they offer important contributions specific to the fishing sector, especially in the context of developing countries. The results obtained provide strategic clues that can be valid not only in the fishing sector in Türkiye but also in other countries with similar economic and environmental conditions. For example, supporting small-scale enterprises and using renewable energy resources can also provide a model that can be applied in other natural resource-based sectors. In this context, the results of the study are an important reference for policy makers at both national and international levels.

Conflict of Interest

The author(s) declare no conflict of interest.

Ethics Approval

Ethics committee permission is not required for this study.

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COMU J Mar Sci Fish 2025 8(1): 32-40
https://doi.org/10.46384/jmsf.1629146

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Otolith Shape Analysis and Otolith Morphometry of Bogue *Boops boops* (Linnaeus, 1758) in the Aegean Sea and the Marmara Sea

Yusuf Şen*[1], İsmail Burak Daban[2], Mukadder Arslan İhsanoğlu[2], Oğuzhan Ayaz[3], Uğur Özekinci[2]

[1]Department of Marine Biology, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0002-0595-4618
[2]Department of Fisheries and Fish Processing, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0002-2973-5698
[2]Department of Fisheries and Fish Processing, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0003-0072-5848
[3]School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0001-8458-4872
[2]Department of Fisheries and Fish Processing, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org/0000-0003-2207-0168

Received: 29.01.2025 /Accepted: 21.05.2025 / Published online: 14.07.2025

Keywords:

Sagittal otolith
Otolith shape indices
Stock discrimination
Çanakkale Strait
Otolith dimensions

Abstract: In the present study, otolith shape indices and some otolith morphometric characteristics in the left and right sagittal otoliths were identified and compared for *Boops boops* in the Aegean Sea and the Marmara Sea. Otoliths of *Boops boops* from 244 individuals in the North of Aegean Sea and 188 individuals from the Southwest of Marmara Sea were analyzed during the 2024 fishing season. The total length-otolith dimensions relationships were evaluated, the best fit was obtained between total length and otolith perimeter in the Marmara Sea and Aegean Sea, while the least fit was obtained among total length and otolith area of right otoliths, total length and otolith length of left otoliths in the Aegean Sea and Marmara Sea. Some similarities of *Boops boops* stocks were found with shape indices and otolith morphometry between the Aegean Sea and Marmara Sea. The otolith area and circularity of the left otolith, roundness and rectangularity of the right otolith, form factor of left and right otolith were significantly different (p<0.05). Principle Component Analysis may explain these differences as arising from variations in the roundness, otolith area and otolith width. Also, Canonical Discriminant Analysis demonstrated significant discrimination of otoliths between the Aegean Sea and the Marmara Sea (p<0.05), and 56.6% of the originally grouped cases were correctly classified. Based on the results of this study, two stocks of *Boops boops* in the North of Aegean Sea and the Southwest of Marmara Sea might be different. However, more detailed studies are required the determine whether this species constitues the same stock in these areas.

Anahtar kelimeler:

Sagittal otolith
Otolit şekil indeksi
Stok ayrımı
Çanakkale Boğazı
Otolit boyutları

Ege Denizi ve Marmara Denizi'ndeki Kupez'in *Boops boops* (Linnaeus, 1758) Otolit Şekil Analizi ve Otolit Morfometrisi

Öz: Bu çalışmada, Ege Denizi ve Marmara Denizi'nde *Boops boops*'un otolit şekil indeksleri ile bazı otolit morfometrisi özellikleri sağ ve sol sagittal otolitlerinde tanımlanmış ve karşılaştırılmıştır. 2024 balıkçılık sezonunda Ege Denizi'nin kuzeyinden 244 birey ile Marmara Denizi'nin güneybatısından 188 bireyin otolitleri analiz edilmiştir. Toplam boy-otolit boyutları ilişkileri değerlendirildiğinde, Marmara Denizi ve Ege Denizi'nde toplam boy ile otolit çevresi arasında en iyi uyum sağlanırken, Marmara Denizi ve Ege Denizi'nde toplam boy ile sağ otolitlerin otolit alanı ile toplam boy ile sol otolitlerin otolit boyu arasında en düşük uyum sağlanmıştır. *Boops boops* stokları arasında Ege Denizi ve Marmara Denizi'nde otolit şekil indeksleri ve otolit morfometrisi açısından bazı benzerlikler bulunmuştur. Sol otolitin alanı ve daireselliği, sağ otolitin yuvarlaklığı ve dikdörtgenselliği, sol ve sağ otolitin form faktörü açısından anlamlı farklılıklar göstermiştir (p<0,05). Temel Bileşen Analizi, bu denizlerdeki farklılıkların yuvarlaklık, otolit alanı ve otolit genişliğinden kaynaklandığını açıklayabilir. Ayrıca, Kanonik Ayırım Analizi, Ege Denizi ile Marmara Denizi arasında otolitlerin anlamlı şekilde ayırt edilebildiğini (p<0,05) ve gruplandırılan örneklerin %56,6'sının doğru şekilde sınıflandırıldığını ortaya koymuştur. Bu çalışmanın sonuçlarına göre, Ege Denizi'nin kuzeyindeki ve Marmara Denizi'nin güneybatısındaki iki *Boops boops* stoğunun farklı olabileceği düşünülmektedir. Fakat bu türün, bu denizlerde aynı stok olup olmadığının incelenmesini içeren daha ayrıntılı çalışmalar gerekmektedir.

*Corresponding author: yusuf.sen.parion@gmail.com

How to cite this article: Şen, Y., Daban, İ. B., Arslan İhsanoğlu, M., Ayaz, O. & Özekinci, U (2025). Otolith shape analysis and otolith morphometry of Bogue *Boops boops* (Linnaeus, 1758) in the Aegean Sea and the Marmara Sea. COMU J. Mar. Sci. Fish, 8 (1): 32-40. doi:10.46384/jmsf.1629146

Introduction

Otoliths are calcareous structures located in the inner ear cavity of teleost fish. They are a function of balance, movement, and sound detection (Popper and Lu, 2000). Teleost fishes have three pairs of otoliths: lapillus, sagitta and asteriscus (Schultz-Mirbach and Reichenbacher, 2006). Among these, sagittal otoliths are used widely in biological studies because of their large size, relative ease of access and their variation in otolith shape and size in different groups of fishes (Tuset et al., 2003a). The otolith shape is often linked to the ecological, taxonomical phylogenetic and functional characteristics of fish species (Vignon and Morat, 2010; Tuset et al., 2016; Van Damme et al., 2024). Also, the morphometry and shape of otoliths can vary in different populations of the same species. Consequently, otoliths can be used in stock discrimination (Popper et al., 2005). The otolith shape analysis accounts for stock-specific differences in the morphometric outline of otoliths, and is a common otolith-based stock discrimination and identification method in fisheries science (Campana and Casselman, 1993; Schade et al., 2019).

The Çanakkale Strait, with a length of 62 km, connects the Marmara Sea and the Aegean Sea. These adjacent seas are in constant interaction through the Çanakkale Strait, as surface currents carry water from the Marmara Sea to the Aegean Sea, and bottom currents carry water from the Aegean Sea to the Marmara Sea (Ozturk, 2021).Many species of fish living in this region are known to migrate in and out of the Aegean Sea and the Marmara Sea. However, only limited studies have been conducted on the otoliths of species until today. In previous studies, otolith shape analysis of *Pomatomus saltatrix* (Bal et al., 2021), *Engraulis encrasicolus* (Durrani et al., 2022), *Pagellus acarne* (Yedier et al., 2023a) were investigated in the Marmara Sea and the Aegean Sea. Therefore, there is a need to investigate the differences in stock structures of fish species living in different areas of constant interaction.

Bogue *Boops boops* (Linneaus, 1758) from the Sparidae family, is widely found in the Mediterranean Sea and adjacent seas. This species lives in the bottom and middle waters of coastal areas with muddy, rocky, sandy bottom structures or seagrass areas (Bauchot and Hureau, 1986). Yields from capture fishing reached a mean of 2317 tonnes in Türkiye seas between 2019- 2023, with 2244 tonnes coming from the Aegean Sea and 73 tonnes from the Marmara Sea (TUIK, 2024). The otolith shape analysis and morphometry of bogue have never been analyzed in the Turkish Seas. Only two studies were conducted of *B.boops* stock discrimination by elliptical Fourier analysis in Algerian and Tunisian coasts (Ider et al., 2017; Ben Labidi et al., 2020). The aims of the present study were to analyse the otolith shape and identify some otolith morphometric characteristics of *B.boops* sampled in the North of Aegean Sea and Southwest of Marmara Sea, Turkiye.

Material and Methods

B.boops samples were obtained using purse seine from January to April 2024 and September to December 2024, and using gillnets from May to August 2024. A minimum of five individuals were sampled from each month. The total length of these individuals were measured (nearest 0.01 mm) and the total weight was recorded using a precision scale (nearest 0.01 g). Sagittal otolith extractions of 244 *B.boops* individuals from the North of Aegean Sea and from 188 *B.boops* individuals from the Southwest of Marmara Sea were performed (Figure 1). These sagittal otolith pairs were cleaned, dried and stored in eppendorf tubes.

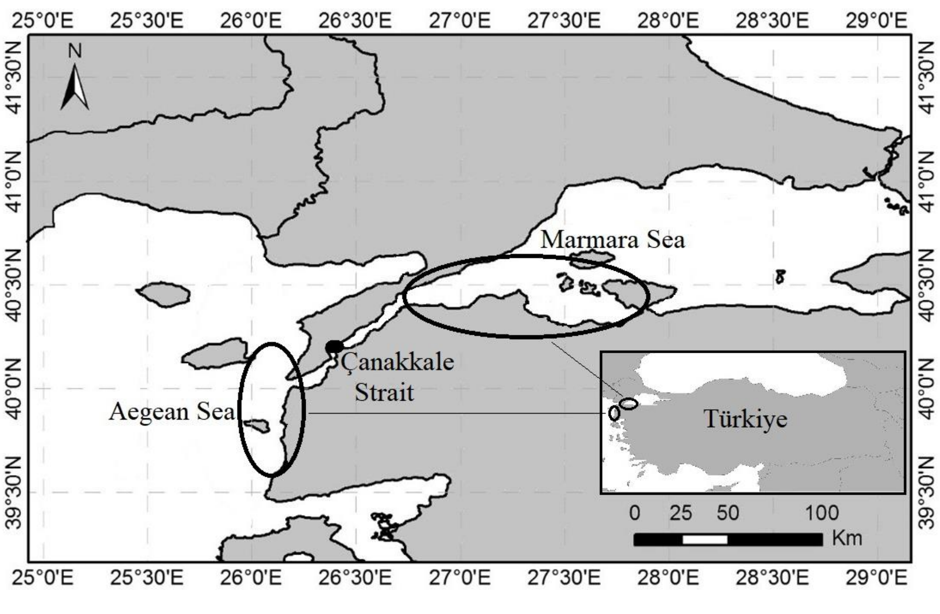

Figure 1. Sampling areas of *Boops boops* in the Marmara Sea and Aegean Sea

Otolith measurements were then performed to determine otolith morphometries of *B.boops*. The length (OL), width (OW), radius (OR), perimeter (OP), area (OA) and weight (W) of the right and left otoliths of each individual were measured to the nearest ±0.0001 mm from the undamaged otolith using a stereomicroscope and Q-Capture digital imaging program (Figure 2). Otolith pairs were weighted with an accuracy of ±0.0001 g sensitiveness. The Independent Samples T-test was used to compare right and left otoliths measurements between Aegean Sea and Marmara Sea.

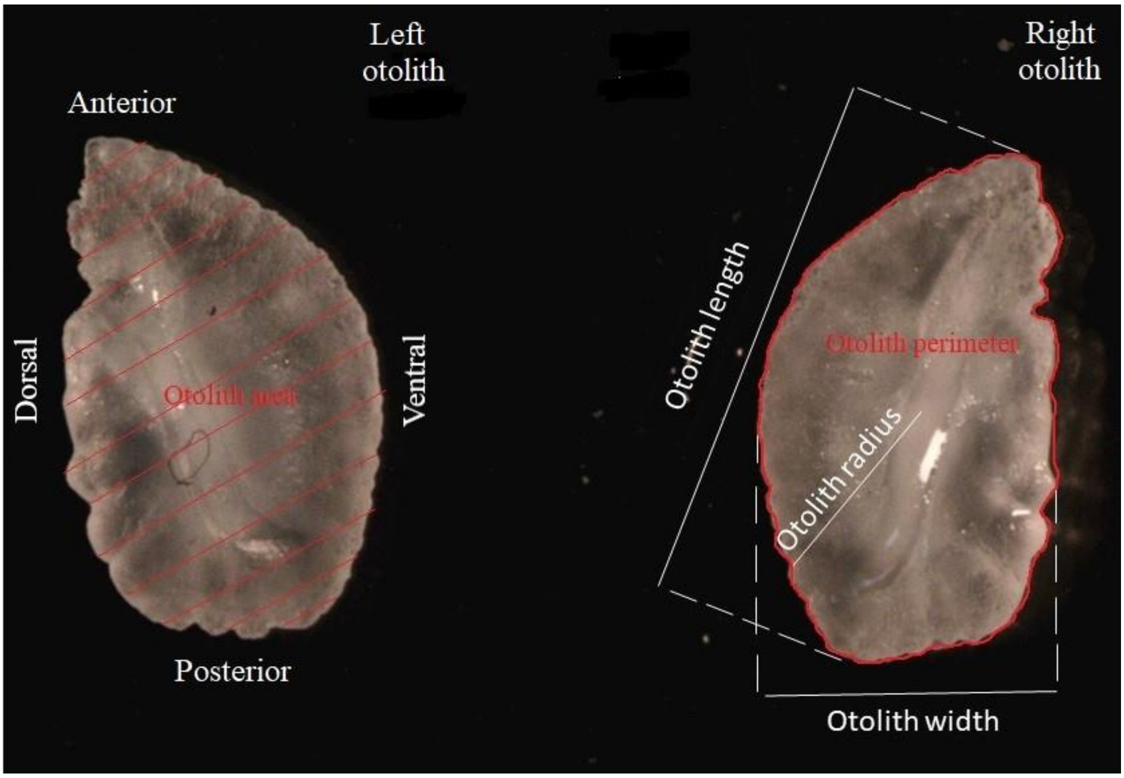

Figure 2. The left and right sagittal otolith measurements of proximal surface for *B.boops*

The relationships between different otolith dimensions were determined using linear regression analysis (Scherrer, 1984). Also, total length-otolith dimensions relationships and coefficient (R^2) were determined with this analysis. Linear regression analysis estimated by using the equation:

$y = bx + a$ (where a is the intercept and b is the slope of the linear regression)

The otolith shape indices were analyzed for *B.boops* individuals using the data obtained from the measured otolith dimensions. The otolith shape indices were calculated separately for both left and right otoliths in the Aegean Sea and Marmara Sea. The otolith shape indices used include (Tuset et al., 2003b; Ponton, 2006):

Aspect ratio (AR) = OL/OW

Elipticity (E) = (OL–OW)/(OL+OW)

Form factor (FF) = $(4\pi \times O \times A)/OP^2$

Roundness (RD) = $(4 \times OA)/(\pi OL^2)$

Circularity (C) = OP^2/OA

Rectangularity (R) = OA/(OL×OW)

Independent sample T-test was used to compare the right and left otolith shape indices between Aegean Sea and Marmara Sea. Principal component analysis (PCA) was applied to show similarities/differences in otolith shapes and morphometry between Marmara Sea and Aegean Sea. Canonical Discriminant Analysis (CDA) was also used to determine the differences between the two stocks. The performance of the discriminant analyses was assessed using Wilk's lambda (λ) test. All analyzes were performed using the PAST (Ver. 4.04) (Hammer et al., 2001) and SPSS 25 (IBM Corp 2017) software.

Results

The mean total length and weight of *B.boops* individuals were 177.6±0.29 mm and 75.97±5.22 g from the Marmara Sea, while they were 179.8±0.72 mm and 63.5 g from the Aegean Sea, respectively. While no significant difference was found for total length ($p>0.05$), a significant difference was found for weight between the Aegean Sea and Marmara Sea ($p<0.05$). The sampling details are given in Table 1.

Table 1. Total length and weight distribution of *B.boops* (N:Number of individual; SE:Standard error; Min:Minimum; Max:Maximum; p:Significant differences)

Measurements	N	Area	Mean	SE	Min	Max	p
Total length (mm)	188	Marmara Sea	177.6	0.29	108	291	0.525
	244	Aegean Sea	179.8	0.72	106	286	
Total weight (g)	188	Marmara Sea	75.97	5.22	13.08	399.88	**0.03**
	244	Aegean Sea	63.5	5.31	8.1	236.86	

Table 2. The otolith morphometric charateristics of *B.boops* from left and right otoliths (Se:Standard error; Min:Minimum; Max:Maximum; p:Significant differences)

Otolith morphometrics	Side	Area	Mean	Se	Min	Max	p
Otolith length (mm)	Right	Marmara Sea	5.8533	0.0647	4.2530	8.2630	
		Aegean Sea	6.0711	0.0501	3.6910	7.7130	0.322
	Left	Marmara Sea	5.8589	0.0648	4.2410	8.3800	
		Aegean Sea	6.0709	0.0501	3.6690	7.9450	0.274
Otolith width (mm)	Right	Marmara Sea	3.4149	0.0246	2.6780	4.9410	
		Aegean Sea	3.5107	0.0227	2.1850	4.4220	0.600
	Left	Marmara Sea	3.4423	0.0280	2.7210	5.0430	
		Aegean Sea	3.5548	0.0245	2.2960	4.4710	0.606
Otolith radius (mm)	Right	Marmara Sea	2.3140	0.0284	1.6250	3.6020	
		Aegean Sea	2.4336	0.0214	1.3940	3.4600	0.067
	Left	Marmara Sea	2.3713	0.0257	1.6440	3.3780	
		Aegean Sea	2.4993	0.0213	1.4020	3.4700	0.449
Otolith perimeter (mm)	Right	Marmara Sea	15.6499	0.1834	10.0780	22.8260	
		Aegean Sea	16.2786	0.1317	9.7780	21.2880	0.172
	Left	Marmara Sea	15.5934	0.1821	11.3420	23.4850	
		Aegean Sea	16.2309	0.1315	9.7670	22.5520	0.166
Otolith area (mm^2)	Right	Marmara Sea	13.6265	0.2637	8.0340	27.5860	
		Aegean Sea	14.3378	0.1950	5.6660	22.9200	0.051
	Left	Marmara Sea	13.5679	0.2709	7.9500	28.3590	
		Aegean Sea	14.4117	0.2020	5.9100	22.6140	**0.039**
Otolith weight (g)	Right	Marmara Sea	0.0194	0.0006	0.0098	0.0552	
		Aegean Sea	0.0217	0.0004	0.0064	0.0436	0.392
	Left	Marmara Sea	0.0192	0.0006	0.0099	0.0551	
		Aegean Sea	0.0217	0.0004	0.0062	0.0442	0.447

Otolith morphometric measurements of *B.boops* in the Marmara Sea and Aegean Sea were presented separately for right and left otoliths in Table 2. The otolith morphometry for right and left otoliths were not significantly ($p>0.05$) different among Marmara Sea and Aegean Sea stocks of *B.boops*, except for the left otolith area ($p<0.05$).

Plots of relationships between total length and right and left otolith dimensions of *B.boops* were presented in Aegean Sea and Marmara Sea in Figure 3. R^2 values were generally higher in the right otoliths than in the left otoliths in the both seas. When the total length-otolith dimensions relationships were evaluated, the best fit was obtained between TL-OP in the Marmara Sea (R^2:0.90) and TL-OP in the Aegean Sea (R^2:0.87), whereas the least fit was obtained among TL-OA of right otoliths (R^2:0.54) and TL-OL of left otoliths (R^2:0.70) in the Aegean Sea, TL-OA of right otoliths (R^2:0.67) and TL-OL of left otoliths (R^2:0.70) in the Marmara Sea.

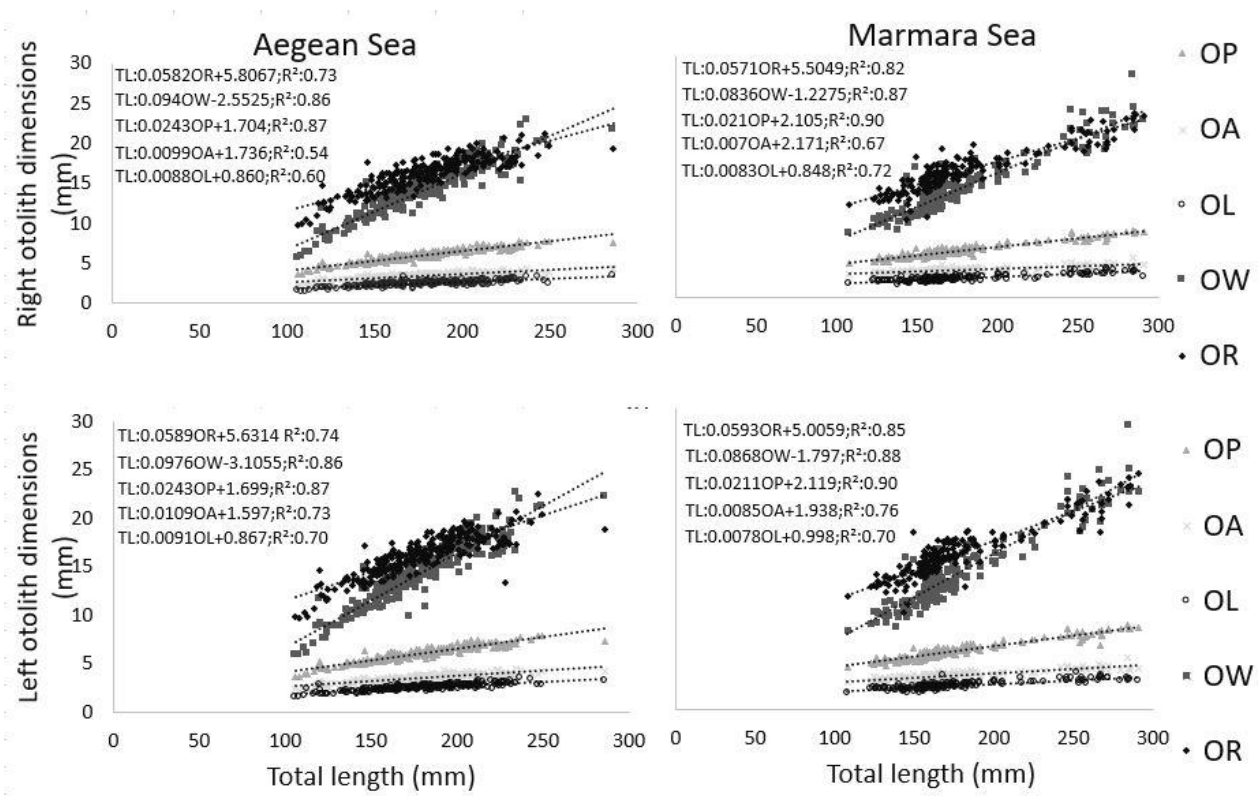

Figure 3. Total length-otolith dimensions relationships for left and right otoliths in *B.boops* in Aegean Sea and Marmara Sea

The otolith dimension relationships of *B.boops* in the Marmara Sea and Aegean Sea were presented separately for right and left otoliths in Table 3. R^2 values were generally higher in the left otoliths than in the right otoliths in the both seas. R^2 values of otolith dimensions with otolith radios relation were generally lower in both sides of otoliths and seas. When the otoliths dimension relations were evaluated, the best fit was obtained among OA-W of right otoliths (R^2:0.92) and OL-OA of left otoliths (R^2:0.93) in the Marmara Sea, and OL-OA of right otoliths (R^2:0.94) and left otoliths (R^2:0.92) in the Aegean Sea. The least fit was obtained among OR-OP and OW-OR of right otoliths (R^2:0.67), OR-OP of left otoliths (R^2:0.65) in the Marmara Sea, OR-OP of right (R^2:0.50) and left (R^2:0.57) otoliths in the Aegean Sea.

The otolith shape indices values are given in Table 4. Statistically significant differences were determined in the FF value of both side otoliths, RD and R values of right otoliths, C value of left otoliths ($p<0.05$) between the right and left otoliths of individuals among the Marmara Sea and Aegean Sea stocks.

Principal component analysis (PCA) explained 65.87 and 75.4% of the model of right and left otoliths, respectively. The left sagittal otoliths were preferred for analysis. The some otolith shape indices and dimensions explained as different between Aegean Sea and Marmara Sea. The PCA performed on RD of otolith shape indice and OA and OW of otolith dimensions were the most effective variables for stock discrimination of *B.boops* (Figure 4). The CDA analysis performed on the RD, OA, and OW demonstrated significant discrimination of otoliths between Aegean Sea and Marmara Sea (Wilks' lambda:0.979, df:1, p:0.003) and 56.6% of the originally grouped cases were correctly classified.

Table 3. Otolith dimension relations with coefficient determination (R^2) for *B.boops* in the Marmara Sea and Aegean Sea (OL:Otolith length; OW:Otolith width; OR:Otolith radius; OP:Otolith perimeter; OA:Otolith area; W:Otolith weight)

Relations	Marmara Sea		Aegean Sea	
	Right otolith	Left otolith	Right otolith	Left otolith
OL-OW;R^2	y:0.3397x+1.4266;0.72	y:0.3927x+1.1412;0.82	y:0.3908x+1.138;0.75	y:0.4315x+0.9353;0.77
OL-OR;R^2	y:0.3789x+0.096;0.74	y:0.3495x+0.3236;0.77	y:0.3341x+0.4052;0.62	y:0.3703x+0.2514;0.75
OL-OP;R^2	y:2.6876x-0.0816;0.90	y:2.698x-0.214;0.92	y:2.4728x+1.2663;0.89	y:2.5071x+1.0107;0.91
OL-OA;R^2	y:3.8963x-9.1796;0.91	y:4.0709x-10.283;0.95	y:3.7693x-8.5457;0.94	y:3.8702x-9.084;0.92
OL-W;R^2	y:0.0084x-0.0299;0.90	y:0.0082x-0.0289;0.89	y:0.0076x-0.0246;0.81	y:0.0076x-0.0241;0.80
OW-OR;R^2	y:0.8952x-07432;0.67	y:0.7981x-0.3761;0.76	y:0.7052x-0.0422;0.56	y:0.7162x-0.0466;0.68
OW-OP;R^2	y:5.9548x-4.685;0.71	y:5.6887x-3.9887;0.77	y:5.1019x-1.6327;0.78	y:4.7311x-0.5876;0.78
OW-OA;R^2	y:9.0545x-17294;0.80	y:9.1506x-17.931;0.90	y:7.9058x-13.417;0.84	y:7.5231x-12.332;0.84
OW-W;R^2	y:0.019x-0.0454;0.74	y:0.0184x-0.044;0.83	y:0.0158x-0.034;0.71	y:0.0147x-0.0306;0.73
OR-OP;R^2	y:5.2669x+3.4626;0.67	y:5.7244x+2.0192;0.65	y:4.3409x+5.7146;0.50	y:5.0364x+3.6435;0.57
OR-OA;R^2	y:7.8856x-4.6205;0.72	y:9.1418x-8.11;0.75	y:7.2251x-3.2451;0.62	y:7.9762x-5.5233;0.71
OR-W;R^2	y:0.0176x-0.0213;0.76	y:0.0186x-0.0248;0.72	y:0.016x-0.0172;0.64	y:0.0165x-0.0196;0.70
OP-OA;R^2	y:0.6361x+6.9821;0.84	y:0.6416x+6.8887;0.91	y:0.6342x+7.1855;0.89	y:0.6197x+7.2994;0.90
OP-W;R^2	y:0.0028x-0.0251;0.83	y:0.0029x-0.0252;0.84	y:0.0027x-0.0222;0.69	y:0.0027x-0.0227;0.73
OA-W;R^2	y:0.0021x-0.009;0.92	y:0.002x-0.0082;0.93	y:0.0076x-0.0246;0.81	y:0.0019x-0.0054;0.81

Table 4. Comparison of descriptive statistics and otolith shape indices for left and right otoliths of *B.boops* in the Marmara Sea and Aegean Sea (SE:Standard error; Min:Minimum; Max:Maximum; Sig:Significant differences)

Otolith shape indices	Side	Area	Mean	SE	Min	Max	Sig
Aspect ratio (AR)	Right	Marmara Sea	1.7100	0.01	1.3794	2.1787	0.066
		Aegean Sea	1.7271	0.0073	1.503	2.0957	
	Left	Marmara Sea	1.6976	0.0081	1.3864	2.0781	0.967
		Aegean Sea	1.7064	0.0067	1.4402	2.0454	
Ellipticity (E)	Right	Marmara Sea	0.6954	0.0069	0.1594	0.3708	0.113
		Aegean Sea	0.2654	0.0019	0.201	0.3539	
	Left	Marmara Sea	0.2574	0.0022	0.1619	0.3503	0.372
		Aegean Sea	0.2599	0.0018	0.1804	0.3433	
Form factor (FF)	Right	Marmara Sea	0.6954	0.0069	0.511	1.286	**0.019**
		Aegean Sea	0.6791	0.0038	0.4932	0.807	
	Left	Marmara Sea	0.698	0.0057	0.4952	1.1025	**0.001**
		Aegean Sea	0.6826	0.0033	0.5225	0.8223	
Roundness (RD)	Right	Marmara Sea	0.504	0.0035	0.4013	0.7558	**0.018**
		Aegean Sea	0.493	0.033	0.3973	0.5857	
	Left	Marmara Sea	0.4992	0.0026	0.4022	0.6024	0.807
		Aegean Sea	0.495	0.0022	0.3431	0.5969	
Circularity (C)	Right	Marmara Sea	18.2527	0.1621	7.8484	24.1044	0.059
		Aegean Sea	18.6941	0.0964	15.1949	22.6632	
	Left	Marmara Sea	18.1709	0.1333	11.3924	25.3642	**0.003**
		Aegean Sea	18.5056	0.0906	15.2744	24.0367	
Rectangularity (R)	Right	Marmara Sea	0.6731	0.0035	0.5703	0.9503	**0.000**
		Aegean Sea	0.6659	0.0016	0.5704	0.751	
	Left	Marmara Sea	0.6627	0.002	0.5591	0.7593	0.531
		Aegean Sea	0.661	0.0021	0.4442	0.7648	

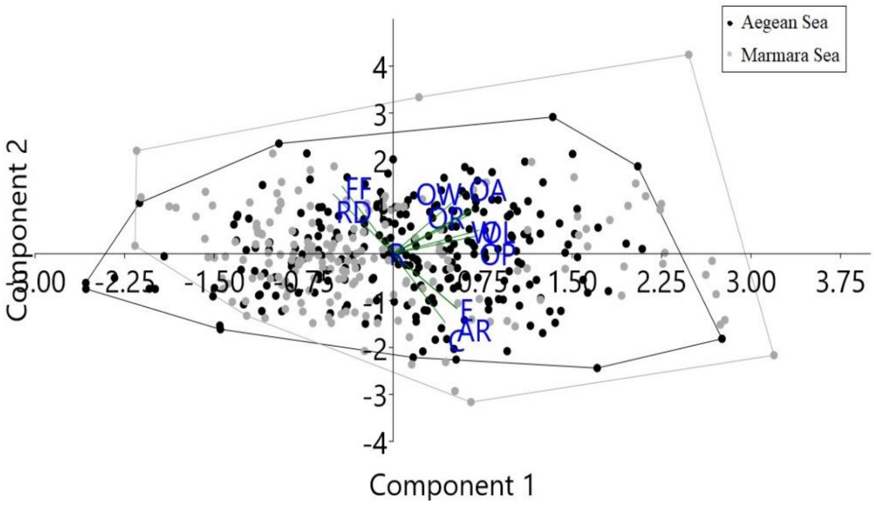

Figure 4. Principal component analysis (PCA) plot showing similarities/differences in otolith shapes and dimensions for the left otoliths for *B.boops* in the Aegean Sea and Marmara Sea.

Discussion

The present study is the first to identify and compare the shape indices of the otoliths and morphometry of *B.boops* between the Aegean Sea and Marmara Sea. This study found some similarities in *B.boops* stocks of the Aegean Sea and Marmara Sea . The otolith area and circularity of the left otolith, roundness and rectangularity of the right otolith, form factor of the left and right otolith in otolith shape indices and morphometry were significantly different ($p<0.05$) between *B.boops* stocks in the Aegean Sea and Marmara Sea . Also, the PCA analysis from the left otolith for otolith shape indices and morphometry varriables may explain differences in roundness, otolith area and otolith width of *B.boops* stocks. Also, CDA analysis performed on the RD, OA, and OW demonstrated significant discrimination. However, different methods such as genetic analysis, analysis of biological markers, stable isotope analysis and otolith microchemistry should be used in stock discrimination (Schade et al., 2019; Morales-Nin et al., 2022; Yedier et al., 2023b). Besides otolith shape indices, also elliptic fourier and wavelet analyses are used to compare otolith shape. So, these analyses are also needed to verify whether *B.boops* belong to the same stock in the Marmara Sea and the Aegean Sea.

Yedier et al. (2023a) reported that *Pagellus acarne* stocks were different according to otolith morphometrics, shape and ecomorphological indices from the Aegean Sea and the Marmara Sea. Durrani et al. (2022) revealed that the body and otolith shapes of *Engraulis encrasicolus* were separated between the Aegean Sea and the Marmara Sea. In these areas, the different populations of *Pomatomus saltatrix* were successfully demonstrated with the morphometrics, meristics, and otolith shape analyses (Bal et al., 2021). Saygılı et al. (2016) showed that *Spicara maena* stocks from the Northern Aegean Sea and the Marmara Sea might be different baed on the otolith shape. Also, *B.boops* stocks sampled from three Algerian locations, and also two Tunisian stations shown that stocks may be different based on the otolith shape analysis (Ider et al., 2017; Ben Labidi et al., 2020). Our findings suggested that, *B.boops* stocks from the Aegean Sea and the Marmara Sea might be different based on comparisons of shape indices and some morphometric characteristics of otoliths.

The Marmara Sea and the Aegean Sea, connected by the Çanakkale Strait, are in constant interaction with each other (Ozturk, 2021). The environmental and ecological factors are different in these areas. Vignon et al. (2008) and Vignon and Morat (2010) emphasized that environmental and ecological factors may have an effect on the otolith shape and morphology. Also, otolith morphology is affected by a complex combination of physiological (sexual maturity, growth, etc.) factors (Vignon and Morat, 2010; Mille et al., 2015). In the present study, in order to account for differences in fish size a large number and various sizes of *B.boops* individuals were sampled monthly to minimize errors. The effects of above mentioned factors should be investigated to discriminate stocks inhabiting the Marmara Sea and the Aegean Sea.

The otolith shape indices AR, E, FF, RD, C and R for *B.boops* were calculated as 0.682, 0.472, 1.812, 18.455, 0.672, 0.289, respectively, by Cicek et al. (2021) in the İskenderun Bay, Northeastern Mediterranean Sea. Although their study was in a different area, the calculated otolith shape indices were similar to those reported in this study.

The relationship between fish length and otolith length of 179 specimens of *B.boops* was estimated as OL=0.233TL+0.971 in the Algerian coast, Southwestern Mediterranean Sea (Ider et al. 2017). Ider et al. (2017) reported that a significant linear relationship (R^2:0.823) existed between fish length and otolith length. In this study, the parameters “a” and “b” in the equation with 244 and 188 individuals for this species were estimated as

0.0243, 1.704 and 0.021, 2.105 for the right otoliths in the Aegean Sea and the Marmara Sea,. The a and b values reported by Ider et al. (2017) were different than those in the present study. The R^2 value of left and right otoliths by their study were mostly lower than in the study. The best fit R^2 value was obtained among TL and OL (R^2:0.945). These differences may be related to changing environmental conditions, sampling sizes and sampling area. Although the sampling locations were different between the Aegean Sea and the Marmara Sea, the TL-OL relationship was found to be mostly similar in this study. When, the dimensions of the otoliths were compared, R^2 values were generally higher in the left otoliths than in the right otoliths in the both seas.

The current results of this study suggested that *B.boops* stocks in the North of Aegean Sea and the Southwest of Marmara Sea may be different based on the right and left otolith shape indices and otolith dimensions. . This is the first study evaluating differences of otolith shape indices and otolith dimensions to discriminate *B.boops* stocks in the Aegean Sea and Marmara Sea. The present study found significant differences in OA of otolith dimension and FF, RD, C and R of shape indices. So, these parameters can be used as alternative and effective parameters to discriminate *B.boops* stocks from the Aegean Sea and the Marmara Sea. However, more detailed studies involving environmental, physicochemical, ecological parameters and genetic traits will be required to better understand the origin of these variations between *B.boops* stocks. In conclusion, this study suggests that *B. boops* stocks in the Marmara Sea and the Aegean Sea should be strategically assessed and managed separately to their sustainable use in the future.

Acknowledgment

The authors would like to thank the research project (FBA-2024-4630) was supported by Çanakkale Onsekiz Mart University Scientific Coordination Unit. The authors are grateful to Çalı Balıkçılık purse seine crew and Ahmet ŞEN for providing the samples.

Conflict of Interest

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

Author Contributions

Yusuf ŞEN: Designing of the study, laboratory study, data analysis, writing original draft preparation. İsmail Burak DABAN: Data analysis, software, checking-original draft preparation. Mukadder ARSLAN İHSANOĞLU: Supported the laboratory study. Oğuzhan AYAZ: Sample collections. Uğur ÖZEKİNCİ: Checking-original draft preparation.

Ethics Approval

Ethics committee approval is not necessary for this study.

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COMU J Mar Sci Fish 2025 8(1): 41-45
https://doi.org/10.46384/jmsf.1636391 e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Genotoxic Effects of Acute Difenoconazole Exposure on *Daphnia magna*

Ayşegül Ergenler*, Funda Turan

İskenderun Technical University, Hatay, Türkiye https://orcid.org//0000-0001-9186-3909
İskenderun Technical University, Hatay, Türkiye https://orcid.org//0000-0002-0257-6009

Received: 09.02.2025 /Accepted: 10.04.2025 / Published online: 14.07.2025

Keywords:

Pesticide
Difenoconazole
Comet Assay
DNA Damage

Abstract: Difenoconazole, a fungicide often used in agriculture, is harmful to aquatic organisms. However, its toxicity to aquatic organisms is not yet well recognized. Among the triazole fungicides, Difenoconazole (PEN) is one of the most extensively used in many countries. In this study, the genetic impacts of different Difenoconazole doses on *Daphnia magna* was investigated. Experiments involving the control group and treatments were executed in compliance with the standard methodology outlined by the Economic Cooperation and Development Organization (OECD) standards 202 and 212 (OECD, 2012, 2009). In the present study, *Daphnia magna* were exposed to a control group (0 mg/L) and three different doses of (1.00, 1.5, 2.5 mg/L) difenoconazole for a period of 10 days. At the end of the experiment, comet assay was used to determine the damage frequency (%), Arbitrary unit (%), and Genetic damage index (%) of tissues. The 2.5 mg L^{-1} group showed notably greater damage frequencies (45.33±1.52) on *Daphnia magna* (p<0.001). Our findings indicated a considerable increase in DNA strand breakage in *Daphnia magna* after exposure to difenoconazole, indicating that the fungicide is genotoxic to daphnids.

Anahtar kelimeler:

Pestisit
Difenokonazol
Komet Testi
DNA Hasarı

Akut Difenokonazol'ün *Daphnia magna* üzerindeki Genotoksik Etkileri

Öz: Difenokonazol, tarımda sıkça kullanılan bir fungisit olup; sucul canlılar için zararlı olabilmektedir. Difenokonazol'un sucul canlılarda olan toksisitesi henüz iyi bilinmemektedir. Triazol mantar ilaçları arasında, Difenokonazol (PEN) birçok ülkede en yaygın kullanılan fungusitlerden biridir. Bu çalışmada, *Daphnia magna* üzerinde farklı dozlarda Difenokonazolün genotoksik etkisi araştırıldı. Ayrıca Kontrol Grubu ve diğer doz grublarıyla yapılan çalışma, Ekonomik İşbirliği ve Kalkınma Örgütü (OECD) standartları 202 ve 212 (OECD, 2012, 2009) tarafından belirlenen standart metodolojiye uygun olarak gerçekleştirildi. Çalışmada, *Daphnia magna* 10 gün boyunca kontrol grubu (0 mg/L) ve üç farklı dozda Difenokonazole (1.00, 1.5, 2.5 mg/L) maruz bırakıldı. Deneyin sonunda, dokuların Hasar Frekansı (%), Arbitrary Birim (%), ve Genetik Hasar İndeksi (%) belirlemek için Comet testi kullanıldı. Doz gruplarından 2.5 mg L^{-1} grubu, *Daphnia magna* üzerinde en yüksek hasar frekansına sahip olduğu(45.33±1.52) (p<0.001) tespit edildi. Elde ettiğimiz bulguların Difenokonazol'un maruziyeti sonrasında *Daphnia magna*'da DNA hasar düzeylerinde önemli bir artış olduğunu göstermesine bağlı olarak kullanılan bu fungisitin daphnidler için genotoksik olabileceğini işaret etmiştir.

Introduction

Pesticides are used worldwide to control and eradicate pests, including microorganisms, fungus, flora, slugs, vermin, rodents, and parasites. They are categorized based on the kind of organism they affect and can be acquired in many configurations, including liquids, granules, compounds, polymer parts, coated pelleted capsules, as well as encapsulated forms. Nonetheless, the increasing use of pesticides to increase agricultural production is raising concerns over environmental contamination and adverse impacts on non-target organisms (Wang *et al*., 2022). Pesticides can move from their target area to other parts of the environment, such as water, air, and soil via various transfer processes, such as volatilization and discharge. Numerous studies have examined the impact of pesticides on aquatic ecosystems owing to their ability to enter the aquatic environment through diverse mechanisms. Certain pesticides may induce oxidative stress, which is closely associated with the growth and development of aquatic organisms. Absorption of pesticides may result in musculoskeletal malformations, vertebral curvature, aberrant growth in fish eggs, and DNA damage (Li *et al*., 2018; Turan and Ergenler, 2022; 2023; Ergenler and Turan, 2023).

*Corresponding author: aergenler@gmail.com

How to cite this article: Ergenler, A. & Turan, F. (2025). Genotoxic effects of acute difenoconazole exposure on *Daphnia magna.* COMU J. Mar. Sci. Fish, 8 (1): 41-45. doi:10.46384/jmsf.1636391

Triazole fungicides are a primary category of pesticides often used for diverse fruits, vegetables, and cereal items. Their elevated characteristics, including chemical and photochemical stability, limited biodegradability, and ease of environmental mobility render them persistent in soil and water. Consequently, concerns about the toxicity of triazole insecticides to aquatic organisms are becoming increasingly prominent in the context of natural ecosystems and human health risk assessment. The compound difenoconazole (cis-trans-3-chloro-4-(4-methyl-2-(1H-1,2,4-triazol-yl methyl)-1,3-dioxolan-2-yl) phenyl 4-chlorophenyl ether) is a representative fungicide with a modified triazole moiety that interacts with the heme component of fungus cytochrome P450 (cyp) 51. (Figure 1).

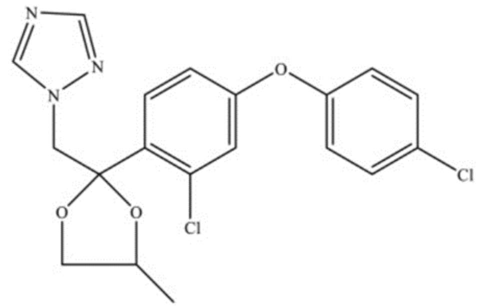

Figure 1. Structure of difenoconazole (Gao *et al.,* 2021).

Difenoconazole impedes the action of fungal lanosterol-14α-demethylase and obstructs ergosterol production, thereby hindering chitin formation in the fungal cell wall and causing cytoplasmic leakage. It has both preventive and therapeutic properties and is extensively used to manage diseases induced by various pathogenic fungus in diverse plant species. For many years, Difenoconazole has been the main pesticide used in China to fight fungal diseases in rice crops. Wang, 2012). This has increased the risk of difenoconazole contaminating the aquatic environment. Many studies have been published on the occurrence of difenoconazole in aquatic systems (Teng *et al*., 2013;Mu *et al*., 2013; 2015; 2016; Wang *et al.,* 2022; Jiang *et al*.,2020). *Daphnia magna* (Ton *et al.,* 2012), and trout (Knudsen et al., 2011), have been used in the toxicity evaluation of environmental contaminants. Aquaculture models, characterized by quick life cycles, have proven valuable across other disciplines such as ecotoxicology, cell biology, and developmental genetics (Strähle *et al*., 2011; Ankley and Villeneuve, 2006).

Daphnia is recognized as a pivotal species in the food webs of several freshwater ecosystems, serving as a crucial foundation for sustainable and progressive ecotoxicological research. Daphnia are widely utilized in research owing to their cyclical parthenogenetic life cycle, which encompasses the previously mentioned model criteria and diapause phases. The uncomplicated growing of identical cells facilitates research focused on separating the impacts of environmental and genetic elements. *Daphnia magna* is often used as a model organism for evaluating contaminants in aquatic environments due to its tolerance to a diverse array of substances (Turan and Ergenler, 2022; Tekin *et al.,* 2024). Identifying the most sensitive period in the life stages of an organism is important in protecting the species from external toxic substances. This study aims to determine the most vulnerable life stages of aquatic organisms by conducting multiple toxicity tests at different stages. Systematic multi-stage assays have been used to determine species' sensitivity to toxic chemicals. However, the mechanisms behind different sensitivities at different life stages are still poorly understood. In the present study, the comet test was used to determine if difenoconazole promotes genotoxicity in the model organism *Daphnia magna*, highlighting the importance of understanding the ecological impact of chemicals.

Material and Methods

The Genotoxicology Laboratory at the Faculty of Marine Science and Technology, University of Iskenderun Technical, Turkey, housed and cared for *Daphnia magna.* The Daphnids were placed in 3-L glass beakers with 2 L of tap water that was aerated and had a 16:8 (light:dark) photoperiod. The results of four different groups (including an observation group and a pair of test groups) were subjected to a toxicity test. Each concentration of difenoconazole was tested using three duplicate samples, and twenty daphnias were used per container, following the Organisation for Economic Co-operation and Development (OECD) Test Guideline 202. The specimens were maintained at a temperature of 20 ± 1 °C, with a dissolved oxygen content of 6.4 ± 0.5 mg/L. The pH value was controlled within the range of 8.2 ± 0.2. Three different fungisite concentrations at 1.0, 1.5, 2.5 mg/L based on levels found in aquatic settings along with a control treatment at 0.0 mg/L were used. Overall, a group of twenty newborns, <1 day-old when were placed in glass containers with a volume of 100 mL each. Solutions of three different difenoconazole levels were then prepared and tranferred to glass containers containing daphnia.

A 10-day experiment was conducted as described by Cavalcante et al. (2008), using gill cell suspension, retained cell pellets, and single-cell gel electrophoresis. The slides were analyzed using a fluorescence microscope and stained with ethidium bromide and neutral Tris solution. 100 cells from each specimen were examined to assess nuclei, categorizing them into five types. Damage percentage, arbitrary values, and DNA damage rating were computed for evaluation. Data were assessed for regularity and homogeneity, and a unidirectional analysis of variance was conducted to identify notable disparities across treatment groups. Observed changes were considered statistically noteworthy at a significance level of $P<0.05$ (Norusis, 1993).

Results and Discussion

The mean as well as the standard deviations of the DNA damage frequency (%), arbitrary units values (AU), and genetic damage index (%) observed in *Daphnia magna* for the groups treated with 1.0; 1.5; 2.5 mg/L of Difenconazole compared to the untreated control group are presented in Table 1.

Table 1. The mean and standard deviations for DNA damage in control and different concentrations of difenoconazole on the *Daphnia magna*.

Groups (mg/L)	Damage Frequency (%)	Arbitrary Unit (AU)	Genetic Damage Index (DI) (%)
Control	24.33±2.08[a]	62.00±5.29[a]	0.62±0.05 [a]
1.0	36.33±4.72[b]	86.66±13.79 [b]	0.86±0.13 [b]
1.5	41.00±1.00[bc]	100.66±4.04 [bc]	1.00±0.04 [bc]
2.5	45.33±1.52[c]	107.33±3.21 [c]	1.07±0.03 [c]
P	***	***	***

The data are shown as arithmetic mean ± standard deviation. *Values with different superscripts in each column indicate significant differences. Indicate significance level between DNA damage D.magna obtained from control and three different concentrations of Difenoconazole (*, P<0.001).

The data are shown as arithmetic mean ± standard deviation. *Values with different superscripts in each column indicate significant differences. Indicate significance level between DNA damage D.magna obtained from control and three different concentrations of Difenoconazole (*, P<0.001).

The lowest damage frequencies was obtained in the control (0 mg/L) group with 24.33% and there was significant change observed in the DNA damage parameters (p<0.001). The highest damage frequencies was obtained in the 2.5 mg/L treatment with 45.33±1.52%. In comparison to the control and the other pencanozole groups, the frequency of DNA damage and other damage metrics (AU and GDI) were greater in the 2.5 mg/L treatment (Table 1).

Difenoconazole, a fungicide used to enhance agricultural productivity, has become a significant pollutant in aquatic ecosystems due to excessive application and resistance to degredation (Na *et al.*, 2024). According to Mu *et al.* (2016), who determined that difenoconazole exposure can cause developmental toxicity in fish, also found that difenoconazole was toxic at concentrations 0.15–5.00 mg/L. They observed body deformities in zebrafish and suggested that the exposed dose harmed the organism which is in accordance to our findings. Similarly, Jiang e*t al.*, (2020) suggested that difenoconazole exposure at 0.400, 1.00, 2.00 mg/L caused toxic effects in zebrafish. In zebrafish, exposure to difenoconazole increased the percentage of perinuclear oocytes, spermatogenesis germ cells, spermatogonia, and cortical alveolus oocytes, and decreased the ratio of early and late vitellogenic oocytes, spermatocytes, late spermatogenesis germ cells, spermatids, and spermatozoa. Additionally, Wang *et al.* (2022), who determined the degree of heart damage in carp (*Cyprinus carpio*) exposed to low doses (0.488 mg/L) and high doses (1.953 mg/L) of difenoconazole for four days showed that although the heart damage indicators CK and LDH were not significantly different in fish exposed to low difenoconazole level, a significant toxic effect and cardiotoxicity was observed when difenoconazole level reached 1.953 mg/L. As a result, the use of high doses of difenoconazole can cause harmful effects in aquatic organisms and lead to damage in living beings. The findings of the present study is in accordance to earlier studies. Given the literature on the genotoxic potential of difenconazole, further studies are necessary to elucidate the long-term ecological and human health impacts of difenoconazole exposure. This research highlights the need of ongoing surveillance and regulatory evaluation of pesticides to safeguard environmental and public health.

Conflict of Interest

The authors guarantee that there is no conflict of interest in publishing this work, and they have rigorously followed ethical guidelines on problems such as plagiarism, informed consent, misconduct, data fabrication, double publication, and redundancy.

Author Contributions

A. Ergenler, Designing of the study, data analysis, supported the laboratory study, and checking-original draft preparation. F. Turan, Data analysis, submission,writing-review and editing, visualization.

Ethics Approval

Ethics committee approval is not need for this study.

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COMU J Mar Sci Fish 2025 8(1): 46-63
https://doi.org/10.46384/jmsf.1639055
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Comprehensive Biometric Study on the Invasive Seaweed, *Caulerpa mexicana*, in the Aegean Sea

Erhan Mutlu[1*], Yaşar Özvarol[2], Barış Akçalı[3], Berivan Elif Aslan[1], Zeynep Narlı[1], Zeynep Zabun[1]

[1]Akdeniz University, Fisheries Faculty, Antalya, Turkey https://orcid.org//0000-0002-6825-3587
[2]Akdeniz University, Kemer Maritime Faculty, Antalya, Turkey https://orcid.org//0000-0002-8308-1189
[3]Dokuz Eylül University, Institute of Marine Sciences and Technology, İzmir, Turkey https://orcid.org//0000-0002-7341-0196
[1]Akdeniz University, Fisheries Faculty, Antalya, Turkey https://orcid.org//0000-0002-6248-8553
[1]Akdeniz University, Fisheries Faculty, Antalya, Turkey https://orcid.org//0009-0009-1764-8587
[1]Akdeniz University, Fisheries Faculty, Antalya, Turkey https://orcid.org//0009-0008-2786-4729

Received: 13.02.2025 /Accepted: 08.04.2025 / Published online: 14.07.2025

Keywords:

Caulerpa mexicana
Non-indigenous species
Invasive
Biometry
Aegean Sea

Abstract: The present study investigates the occurrence and biometry of the invasive seaweed *Caulerpa mexicana* in the Gulf of İzmir, Aegean Sea, thereby highlighting its ecological implications in a region increasingly affected by biological invasions. The Mediterranean basin, especially the eastern and Aegean parts, is becoming a hotspot for non-native species, and the region may be a place of interest with the occurrence of the *C. mexicana* species. This research represents the first detailed assessment of its biometric characteristics in the Levant-Aegean Basin. The study's sampling was conducted from May to August 2024 at 321 stations along the Aegean coast, and *C. mexicana* was identified at only two specific sites. The species was found in shallow coastal waters at depths of 10 m and 15 m with densities of 630 shoots/m² and 469 shoots/m², respectively. Morphometric analysis revealed frond lengths ranging from 10 to 12.5 centimeters and widths from 7 to 9 millimeters, with a significant correlation between frond length and number of pinnae. Environmental parameters such as temperature, salinity, and dissolved oxygen were measured, providing a context for the species' habitat preferences. The findings underscore the invasive potential of *C. mexicana* and its capacity to perturb local marine ecosystems, highlighting the necessity for persistent monitoring and management strategies to mitigate its deleterious effects.

Anahtar kelimeler:

Caulerpa mexicana
Yerli olmayan tür
İstilacı
Biyometri
Ege Denizi

Ege Denizi'ndeki Istilacı Deniz Yosunu *Caulerpa mexicana* Hakkında Kapsamlı Biyometrik Çalışma

Öz: Mevcut çalışma, İzmir Körfezi, Ege Denizi'ndeki istilacı deniz yosunu *Caulerpa mexicana*'nın bulunmasını ve biyometrisini araştırarak, biyolojik istilalardan giderek daha fazla etkilenen bir bölgedeki ekolojik etkilerini vurgulamaktadır. Akdeniz havzası, özellikle doğu ve Ege kesimleri, yerli olmayan türler için bir sıcak nokta haline gelmektedir ve *C. mexicana* türünün görülmesi ile bölge dikkat çekebilir. Bu araştırma, Ege-Levant Havzası'ndaki biyometrik özelliklerinin ilk ayrıntılı değerlendirmesini temsil etmektedir. Çalışmanın örneklemesi Mayıs-Ağustos 2024 arasında Ege kıyısı boyunca 321 istasyonda yürütülmüş ve *C. mexicana* yalnızca iki belirli noktada tanımlanmıştır. Tür, sırasıyla 630 sürgün/m² ve 469 sürgün/m² yoğunlukta, 10 m ve 15 m derinlikteki sığ kıyı sularında bulunmuştur. Morfometrik analiz, yaprak uzunluklarının 10 ila 12,5 santimetre ve genişliklerinin 7 ila 9 milimetre arasında değiştiğini ve yaprak uzunluğu ile pinnae sayısı arasında önemli bir korelasyon olduğunu ortaya koydu. Sıcaklık, tuzluluk ve çözünmüş oksijen gibi çevresel parametreler ölçülerek türün habitat tercihleri için bir ilişki sağlandı. Bulgular, *C. mexicana* türünün istilacı potansiyelini ve yerel deniz ekosistemlerini bozma kapasitesini vurgulayarak, zararlı etkilerini azaltmak için sürekli izleme ve yönetim stratejilerinin gerekliliğini belirtmiştir.

Introduction

The Mediterranean basin, particularly the eastern basin and seas such as the Levant and Aegean, has become a significant nexus for the introduction and subsequent invasion of exotic species. These biological invasions present a substantial threat to the conservation of endangered species in natural plant and animal communities, with repercussions for seagrass on submerged algal communities (Ceccherelli and Cinelli 1998). Fish, benthic fauna, and macrophytes have been introduced intentionally and accidentally in the Eastern

*Corresponding author: emutlu@akdeniz.edu.tr
How to cite this article: Mutlu, E., Özvarol, Y., Akçalı, B., Aslan, B.E., Narlı, Z. & Zabun, Z. (2025). Comprehensive biometric study on the invasive seaweed, *Caulerpa mexicana*, in the Aegean Sea. COMU J. Mar. Sci. Fish, 8 (1): 46-63. doi:10.46384/jmsf.1639055

Mediterranean. The majority of these invaders are Indo-Pacific species and temperate and tropical species. Invasive organisms alter the established Mediterranean ecosystem in space and time. The dynamics of abundance and biomass of invaders, such as seaweeds, provide insights into their interaction with variations in their life history between isolated and mixed populations (Schemske et al., 1994). Clonal vegetative growth is prevalent, leading to the formation of dense aggregations, foraging, and movement to adjacent suitable areas, followed by rapid spread and low mortality (Wright, 2005).

A total of 98 species of marine algae have been introduced into the Mediterranean Sea, of which nine species were invasive and had ecological and economic impacts (Siguan & Ribera, 2002). These nine species include *Caulerpa taxifolia*, *Caulerpa racemosa*, *Sargassum muticum*, *Laminaria japonica*, *Asparagopsis armata*, *Undaria pinnatifida*, *Womersleyella setacea*, *Acrothamnion preissii*, and *Lophocladia lallemandii*. More recently, the species *Caulerpa cylindracea* and *Rugulopteryx okamurae* have also been introduced. It is noteworthy that the number of species has increased by fifty fold over the past two decades. In the western Mediterranean, sixty-seven species were identified as non-indigenous, with their geographical origins being Japanese or Pacific waters (Siguan & Ribera, 2002). Additionally, twenty-nine non-indigenous species of Indo-Pacific origin were documented in the eastern basin (Siguan & Ribera, 2002). Zenetos & Galanidi (2020) updated the list of non-indigenous seagrass species in the Mediterranean, and Cinar et al. (2021) revised the phytobenthos in the Turkish waters of the eastern Mediterranean. Of the 253 alien species that Cinar et al. (2021) found in Turkish Aegean waters, 28 were Phytobenthos. In the Turkish marine water system, 47 invasive non-indigenous macrophyte species were reported: they are mainly *Caulerpa cylindracea* Sonder, 1845, *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini, 2013, *Codium fragile fragile* Suringar Hariot, 1889, *Stypopodium schimperi* (Kützing) Verlaque & Boudouresque, 1991 and *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Çinar et al. 2025).

Caulerpa species constitute a taxonomic group of significant seaweeds, exhibiting properties such as a siphonous thallus with multinucleate cells, serving as food sources for marine organisms, and demonstrating high invasion and expansion capacity in space (Paul and Fenical 1987; Ceccherelli et al. 2002). Furthermore, these species possess a remarkable capacity for tolerating diverse temperatures and light conditions (Uchimura et al. 2000; Ljiljana et al. 2006), which enables their presence in various marine environments, each of which is adapted to its specific characteristics. These characteristics include vegetative propagules, fragmentation, and running reproduction, as well as phenotypic plasticity derived from the different environments (Ceccherelli and Cinelli 1999a, b, c; Ceccherelli and Piazzi 2001; Wright 2005). The distribution of their densities and traits in space and time provides information on the persistence of organisms, especially for a species that responds rapidly to changing environmental parameters such as seawater surface temperature and surface photon flux density in the visible spectrum (Tuya et al. 2006).

A reevaluation of the global distribution of *Caulerpa mexicana* Sonder ex Kützing 1849 is necessary due to its erroneous placement within the synonymy of *Caulerpa taxifolia*. This species is widespread in warmer marine environments, especially in the western Atlantic, where it was first described from Mexico, the Caribbean, and Brazil. In contrast, in the eastern Atlantic, it has been documented in the Canary Islands and Mauritania, as well as in the Red Sea and the Indian Ocean, extending as far as Australia and the western Pacific, including regions such as Vietnam, the Philippines, Papua New Guinea, and various Pacific islands such as Fiji (Verlaque et al. 2015; Fernandez-Garcia et al. 2016) and Canary Islands and Porto Santo in the Atlantic Ocean (Pereira 2024). Notably, there have been reports of its presence in the Mediterranean, particularly in Syria, and reports from Sicily have been attributed to misidentifications of *C. taxifolia* (Rayss 1941). According to Rayss (1941), *C. mexicana* may be native to the Mediterranean. Its initial identification in the Mediterranean occurred in 1939, specifically in Palestine and Lebanon, where it was initially classified as *C. crassifolia* (Rayss 1941). Subsequent reports of the species include a 1976 finding by Mayhoub in Syria (Bitar et al. 2003) and, in 1991, a single specimen discovered in Lebanon (Bitar 1999, 2010). However, Bitar (2017) reports the disappearance of this specimen.

The following characteristics distinguish congeneric species of *Caulerpa*: the genus exhibits clonal propagation through fragmentation and frequently displays invasive tendencies when introduced outside its native range, particularly in competition with seagrasses (Varela-Alvarez et al. 2012). A distinctive anatomical characteristic of Caulerpales is the absence of internal cell membranes, which results in the nuclei being separated within the continuous cytoplasm. These algae are further distinguished by the presence of numerous internal trabeculae, which are branching ingrowths of the cell wall. Noteworthy is the observation that individuals of *C. taxifolia* have been found to reach lengths of up to 2.8 m, making it the largest known single cell of its kind (Varela-Alvarez et al. 2012). *C. mexicana*, a species of green algae in the genus *Caulerpa*, exhibits the following characteristics: it possesses a branched horizontal axis (stolon) that can reach up to 1 m in length, affixed by numerous short rhizoidal outgrowths (columns). Its photosynthetic axes (fronds) manifest a flattened, pinnately branched morphology evocative of feathers, with heights reaching up to 15 centimeters and widths ranging from 10 to 15 millimeters. The narrow midrib, which is less than the length of the ramules, is observed to be flattened in its cross-section. The branches (ramuli) are distichous, flattened, clavate-shaped and membranous, with slightly or no constriction at the base, upward curvature, and tapering, pointed tips. They are coenocytic throughout, but with slender cylindrical and branched wall projections (trabeculae) that cross the lumen. The chloroplasts lack pyrenoids, and the presence of amyloplasts is notable. Notably, the species is distinguished by its holocarpic reproduction, a characteristic that has been documented (Fagerberg et al. 2010, 2012; Guiry and Guiry 2024; Verlaque et al. 2015).

According to the published literatures, in the eastern Mediterranean basin and Turkish seas *C. taxifolia* var. *distichophylla* is a common species of non-indigenous species (Jongma et al., 2013; Mutlu et al., 2022a; Taşkın et al., 2023; Mutlu et al., 2025; Verlaque et al 2015) among *Caulerpa* genus species, expanding the western Mediterranean Sea, followed by *C. taxifolia* (Çevik et al., 2007). *C. mexicana* was reported in the Iskenderun bay, eastern Mediterranean Sea (Erduğan et al., 2009; Verlaque et al 2015), but the species could not be conspicuous. The present study was the first attempt to outline the biometric dynamics of *C. mexicana* with an emphasis on comparison with biometrics of other two species found in a survey conducted in the Aegean Sea.

Material and Methods

Specimens-environment sampling

A research cruise was conducted to investigate the distribution and species composition of seagrasses and seaweeds as submerged vegetation along the Turkish coast of the Aegean Sea from May to August of 2024 (Fig. 1). During the study, a total of 321 stations were surveyed during daylight hours, and two stations were found to contain specimens of *C. mexicana* in July 2024. These stations were situated in the Gulf of Izmir, within the Aegean Sea of Turkey (Fig. 1). At both stations, a small number of specimens were collected by SCUBA divers, who identified them within a quadrat measuring 0.4 x 0.4 m. The first station (I1) was sampled at approximately 3:00 p.m., and the second station (I2) was sampled at approximately 5:00 p.m.

Aboard the R/V "Akdeniz Su," the fronds, stolons, and rhizoids were meticulously disentangled for biometric measurements (Fig. 2). The measurements were obtained from fresh, unpreserved specimens, which were subsequently preserved in a 10%-formalin solution due to the survey's duration of 3.5 months. This constraint precluded the possibility of conducting a genetic analysis.

During the shipboard sampling process, physicochemical parameters (temperature, salinity, pH, oxygen, and total suspended solids) and optical parameters (Sechhi disk depth and photosynthetic active radiation) were measured from surface and near-bottom waters. Water samples were collected on board using a 5-liter Niskin bottle, and the physicochemical parameters were measured using multiparameter probes (AZ Combo, model 84051). Photosynthetically active radiation (PAR) was measured using an ampoule (Spherical SPQA-4671 model, Li-Cor Inc.) and a multiparameter recorder (LI-1400 model). The ampoule was poured from the surface to the near-bottom depth, and the profiled PAR values were then converted to percent values with the sea surface value as 100% over the water depth.

Biometrical measurements

The biometry of the specimens was characterized by the density (number of shoots/m2 and per quadrant; TS, number of fronds: buds; BNo) on a frond branched from the stolon and the number of paired rachis or pinnae per rachis and 1 cm of rachis. The morphometry of the specimens was characterized by rachis length; rachis 1: RL1 and rachis 2: RL2, frond length: FL, and frond width: FW) of the specimens (Fig. 2). The morphometric parameters were measured with an accuracy of µm using a capillary balance.

After a significant subsampling of the clusters of specimens, a total of 25 and 38 shoots branched from the stolon and 68 and 114 fronds were measured at I1 and I2, respectively, since a shoot contained fronds in numerous more than one frond. The number of pinnae per frond was determined by meticulously enumerating a total of 19 and 17 fronds at I1 and I2, respectively. The number of buds per frond was subsequently enumerated and measured to ascertain the biometric parameters.

Statistical interpretation of the biometry

In order to ascertain the biometric characteristics of the species, the frond length-width and frond length-number of pinnae relationships were determined using the Pearson correlation and regression model. Differences in frond length-number of pinnae relationships among bottom depths were tested using ANOCOVA. Furthermore, the normality of each biometric variable dataset was assessed using the dispersion index, randomness test, and FAO (1991) criteria, and the distribution of these variables was then tested for significance using ANOVA. The confidence level chosen for the significance of the tests was $p<0.05$. Furthermore, the number of shoots per quadrant (*c.a.* 0.16 m^2 at station I1 and I2) was calculated as shoots/m^2. All analyses were performed using the statistical tools of MatLab (vers. 20221a, Mathworks Inc.).

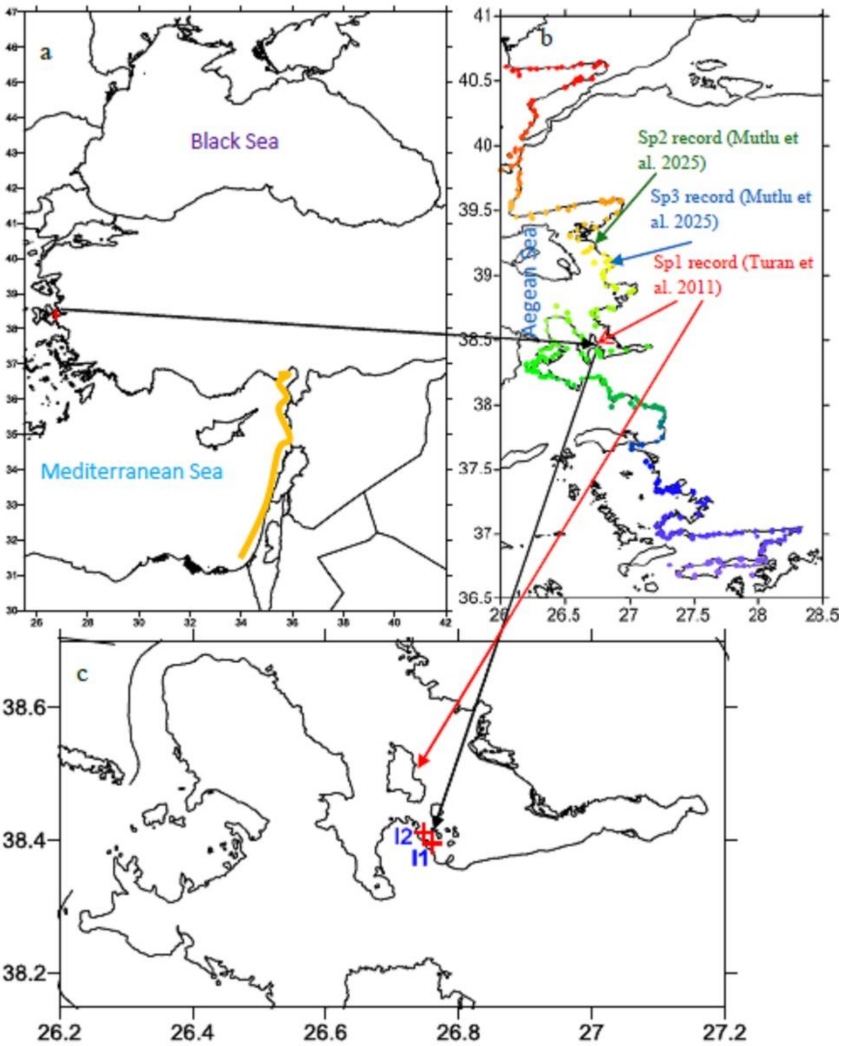

Figure 1. Study area (a, b) and sampling stations (b) and stations where *C. mexicana* occurred (+, c). Previous Mediterranean records of *C. mexicana* (brown line from Verlaque et al 2015) (a), and sampling stations colored with the latitudes during the present study (b). Red arrow denotes the fist occurrence of Sp1: *C. taxifolia* in the Aegean Sea (Turan et al., 2011), dark green Sp2: *C. taxifolia* var. *distichophylla* (Mutlu et al., 2025) and blue Sp3: *C. taxifolia* found during the present survey (Appendix Fig. A1, A3).

Results

Study environment

The hydrograph of the entire study area was described by (Mutlu et al., 2025) as follows: A total of 321 sampling stations were surveyed, and two of these stations provided evidence for the presence of *C. mexicana* specimens (Fig. 2). The stations were located in coastal waters around Urla province in the Gulf of Izmir (station I1 at 10 m and station I2 at 15 m, Table 1). The area is frequently visited by recreational boats and tourists. Notably, the Gulf of Izmir is home to one of Turkey's largest seaports, Alsancak Harbor, which serves as a gateway for international maritime traffic.

The sea surface temperature ranged from 20.5 to 28.5°C, while the temperature of the near-bottom waters exhibited a range of 18 to 28°C (Fig. 2). The Gulf of Izmir was found to have the highest temperatures of the two layers. The salinity levels exhibited a range from 30.5 to 38 parts per thousand (PSU) at the sea surface and from 33 to 37.5 PSU in the near-bottom waters (Fig. 2). The pH values demonstrated a comparable range at both stations, both at the sea surface and in the near-bottom waters (Fig. 2). The salinity levels exhibited a decreasing gradient from south to north within the study area, a trend that was more pronounced in the near-bottom waters. Conversely, oxygen content and pH exhibited a slight increase from south to north within the study area, contrasting with the variation in total suspended solids (Fig. 2). The Gulf of Izmir was characterized by the highest pH value, the lowest oxygen levels, and moderate total suspended matter values in the near-bottom waters. The Black Sea and the Sea of Marmara experienced cold water in the Dardanelles Strait exit within the Aegean Sea, while the northern part of the Aegean Sea was marked by warmer water. The influence of the river Meriç resulted in less saline water in this specific region.

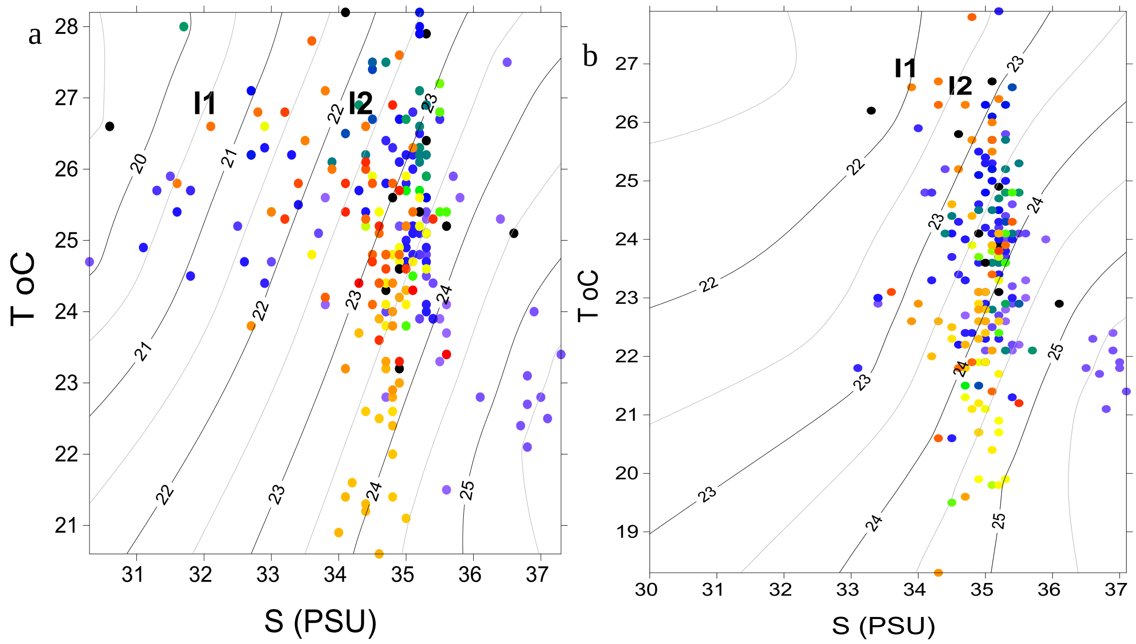

Figure 2. Sea surface water and near-bottom water T-S diagram with isoline of density, σ_t at the stations colored with geographical latitudes (see Fig. 1b for the station locations).

In contrast to the sea surface water, the TS diagram revealed that stations I1 and I2 exhibited distinct characteristics compared to the other nearby stations in the Gulf of Izmir and the study area. These two stations were characterized by lower salinity and higher temperatures compared to the other stations (Fig. 2, Table 1). Water temperatures were measured as warm, ranging from approximately 26°C at the sea surface to near bottom waters at both stations (Fig. 2). However, a notable disparity in salinity was observed between the two stations, with I2 exhibiting a lower salt content compared to I1 (Fig. 2). Furthermore, the dissolved oxygen levels varied significantly, with I1 recording a maximum of 8.5 mg/L in the near-bottom water and a minimum of 10 mg/L in the surface water (Fig. 2, Table 1).

Table 1. Physicochemical properties of the sea surface and near-bottom waters at station I1 and I2.

Sta.	WD	T (ºC)	S (ppt)	Ph	TSM (mg/l)	DO (mg/l)	C (ms)	SD (m)	BD (m)
I1	SS	26.6	34.4	8.23	25.4	10	50.7	9	10
	NB	26.3	34.7	8.31	25.6	8.5	51.1		
I2	SS	26.6	32.1	8.34	23.9	9.2	47.2	13.5	15
	NB	26.6	33.9	8.39	25.1	8.8	50.1		

The Secchi disk recorded a reading of 9 meters at I1 and 13.5 meters at I2. Due to the discrepancy in the hourly sampling times between the I1 and I2 stations, the PAR values were measured in units of µmol photons/cm²/s (Fig. 3a). However, the percentage of light reaching the near-bottom waters was estimated to be approximately 99.9% of the surface PAR (Fig. 3b).

Plant traits

The following materials were examined: unpreserved specimens; one stolon for each station of I1 and I2; 25 shoots at I1, 38 shoots at I2 for rachis length measurements; 68 fronds at I1 and 114 fronds at I2 for measurements of frond length and width; and 19 fronds. at I1 and 17 fronds at I2 for the enumeration of pinnae on the fronds, collected at location of 38.395003 N and 26.759612 E (I1), and 38.412348 N and 26.746 E (I2). The samples were collected from depths of 10 and 15 meters by SCUBA diving on July 9, 2024, by Yaşar Özvarol and Barış Akçalı at locations designated as I1 and I2, respectively. The specimens were identified by Erhan Mutlu and Barış Akçalı.

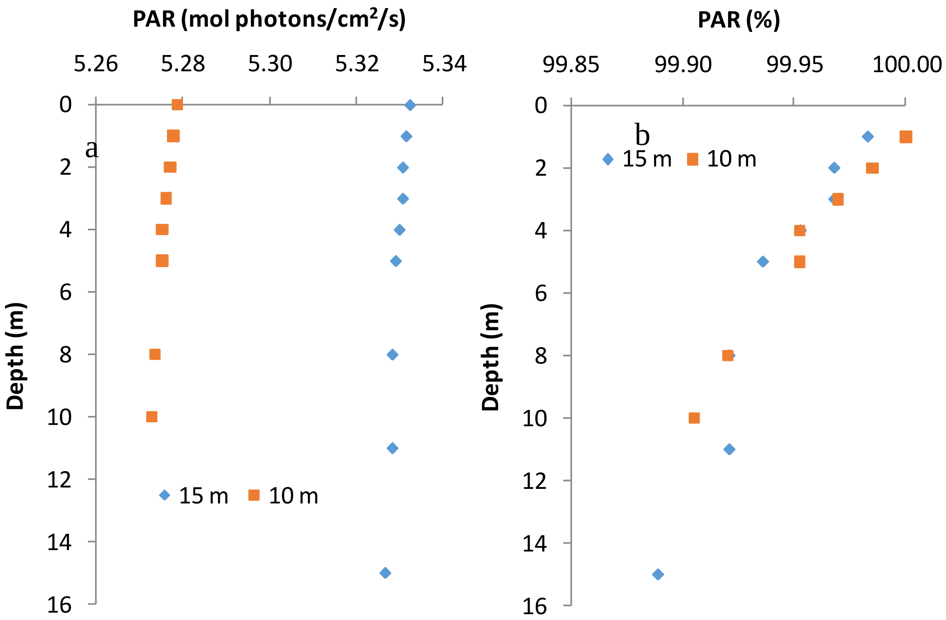

Figure 3. PAR profile in mol photon/cm^2/s (a) and percent based on sea surface PAR (b) along the water depth from the surface to bottom at 10 and 15 m.

Figure 4. *C. mexicana*: appearance of entire specimens (a and b) and close-up view of fronds (c and d). F: frond, St: stolon, Rh: rhizoid, FL: frond length, FW: frond width, P: pinnae, R1: rachis 1, and R2: rachis 2

Description

C. mexicana is characterized by its erect fronds, which are distinguished by their flattened branches. These fronds emerge from a creeping stolon, a specialized root-like structure, that is attached to the substrate by rhizoidal branches, which are specialized structures that facilitate nutrient uptake. The branchlets of *C. mexicana* are typically grassy green in color and are arranged in opposition along the midrib of the frond. This midrib is flat, and the branchlets are positioned in a manner that appears to be overlapping and pointing upward, contributing to the frond's distinctive feathery appearance. The length of frond 1 varied from 0.3 millimeters to 8.3 millimeters, with a maximum frond length ranging from 10 to 12.5 centimeters and a width ranging from 7 to 9 millimeters. The number of branchlets exhibited a range from 10 to 28 and from 120 to 194, with an average of 12 to 15 to 20 to 27 branchlets per 1 cm frond length (Fig. 4).

Remarks

C. mexicana exhibited structural similarities *to C. taxifolia*, yet *C. taxifolia* was distinguished by branchlets with constricted bases and a compressed midrib (Loos et al., 2023).

This species possesses a substantial, branched horizontal axis (stolon) that can attain lengths of up to 1 m, anchored by a multitude of short rhizoidal outgrowths (columns).Fronds: The photosynthetic axes (fronds) are characterized by a flattened structure, with lengths reaching up to 15 centimeters (6 inches) and widths ranging from 10 to 15 millimeters (0.4 to 1.5 inches). These fronds exhibit a pinnate branching pattern, akin to feathers, and possess a narrow midrib that is flattened in its cross-section. Branchlets (ramuli): The branchlets are distichous (arranged in two rows), flattened, and membranous in nature. The samples were found to be slightly or not constricted at the base and were observed to be curved upward, tapering to pointed tips.

The slope of the relationship between frond length and number of pinnae was estimated to be higher (2.15) than 2 for *C. taxifolia* var. *distichophylla* and more or less (0.91-1.19) than 1 for *C. taxifolia* (Mutlu et al., 2025) (Fig. A2).

Distribution

In addition to previous records in the easternmost Mediterranean, *C. mexicana* has been found in two different locations in the shallow and coastal waters of the Aegean Sea (Gulf of Izmir, Turkey). The species is primarily found in subtidal marine environments and has been observed to thrive in the following habitats: Depth range: It is typically found at depths ranging from 0.5 to 33 meters. Substrate: This species demonstrates a marked preference for rocky substrates and sandy bottoms, where it can anchor itself with its rhizoids. Its ecological role is to form lush underwater communities that provide shelter and grazing for various marine organisms, making it an important component of its ecosystem.

Biometrics

The presence of *C. mexicana* specimens at each station was evidenced by their location in wispy bundles of rachis, exhibiting a reticular stolon organization attached to the bottom substrates (Fig. 4). This occurrence was analogous to that of the species found at the bottom, contrasting with the highly elongated stolons characteristic of *C. taxifolia*. The biometric parameters of the species were identified as density and morphometric variables to characterize the recent measurements made from the living specimens that occurre for the first time in the Aegean Sea.

Density

Following the conversion of the number of shoots (fronds) to abundance, it was determined that the shallow station exhibited a higher abundance (630 shoots/m^2 at 10 m bottom depth) in comparison to the deep station (469 shoots/m^2 at 15 m bottom depth). Among the density variables, the number of shoots per sample (TS) and the leaf area index (frond length*width in cm^2 per square sampling area) exhibited a significant relationship with bottom depth, contrasting with the number of pinnae per 1 cm frond length (BNo) at $p < 0.05$ (Fig. 5). Analogous to abundance, the number of shoots per sample demonstrated a higher value in the shallower water (Fig. 5).

Conversely, the leaf area index (frond length*frond width in cm per square sampling area) exhibited a contrasting relationship with the number of shoots, suggesting that the LAI was elevated at greater depths (Fig. 5).

The mean BNo remained at approximately 2.57 and 2.82 pinnae/cm frond length (Fig. 5). No statistically significant differences in BNo were observed between the two sampling depths. The number of pinnae exhibited variability, ranging from 28 to 194, with an average of 18 ± 3 pinnae/cm FL at 10 m and 10 to 130, with an average of 16 ± 2 pinnae/cm FL at 15 m (Table 2).

Morphometry

The characterization of species morphometry was conducted by measuring rachis length (R1 in millimeters) and frond length (R2 in millimeters), frond width (in centimeters), and unilateral leaf area (in square centimeters), independent of surface area per sampling unit (Fig. 6). A statistically significant difference was observed among all morphometric variables between the two sampling depths of 10 and 15 meters at a $p < 0.05$ significance level.

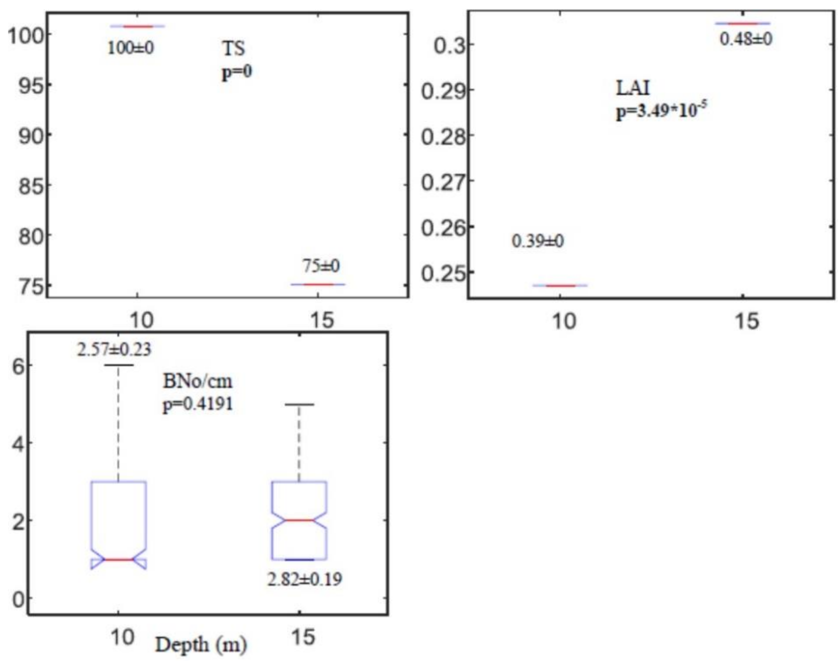

Figure 5. Notch plots of density variables of *C. mexicana* at the sampling depths. TS: number of shoots per sampler, LAI: single-sided leaf area index (cm^2/m^2), and BNo: number of pinnae per 1 cm frond length. The bold p value denotes a significant difference in the variable between two bottom depths, and the average value ± SD at each bottom depth.

Table 2. Minimum, maximum, mean and standard deviation values of density variables of *C. mexicana* at the sampling depths. TS: number of shoots per sampler, LAI: single-sided leaf area index (cm^2/m^2), and BNo: number of pinnae per 1 cm frond length.

	10 m (I2)			**15 m (I1)**		
	FL	**NP**	**BNo**	**FL**	**NP**	**BNo**
Min	1.7	28	15	0.5	10	12
Max	12.4	194	27	7.9	130	20
Mean	5	90	18	4	70	16
SD	3	48	3	2	42	2

Rachis 1 length exhibited a range from 0.3 mm to 8.3 mm, with an average of 3.5 mm at 10 m and 1.9 mm at 15 m. Frond length demonstrated a range from 3.6 to 91 mm at 10 m and from 3.9 to 81.6 mm at 15 m, respectively. The frond length at 10 m was 3.7 mm greater than that at 15 m (Table 3, Fig. 6). Similarly, the mean frond width was narrower at 10 m than at 15 m (Table 3, Fig. 6).

Table 3. Minimum, maximum, mean and standard deviation values of morphometric variables of *C. mexicana* at the sampling depths. RL: Rachis 1 length in mm, FL: frond length in mm, and FW: frond width in mm.

	10 m (I2)			**15 m (I1)**		
	R1	**FL**	**FW**	**R1**	**FL**	**FW**
Min	1	3.6	1.8	0.3	3.9	3
Max	7.4	91	7	8.3	81.6	9
Mean	3.5	31.1	4.37	1.9	34.8	5.76
SD	21.3	19.6	7.32	22.3	20.2	10.16

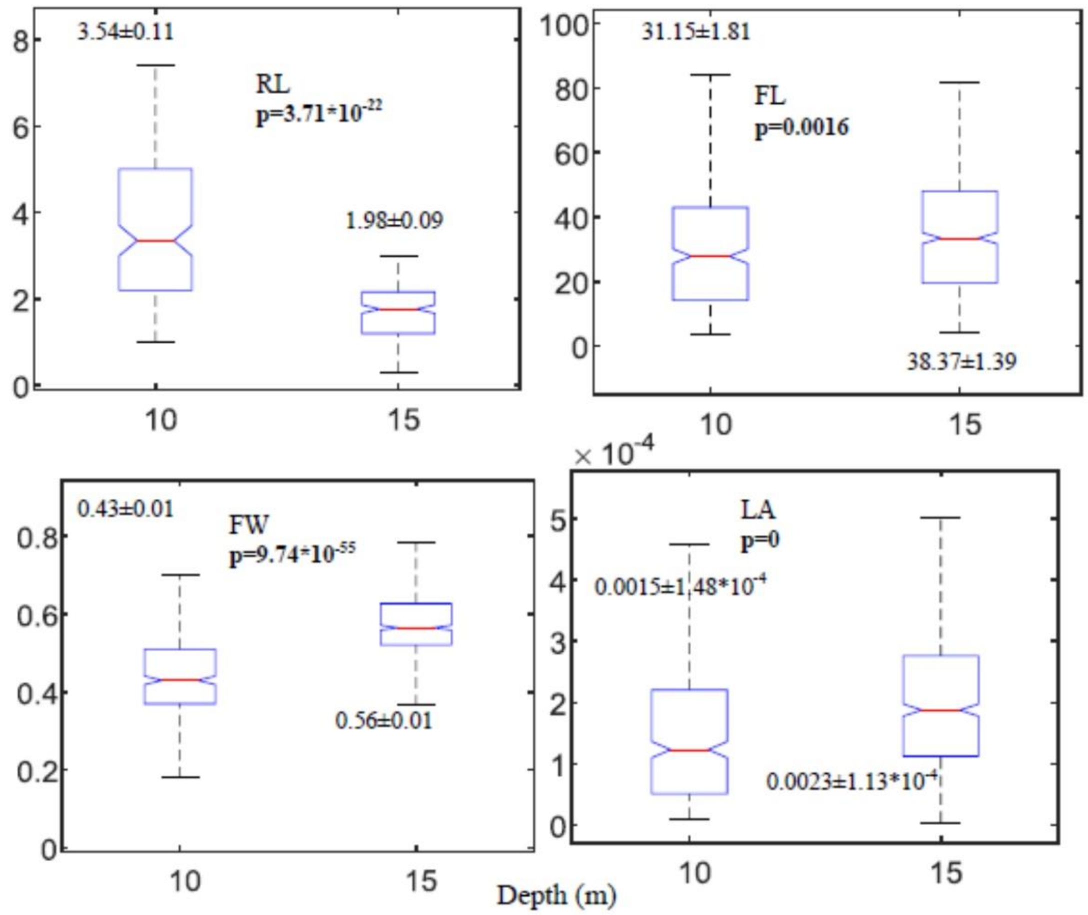

Figure 6. Notch plots of morphometrical variables of *C. mexicana* at the sampling depths. RL: Rachis 1 length in mm, FL: frond length in mm, FW: frond width in mm, and LA: leaf area in m^2. Bold p value denotes significant difference in the variable between two bottom depths, and average value ± SD at each bottom depth.

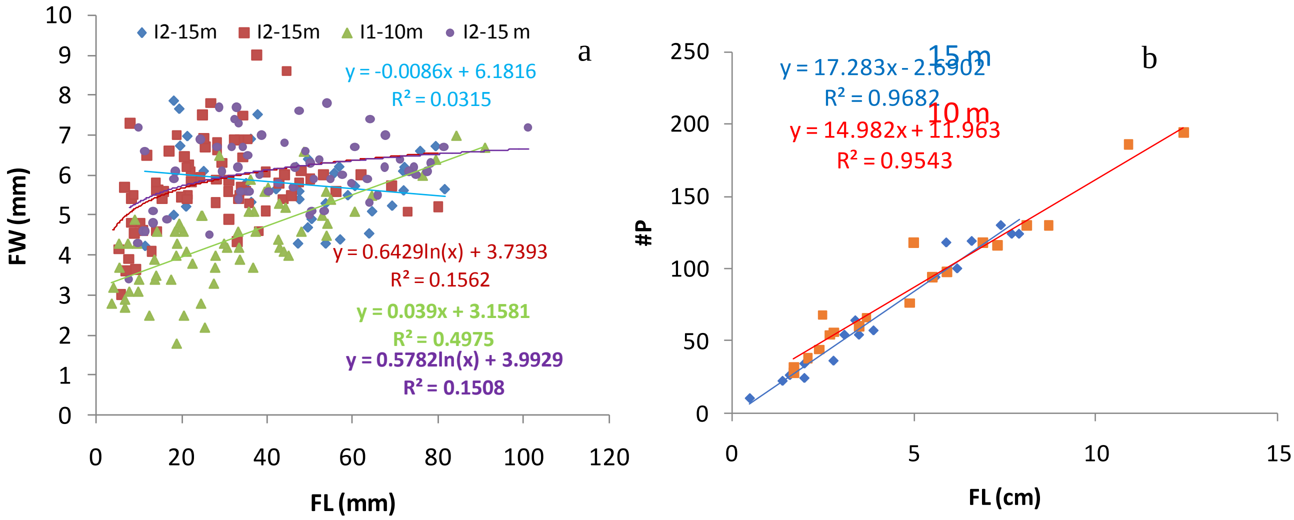

Figure 7. Relationships between frond length (FL) and frond width (FW) at 10 and 15 m depth (a) and between frond length and number of pinnae (#P) at 10 m and 15 m bottom depth (b). It is noted that the marked diamond showed the relationship of the fronds without buds, square with buds, and circle with a new measurement data set with and without buds after irregular relationship obtained at 15 m.

Biometrical relationship

The relationship between frond length and frond width, as well as frond length and number of pinnae, is demonstrated in Figure 7.

The FL-FW relationship proved to be significant for specimens found at 10 m, yet it was non-significant at 15 m. At 15 m, the relationship was plotted separately for either the main frond or buds, and the main frond exhibited a decreasing trend in the number of pinnae with frond length. However, buds demonstrated a bifurcated, non-significant linear relationship between frond length and number of pinnae. Given the irregular relationship between FL and #P at the 15-m depth, the re-established relationship with repeated measurements of the variables exhibited a similar insignificant logarithmic regression line as the previous fitting lines (Fig. 7).

The FL-#P relationship was evaluated significantly for samples taken at both 10 m and 15 m (Fig. 7). A subsequent analysis revealed no statistically significant variation in the FL-#P relationship between the 10-m and 15-m bottom depths ($p = 0.0648$).

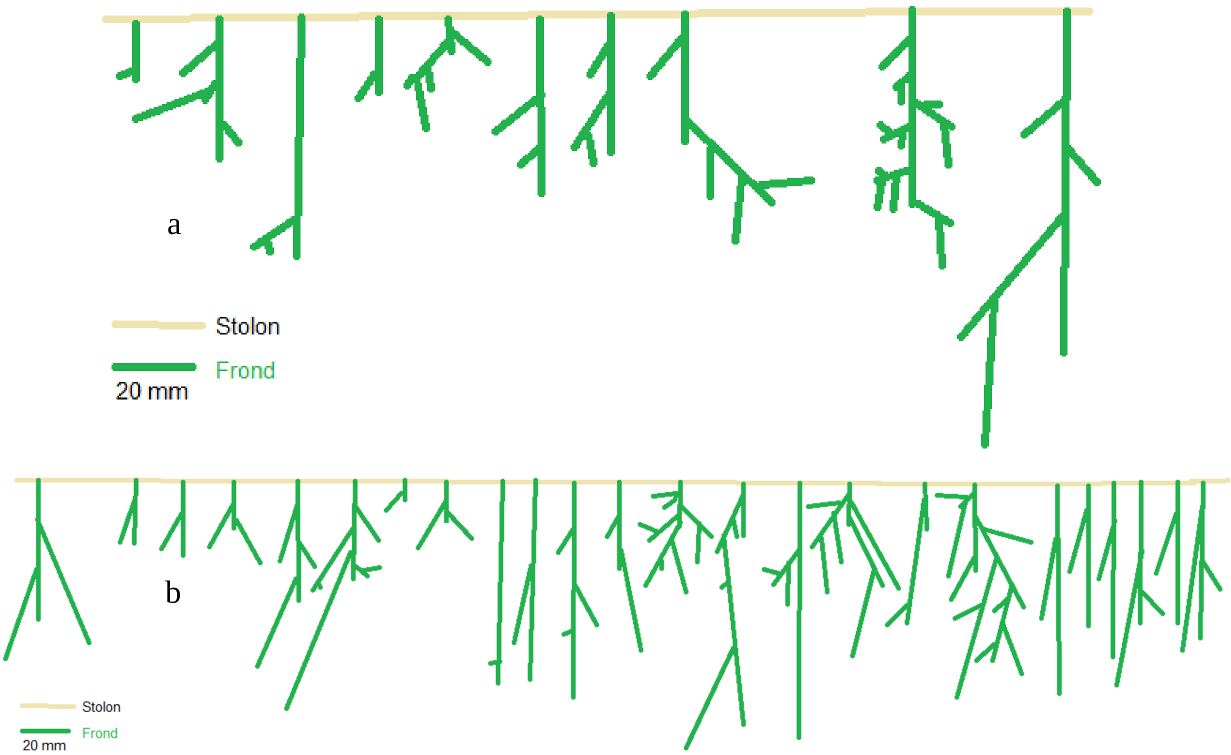

Figure 8. Schematized frond budding (see Fig. 5 for real appearance of budding reticulated with branches) of *C. mexicana* found at 10 m (a) and 15 m (b).

Budding

The stolons of this species manifested in a reticulated form with budding (see Fig. 4). A total of 26 shoots were examined at the 10 m, and 40 shoots at the 15 m to assess the budding patterns of *C. mexicana* specimens. Regardless of the reticulated stolons, the number of buds and buds of buds appeared to be more pronounced at the 15-meter compared to the 10-meter (Fig. 8). Furthermore, the branching of the buds was found to be more forked at 15 m. The maximum number of buds of buds observed varied between 3 buds found at 10 m and 8 buds found at 15 m (Fig. 8). Additionally, 38% of the fronds were found to be bifurcated at 10 m, while it was more doubled at 15 m, with 60% of the fronds examined showing this characteristic

Discussion

Intra/inter-biometric relationships have the potential to assist taxonomists in identifying species at the lower taxonomic level. Depending on the differing growth conditions in various environments, such as the water mass characteristics depicted in the T-S diagram, these relationships can be distinguished by the specific water mass. Consequently, variations in these relationships were observed across different depths of the Izmir Gulf, the study area, which exhibited oligotrophic-eutrophic characteristics in its various sectors. Throughout the year, the near-bottom and sea surface water salinity maintained a consistent value of approximately 39 PSU, with the exception of a decline to 33 PSU in February, where sea surface salinity registered at around 19 PSU. This observation indicates that sea surface salinity exhibited a decrease in February when compared to other months.

Additionally, the water temperature fluctuated seasonally, ranging from a minimum of 15 °C in February to a maximum of 27 °C in July. The maximum recorded temperature in the near-bottom water was 23 °C, while the sea surface temperature ranged from 14-15 °C in February to a peak of 27 °C in July, subsequently dropping to 18 °C in November. A study by Mutlu (2021) and Mutlu and Akçalı (2022) revealed that the temperature of the sea surface and bottom waters were equivalent in November.

In relation to the nutrients necessary for the rapid growth of *C. mexicana*, the levels of surface dissolved inorganic nitrogen fluctuated between 0.5 and 0.7 μM, maintaining a consistent level of 0.5 μM in July and November (extreme 10 μM), and ranging from 0 to 0.5 μM (8 μM) in February. The levels of PO_4 in the outer gulf remained consistent throughout the year, ranging from 0 to 0.1 μM. However, in the inner and middle gulf, PO_4 levels increased to 2 μM in April, reaching a maximum of 3 μM in the inner gulf in July and November, and in the inner and middle gulf in February 2010 (Yelekçi et al. 2021). Furthermore, the 15-meter depth zone served as an intermediate zone for the adaptation of seagrasses to shallower and greater depths, exhibiting high variability in biometric measurements. This suggests that there were irregular and insignificant relationships between frond length and width measured at the 15-meter depth. In this transition zone, the fundamental environmental requirements (temperature, photoperiod, and light intensity, among others) exert a pivotal influence on biometric disparities and variations, as well as on the presence or absence of species (Catucci and Scardi 2020; Gnisci et al. 2020; Mutlu et al. 2022b).

The establishment of a suitable environment by such invasive algae could facilitate their colonization and subsequent spread in space. This species is one of the Lessepsian species in the Mediterranean Sea and could most likely have been introduced to the Aegean Sea via the ballast water of commercial ships that often visit the Gulf of Izmir (Çinar et al (2025). However, the species has not been observed along the Turkish Mediterranean coasts on the path of species introduction first observed at several loci, including the Israeli, Lebanese, and Syrian waters (Rayss 1941; Mayhoub 1976).

In the Mediterranean basin, *C. mexicana* was first documented in the Aegean Sea, following prior records that were exclusively reported in the easternmost Mediterranean waters (Fig. 1a). A comparison of *C. mexicana* and *C. taxifolia* reveals several distinguishing characteristics. The rachis (ramuli) of *C. taxifolia* is flattened and arranged with pinnules in a sickle shape, while the branchlets are characterized by flat blades with broad, flat marginal pinnae (Verlaque et al. 2015). In contrast to *C. taxifolia*, the ramuli of *C. mexicana* are wide and overlap one another, and the blades are clavate in shape. The ramuli were abruptly tapered towards the curved end spine (Coppejans and Beeckman 1990; South and N'Yeurt 1993; Aysel and Dural 1998), as seen in our materials (Figs. 4, 1A). However, the basal part of the ramuli of the specimens published by Erduğan et al. (2009) exhibited no contraction, and the frond blade was sickle-shaped. According to the descriptions made by Verlaque et al. (2015), the species could be identified as *C. taxifolia* var *distichophylla*. In contrast, the specimen reported from the Turkish coast of the Aegean Sea by Turan et al. (2011) was identified as *C. taxifolia* due to the contracted basal part of the ramuli. However, both studies did not establish the biometric relationship. The rachis with frond exhibited an almost ellipsoid shape in both *C. mexicana* and *C. taxifolia*, while in *C. taxifolia* var *distichophylla*, it was more or less cylindrical (Verlaque et al. 2015). The specimen of *C. mexicana* exhibited a unique morphology, characterized by a hybrid form between a clavate structure and a club-shaped structure (Fig. 1A). The latter component of the specimen was wider than the tip parts of the ramuli.

The budding structure of the three species was found to vary significantly. *C. mexicana* exhibited a reticulate structure (Figs. 4, A3) as determined by Verlaque et al. (2015). Çevik et al. (2007) conducted a molecular analysis to identify specimens found in Iskenderun Bay (Turkey) by comparing them with specimens from other regions and aquariums in Izmir. This analysis led them to recognize the Iskenderun specimens as *C. taxifolia*. The Iskenderun specimens exhibited an absence of a contracted structure in the basal part of the ramuli attached to the rachis, suggesting that this species differed from *C. taxifolia* collected from the aquarium. Indeed, both locus specimens (Iskenderun and Izmir) exhibited biometric differences during the description performed by Çevik et al. (2007) who performed molecular analyses for identification of the specimens. Therefore, subsequent analysis by Jongma et al. (2013) addressed the genetic misidentification of the Iskenderun Bay specimens, emphasizing the challenges and limitations of integrating genetic and morphological studies in determining species identity. They concluded that the Australian endemic green alga, *Caulerpa distichophylla*, was present along the Sicilian coast of the Mediterranean Sea. The slender *Caulerpa* previously reported as *C. taxifolia* from southeastern Turkey (Gulf of Iskenderun) also belongs to *C. distichophylla*. Morphologically, *C. distichophylla* clearly differs from *C. taxifolia* in its slender thallus and the lack of large rhizoidal pillars. However, genetic data do not provide undisputed evidence that the species are distinct. The analysis of the tufA cpDNA gene and the cp16S rDNA intron-2 sequences revealed a single nucleotide mutation that demarcated the two taxa, while the ITS rDNA sequences did not provide unequivocal distinction between them.

Biometric measurements have been identified as a primary factor in distinguishing the identity of closely related species and monitoring growth patterns. Consequently, the size and dimensions of three congeneric species of *Caulerpa* vary, and the location or sea-based location affects its biometry (density and morphometry) (Benzie et al., 2000). The potential for species *C. taxifolia* to exhibit ecomorphic variants of *C. mexicana*, or vice versa, as a result of misidentification, has been observed in different environments (Chisholm et al. 1995; Olson et al. 1998). However, there is a paucity of detailed studies examining the occurrence of these species with biometric variation in space and time for the Mediterranean Sea. The present study could be important to provide their density and morphometry for the Mediterranean Sea for future studies, since the species has been restricted to the easternmost corner of the Mediterranean Sea in terms of its distribution. The density of *C. taxifolia* species has increased very rapidly, reaching the number of fronds up to 5000 fronds/m^2 (Pereira et al. 2016; Loos et al. 2023). The length-width relationship of the frond of *C. taxifolia* var. *distichophylla* specimens collected from Antalya Gulf of the Turkish Mediterranean coast (Mutlu et al., 2022a) exhibited a comparable relationship with specimens of the Aegean Sea (Fig. A2). The relationships between the seas were not significantly different at $p < 0.05$ (ANOCOVA, p: 0.457).

In addition to environmental measurements (Tuya et al. 2006), the depth of the seabed has affected the size of the algae and seagrasses. For example, the photosynthetic activity of *C. mexicana* was at its highest in the coldest season and preferred low photosynthetic irradiance (Robledo and Freile-Pelegrín 2005). However, *C. taxifolia* has been observed to exhibit a preference for higher light intensity in comparison to *C. mexicana* (Gayol et al. 1995; Chisholm and Jaubert 1997). Furthermore, the shallower waters have been found to harbor denser shoots of various seaweed and seagrass species compared to those at greater depths. The length of the rachis (the part that holds the leaves or shoots) of these algae or the vertical rhizome length (the root-like structure that helps the plant take in water) of the seagrass was measured to be longer in the shallow waters. Conversely, the frond length, leaf length, and leaf width exhibited a decrease with increasing depth. The environmental parameters exhibited a variation with respect to bottom depth, with the density and size measurements of two species of seaweed, *Caulerpa prolifera* and *C. taxifolia* var. *distichophylla*, demonstrating distinct variations. These measurements varied in both time and space along the Turkish Mediterranean coast (Mutlu et al., 2022a). For instance, in contrast to *C. mexicana*, *P. oceanica* biometrics exhibited a response to numerous predicted environmental variables in the Mediterranean. The shoot density was predicted in response to geographic coordinates and seafloor depth, followed by prevailing wind, bottom gradient, and species in order of importance (Catucci and Scardi 2020; Gnisci et al. 2020; Mutlu et al. 2022b). Additionally, the seagrass *Cymodocea nodosa* exhibited distinct density and plant characteristics between the cold and warm water months, contingent on the physical, optical, chemical, and sedimentary characteristics of the environment (Mutlu et al. 2022c).

The number of pinnae on the frond increased with frond length; however, it remained constant with frond length per 1 cm of frond or rachis length. Furthermore, it did not differ with bottom depth, a phenomenon that has also been observed in the number of leaves of certain seagrass species. The measured frond length reached higher (15-25 cm) in the national Spanish waters (Canary Islands) fed with the riverine environment (Pereira, 2024) than our estimates.

In conclusion, the distribution of the invasive non-indigenous seaweed *C. mexicana* has extended its range northward from its initial occurrence in the Aegean Sea to the eastern basin of the Mediterranean Sea. This phenomenon is a consequence of the ongoing tropicalization resulting from global warming in the Mediterranean Sea, which has led to the proliferation of tropical species into previously unoccupied ecological niches. Consequently, the introduction of non-indigenous and invasive species, such as *C. mexicana*, poses a significant threat to the endemic biodiversity of the eastern Mediterranean basin. The biometry of *C. mexicana* can serve as a baseline for comparison with the characteristics of other congeneric species of *Caulerpa* throughout the Mediterranean basin and their native localities. Given its reproductive characteristics comparable to *C. taxifolia* and the elevated nutrient levels characteristic of the Urla region where it was identified, *C. mexicana* is likely to spread rapidly in this area. The turbidity resulting from high nutrient levels and the degradation of sediment quality leads to the removal of seagrasses, which can act as a barrier to invasive species, thereby providing an advantage to invasive species. In this study, we seek to elucidate the significance of biometric variations among organisms in facilitating species identification, in addition to their structural and plant characteristics. However, molecular analysis was not conducted on the specimens of all three *Caulerpa* species encountered in various locations (Fig. 1) during the present study, as they were preserved in formalin solution following measurement. Biometric measurements and analyses are more expeditious and economical than genetic analysis and can swiftly identify species (Figs. A1-A3). These relationships or morphometric analyses and ratios have been applied to the identical characters of many organisms, e.g., fish (e.g., Geladakis et al., 2017; Currie et al., 2024), zooplankton (e.g., Mutlu et al., 2020; Duman et al., 2025), and zoobenthos (e.g., Strafella et al., 2021) across various marine environments. The genetic analysis can discern slight variations in specimens exhibiting plasticity in their plant traits. However, the practical application of genetic analysis to each specimen during the survey, whether in the same or different area, was not feasible. Further studies are recommended to explore the potential of genetic analysis. Nonetheless, this comparative study underscores the significance of biometric relationships and plant traits among the three species examined in this study (Figs. A1-A3).

Acknowledgments

The authors would like to express their sincere gratitude to the Scientific and Technological Research Council of Turkey (TÜBİTAK) for providing financial support through project number 124Y031. Special thanks are extended to İdil Akçalı for meticulously reviewing and controlling the English language used in the paper. The authors also wish to acknowledge the dedicated efforts and support provided by the crew of the R/V Akdeniz Su during this project.

Conflict of Interest

On behalf of all authors, the corresponding authorstates that there is no conflict of interest.

Author Contributions

Erhan Mutlu: Onboard works, Project administration,Supervision, Software, Data analyzes, Writing, Funding acquisition. Yaşar Özvarol: Onboard works, Laboratorial works, Measurements, Data entry. Barış Akçalı: Onboard works, Laboratorial works, Measurements, Data entry, Writing. Berivan Elif Aslan: Onboard works, Laboratorial works, Measurements, Data entry. Zeynep Narlı: Onboard works, Laboratorial works, Measurements, Data entry. Zeynep Zabun: Onboard works, Laboratorial works, Measurements, Data entry.

Ethics Approval

Not applicable.

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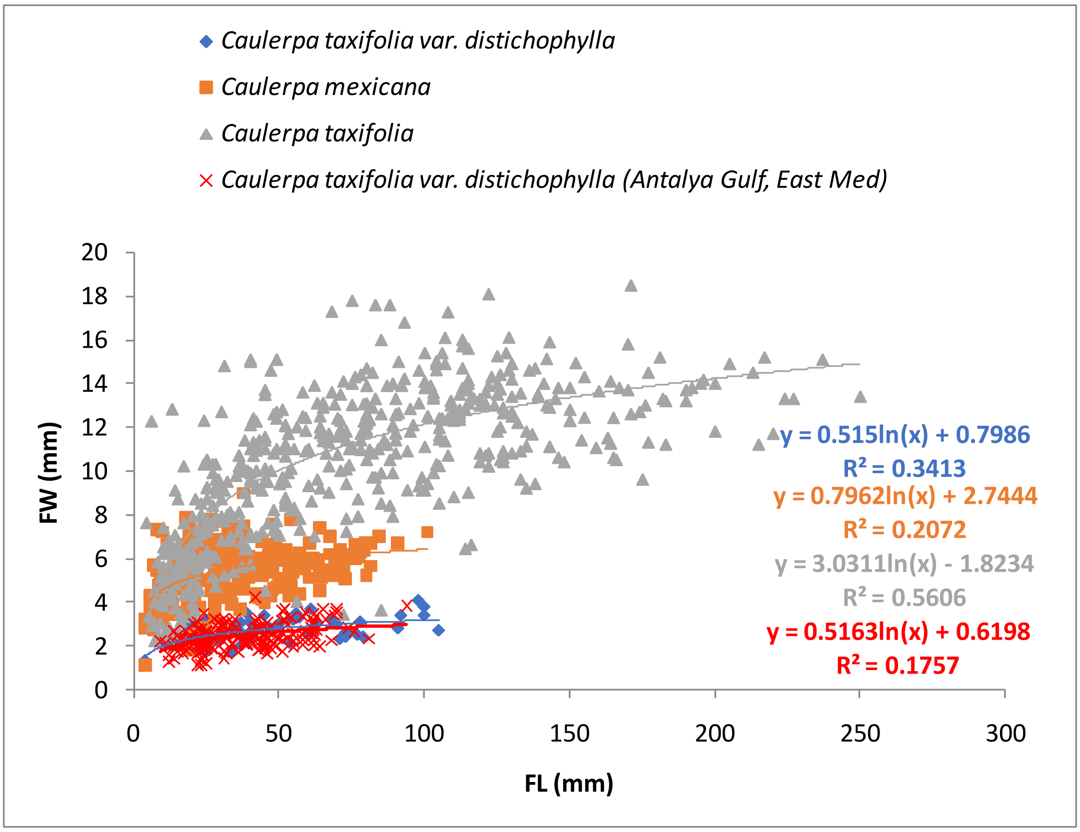
Appendix

Fig. A1. Fronds, rachises and pinnae of *Caulerpa mexicana* (a, d), *C. taxifolia* (b, (unpublished material) and *C. taxifolia* var. *distichophylla* (c) from the Turkish Aegean waters obtained during the present study (Mutlu et al. 2025).



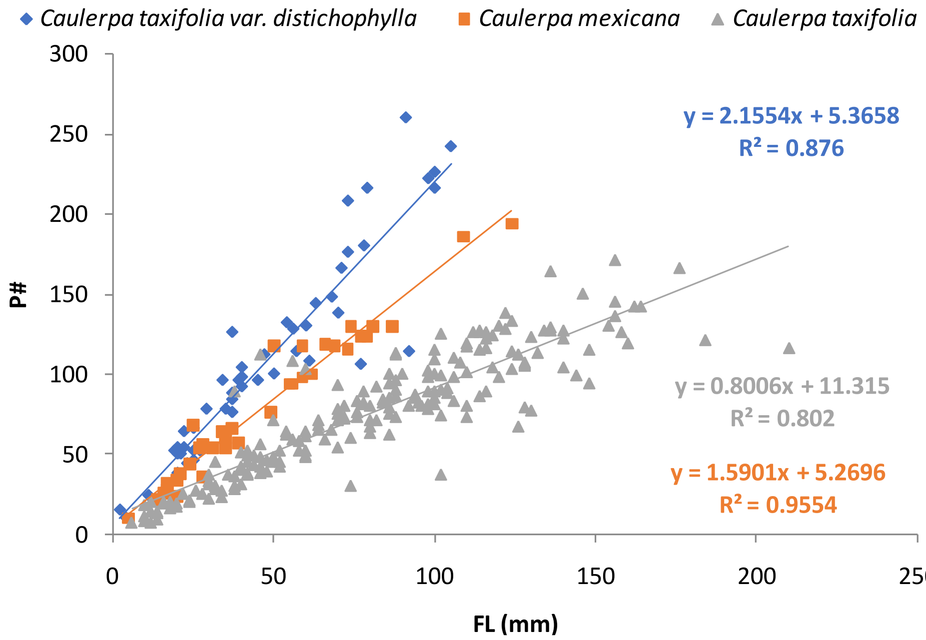


Fig. A2. Frond length (FL)-width (FW) and frond length (FL)-number of pinnae (P#) of *Caulerpa mexicana*, *Caulerpa taxifolia*, *C. taxifolia* var. *distichophylla* specimens collected during the present study conducted in the Turkish coast of the Aegean Sea (Mutlu et al. 2025) and *C. taxifolia* var. *distichophylla* from Antalya Gulf, Eastern Mediterranean Sea (data from Mutlu et al. 2022a).

Fig. A3. Plant traits of fresh specimens of *Caulerpa taxifolia* (a, b), *C. taxifolia* var. *distichophylla* (c, Mutlu et al. 2025) and *C. mexicana* (d) collected during the present study (See Fig. 1 for locations of the species occurrence)

COMU J Mar Sci Fish 2025 8(1): 64-74
https://doi.org/10.46384/jmsf.1652214
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Hydromorphological Status Assessment of Lake Mogan

Damla Coşkun*[1], Muhammet Azlak[2], Alper Uğurluoğlu[2], Nilsun Demir[1]

[1]Ankara Üniversitesi, Fen Bilimleri Enstitüsü, 06110, Ankara, Türkiye https://orcid.org//0000-0001-7954-194X
[2]T.C. Tarım ve Orman Bakanlığı, Su Yönetimi Genel Müdürlüğü, Ankara, Türkiye https://orcid.org//0000-0002-5941-2371
[2]T.C. Tarım ve Orman Bakanlığı, Su Yönetimi Genel Müdürlüğü, Ankara, Türkiye https://orcid.org//0000-0002-8000-7688
[1]Ankara Üniversitesi, Ziraat Fakültesi, Su Ürünleri Mühendisliği Bölümü, 06119, Ankara, Türkiye https://orcid.org//0000-0002-3895-7655

Received: 05.03.2025 /Accepted: 17.04.2025 / Published online: 14.07.2025

Keywords:

Hydromorphological status
Lake Mogan
Hydromorphology
Assessment index

Abstract: Hydromorphology is a discipline that examines the physical, morphological, and hydrological characteristics of aquatic ecosystems and their impacts on ecosystem functioning. In this study, The Lake Hydromorphology Assessment Index (GHDI) was used to examine the current hydromorphological features of Lake Mogan to assess its hydromorphological status. Within the scope of the study, two periods of field work were carried out to represent the wet and dry periods and hydromorphological data were collected from the field. Based on the collected data and using satellite imagery, hydromorphological changes and anthropogenic interventions in the lake were digitized. Subsequently, Geographic Information System (GIS)-based analyses were performed by integrating the digitized datasets with field observations. Our evaluations and analysis revealed the hydrological and morphological modifications, habitat quality status and the overall hydromorphological status of Mogan Lake. According to the five-class evaluation system, the overall hydromorphological status of the Lake Mogan was classified as "moderate". This study represents the most comprehensive and up-to-date hydromorphological assessment of Lake Mogan and provides a scientific basis for the conservation and management for the lake.

Mogan Gölü'nde Hidromorfolojik Durumun Değerlendirilmesi

Anahtar kelimeler:

Hidromorfolojik durum
Mogan gölü
Hidromorfoloji
Değerlendirme indeksi

Öz: Hidromorfoloji, su ekosistemlerinin fiziksel, morfolojik ve hidrolojik yapı ile özelliklerini ve bu özelliklerin ekosistem üzerindeki etkilerini inceleyen bir disiplindir. Bu çalışmada, Mogan Gölü'nün güncel hidromorfolojik özelliklerini incelemek ve hidromorfolojik durumunu ortaya koymak amacıyla Göl Hidromorfolojisi Değerlendirme İndeksi (GHDİ) kullanılmıştır. Çalışma kapsamında, ıslak ve kuru periyodu temsil edecek şekilde iki dönem arazi çalışmaları yürütülmüş ve hidromorfolojik veriler toplanmıştır. Arazi çalışmaları ile toplanan bilgiler çerçevesinde uydu görüntüleri kullanılarak gölde meydana gelen hidromorfolojik değişiklikler ve insan kaynaklı müdahaleler sayısallaştırılmıştır. Ardından, söz konusu sayısal altlıklar kullanılarak Coğrafi Bilgi Sistemleri (CBS) tabanlı analizler yapılmış, araziden elde edilen veri ve bilgiler CBS tabanlı analizlerle bütünleştirilmiştir. Yapılan analiz ve değerlendirmeler sonucunda, Mogan Gölü'nün hidromorfolojik durumu, hidrolojik modifikasyon, morfolojik modifikasyon ve habitat kalitesi ve niteliği olarak ortaya konulmuştur. Mogan Gölü'nün genel hidromorfolojik durumu beş sınıflı değerlendirme sistemine göre "orta" olarak belirlenmiştir. Bu çalışma, Mogan Gölü'ne yönelik en güncel ve kapsamlı hidromorfolojik değerlendirme olması bakımından önem arz etmekte olup Mogan Gölü'nün ekosisteminin korunması ve yönetimi için bir bilimsel temel oluşturmaktadır.

Giriş

Kullanılabilir su miktarının oldukça az olmasına karşın, su kaynakları üzerinde gerek insani faaliyetler gerekse de doğada küresel iklim değişikliği etkisiyle meydana gelen değişimler nedeniyle oluşan baskı hidrolojik döngünün tüm evrelerini etkilemektedir (Tatar, 2019; Vasistha ve Ganguly, 2020). Bu etkenler sebebiyle su kaynakları hem miktar hem de kalite açısından her geçen gün değişime uğramaktadır. Suya duyulan ihtiyacın üstünde oluşan tüketim talebi, su kaynaklarının sürdürülebilir yönetimine ilişkin sorunları artırmaktadır. Su kaynaklarının sürdürülebilirliğinin sağlanmasında, tüm canlıların ve ekosistemin ihtiyacını karşılayacak yeterli miktarda ve kalitedeki suyun temini ve bu kapsamda su kaynaklarının korunması büyük önem taşımaktadır.

Hidromorfoloji, hidroloji ve jeomorfolojiyi birleştiren ve tatlı su ekosistemlerinde yönetim ve restorasyon çalışmalarında ele alınan bir kavramdır (Kemp ve Sandin, 2022). İklim değişikliği nedeniyle değişen hidrolojik rejimler, artan tarımsal faaliyetler vb. ihtiyaçlar sebebiyle

*Corresponding author: coskund@ankara.edu.tr
How to cite this article: Coşkun, D., Azlak, M., Uğurluoğlu, A. & Demir, N (2025). Hydromorphological status assessment of lake Mogan. COMU J. Mar. Sci. Fish, 8 (1): 64-74. doi:10.46384/jmsf.1652214

artan su tüketimi ile hidroelektrik santrallerin kurulması gibi amaçlarla su kaynakları üzerindeki antropojenik faaliyetlerin artması, göl ekosistemlerinde değişimlere sebep olmaktadır. Özellikle, sığ göllerde çevresel değişikliklere daha duyarlı olduğundan göllerin hidrolojik ve morfometrik özelliklerinde önemli değişiklikler görülmektedir (Lawniczak vd., 2011). Örneğin, bir gölün su seviyesinin düşmesi, kıyı bölgelerindeki bitki örtüsünün azalmasına ve hatta gölün tamamen kurumasına neden olabilir. Su seviyesindeki artışlar ise kıyı bölgelerinde yeni bitki türlerinin gelişmesine ve göl ekosisteminin yapısal ve işlevsel olarak değişmesine yol açabilir (Lawniczak vd., 2011; Poikane vd., 2020).

Avrupa ülkelerinde, hidromorfolojik baskılar, sucul ekosistemlerdeki en yaygın baskı türleri arasında yayılı atmosferik kirlilikten sonra ikinci sıradadır (AÇA, 2024). Avrupa Birliği'nde 2000 yılında yürürlüğe giren Su Çerçeve Direktifi (SÇD)'ne göre yerüstü sularının doğal durumuna ulaşmasında temel gösterge olarak biyolojik kalite unsurlarının yanısıra ekolojik durumun belirlenmesinde biyolojik kalite elementlerini desteklemesi açısından hidromorfolojik ve fizikokimyasal kalite elementlerinin izlenmesi gerekmektedir (Azlak vd., 2017; EC, 2000; Meier vd., 2013). Bu nedenle, rutin izleme programlarında hidromorfolojik izleme çalışmalarında kullanılan değişkenlerin yer alması önem taşımaktadır (EC, 2000; Poikane vd., 2020).

Hidromorfolojik analizlerde kullanılan yöntemler hidrolojik değişimler, nehir morfolojisi ve kıyısal yapılar olmak üzere genellikle üç temel bileşene dayanır (Gurnell vd., 2016). Morfolojik değerlendirmeler, nehir yatak yapısı, kıyı stabilitesi, sediment hareketleri ve su kütlesinin zaman içinde gösterdiği şekil değişikliklerini içermektedir (Belletti vd., 2015). Hidrolojik değerlendirmeler ise debi değişimleri, akış sürekliliği ve taşkın gibi su hareketleri ile ilgili parametreleri kapsamaktadır (Poff vd., 1997).

Son yıllarda gelişen teknoloji ile birlikte uzaktan algılama ve Coğrafi Bilgi Sistemleri (CBS) gibi teknolojik yöntemler, hidromorfolojik analizlerin daha kapsamlı ve doğru yapılmasını sağlamaktadır. Bu yöntemler, su kütlelerinin uzun vadeli değişimlerini belirleme ve sürdürülebilir yönetim stratejileri geliştirme açısından önemli katkılar sunmaktadır (Bizzi ve Lerner, 2015).

Hidromorfolojik değerlendirmeler, ekolojik restorasyon çalışmalarından su kalitesi yönetimine kadar geniş bir kullanım alanına sahiptir. Bu nedenle, su ekosistemlerinin korunması ve sürdürülebilir yönetimi için bu alandaki yöntemlerin sürekli olarak geliştirilmesi ve uygulanması büyük bir önem taşımaktadır (Poppe vd., 2016).

Türkiye'de son yıllarda su kaynaklarının sürdürülebilir yönetiminin sağlanması amacıyla oldukça önemli adımlar atılmış ve SÇD'de yer alan uygulamalara yönelik çok sayıda çalışma yapılmıştır. SÇD'ye göre durgun su kütlelerinin belirlenmesinde en önemli adımlardan biri olan tipoloji ve hidromorfolojik durum kavramları, su kaynaklarında yapılması planlanan yönetimsel çalışmalarda kullanılmaktadır (EC, 2000).

Hidromorfolojik izleme çalışmaları, yerüstü suları ile ilişkilendirilen su ekosistemlerinin yapı ve işleyişi olarak tanımlanan ekolojik durumun ortaya konulmasında destekleyici unsur olarak kullanılmaktadır (EC, 2000). Hidromorfolojik izleme; nehir, doğal ve yapay göl, kıyı ve geçiş sularında biyolojik ve kimyasal izleme çalışmaları ile eş zamanlı olarak, aynı alan veya temsil eden yakın alanlarda yapılmaktadır (Azlak, 2015; EC, 2000). Hidromorfolojik izleme çalışmalarında doğal ve yapay göl kütlesine ilişkin hidrolojik rejim ve morfolojik koşulları ortaya koyan parametreler izlenmektedir (Anonim, 2023; Azlak, 2015; EC, 2000).

Türkiye'de ise Hidromorfolojik İzleme Tebliği (RG, 2023) kapsamında hidromorfolojik durum belirleme çalışmaları yürütülmektedir. Türkiye'de göllerde ekolojik durumun belirlenmesine yönelik yürütülen çalışmalar dahilinde hidromorfolojik izleme yapılmakla birlikte doğrudan hidromorfolojik durumun belirlenmesine yönelik olarak yapılmış çalışma oldukça sınırlıdır.

Göllerde hidromofolojik durumun tespit edilmesi amacıyla yapılacak uygulamalar için temel adım çalışma alanının tanımlanmasıdır. Göllerde hidromorfolojik çalışmalar göl havzası boyutu ile göl ve gölün etrafında yapılan ve SÇD'de verilen morfolojik koşullar kapsamında yapılan izleme ve değerlendirme çalışmaları olmak üzere iki mekânsal boyutta yürütülmektedir (Azlak, 2015; EC, 2000). SÇD'ye göre, göl hidromorfolojisinin izlenmesi ve değerlendirilmesi çalışmalarında, Tablo 1'de yer alan izleme parametrelerinin kullanılması gerekmektedir (Azlak, 2015; EC, 2000). Göl hidromofolojisi hidrolojik rejim, morfolojik koşullar olmak üzere 2 temel parametreye dayanmaktadır (Azlak, 2015).

Tablo 1. SÇD'ye göre hidromorfolojik izleme parametreleri (Azlak, 2015; EC, 2000)

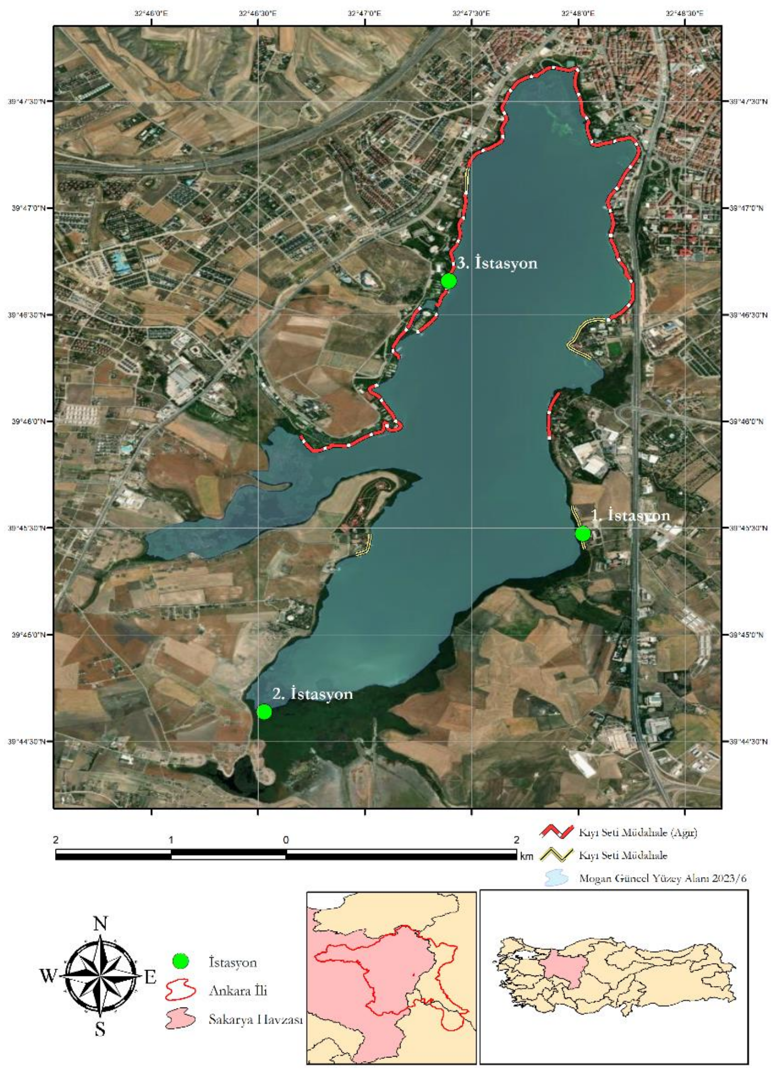
Göl Hidromorfolojisi	
Hidrolojik Rejim	Suyun miktar ve dinamikleri • Karışım ve sirkülasyon deseni
	Yenilenme (bekleme) süresi
	Yeraltı suyu ile bağlantı
Morfolojik Koşullar	
Göl derinlik değişimi • Göl yüzeyi Göl derinliği / hacmi	
Göl yatağının yapısı, dip materyali (substratı) ve miktarı • Materyal boyutu • Su içeriği / yoğunluğu • Element kompozisyonu Sediment yaşı ve oranı	
Göl kıyısının yapısı • Uzunluğu • Kıyı tür kompozisyonu • Bitki kaplaması • Kıyı seti özellikleri	

Hidromorfolojik faktörler ve insan kaynaklı değişiklikler nehirler ve göller üzerinde güçlü etkilere sahip olduğundan, bu değişkenlerin ve etkileşimlerinin anlaşılması tatlı su yönetimi ve restorasyonunda oldukça önemlidir (Kemp ve Sandin, 2022). Mogan Gölü, Ankara'nın yakınında olması dolayısıyla rekreasyonel önemi yüksek olan bir göldür. Eymir Gölü ile birlikte oldukça geniş bir su toplama havzası olan Mogan Gölü, havzadaki değişimlerden ve antropojenik baskılardan etkilenmektedir. Bu çalışmada, Mogan Gölü'nde SÇD'ye göre ekolojik kalitenin belirlenmesine temel teşkil edecek şekilde hidromorfolojik durumun indeks kullanılarak belirlenmesi amaçlanmıştır.

Materyal ve Yöntem

Çalışma alanı

Mogan Gölü, Sakarya Havzası'nda Ankara İl sınırları içinde İl Merkezi'nin 20 km güneyinde, 39˚44'45" ve 39˚47'45" kuzey enlemleri ile 32˚46'30" ve 32˚49'30" doğu boylamları arasında, deniz seviyesinden 973 m yükseklikte yer alır. Alüvyon bir set gölü olan Mogan Gölü yaklaşık 7 km² göl alanına ve ortalama 2,8 m derinliğe sahiptir. Gölün en derin yeri 4 m olup göl çevresinin uzunluğu ise yaklaşık 14 km'dir (Şekil 1) (Anonim, 1995; Pulatsü vd., 2008; Şanal ve Demir, 2018). Mogan Gölü yaz aylarında genellikle kuruyan küçük derelerle beslenmekte olup suyu kuzeydoğusunda bulunan regülatörler ile Eymir Gölü'ne akış göstermektedir. Bu derelerin en önemlileri havzanın doğu-güney-batı kesimlerinde yer alan Sukesen, Başpınar, Gölova, Yavrucak, Çolakpınar, Tatlım, Kaldırım ve Gölcük dereleridir. Mogan Gölü çevresinde geniş sazlıklar ve çok sayıda restoran bulunmaktadır. Göl suyu, beton bir kanal aracılığıyla Eymir Gölü'ne aktarılmakta ve daha sonra İmrahor Deresi ile Ankara Çayı'na boşalmaktadır. Havza genelinde kuru tarım ve mera faaliyetleri yaygın olup, ayrıca çeşitli sanayi tesisleri bulunmaktadır. Bu tesislerin tamamı Mogan Gölü'nün kuşaklama kolektörüne bağlıdır ve zaman zaman kontrolsüz deşarjlar gerçekleşmektedir (Karaaslan vd., 2010; Şanal ve Demir, 2018; Velioğlu ve Kırkağaç, 2017).



Şekil 1. Mogan Gölü kıyı seti modifikasyonları ve izleme istasyonları

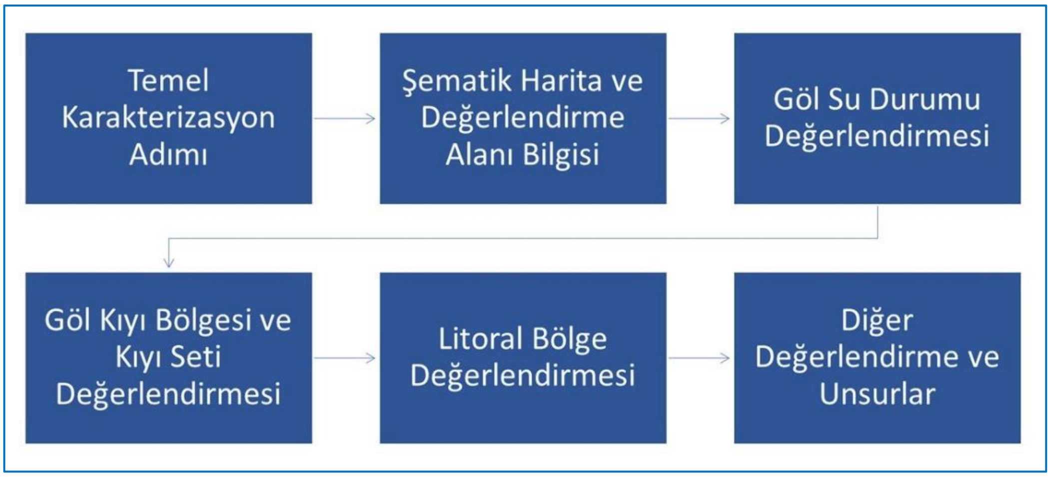
Mogan Gölü çevresindeki sulak ve bataklık alanlar, yoğun kentsel-endüstriyel kirlilik baskısı altında olduğundan ekolojik ve rekreasyonel önemleri göz önüne alınarak, 22 Ekim 1990 tarihinde alınan 90/1117 sayılı Bakanlar Kurulu Kararı ile "Gölbaşı Özel Çevre Koruma Bölgesi" olarak belirlenerek ilan edilmiştir (Anonim, 2024).

Hidromorfolojik izleme

Hidromorfolojik izleme kapsamında, kuru ve ıslak dönemleri temsil edecek şekilde Ekim 2022 ve Mayıs 2023 olmak üzere iki farklı dönemde arazi çalışmaları gerçekleştirilmiştir. Arazi çalışmalarına başlamadan önce, Göl alanının >500 ha olması nedeniyle; gölün genel hidromorfolojik özelliklerini ve rekreasyon ile tarımsal faaliyetlerden kaynaklanan baskıları temsil edecek şekilde üç izleme istasyonu seçilmiştir (Şekil 2).

İzleme çalışmaları kapsamında, hidromorfolojik değerlendirmeye esas veriler standart arazi gözlem formları kullanılarak toplanmıştır (RG, 2023). Ayrıca, izleme noktalarının dışında, göl çevresinin tamamı dolaşılarak yapılan gözlemler ile mümkün olan yerlerde ek veri toplanarak analizlere destek sağlayacak bilgiler elde edilmiştir. Türkiye Göl Hidromorfolojisi Değerlendirme Arazi Formu'nda yer alan hidromorfolojik değerlendirmeye esas bölümler aşağıda verilmiştir (Şekil 3).

Şekil 2. Hidromorfolojik izleme istasyonları sırasıyla 1. İstasyon, 2. İstasyon ve 3. İstasyon



Şekil 3. Türkiye göl hidromorfolojisi değerlendirme indeksi bölümleri (SYGM, 2024)

Coğrafi bilgi sistemleri ile yürütülen hidromorfolojik izleme

Hidromorfolojik izleme çalışmaları kapsamında Mogan Gölü'nde CBS analizleri Göller için Hidromorfolojik İzleme ve Değerlendirme Rehber Dokümanına göre (SYGM, 2024) gerçekleştirilmiştir.

Uzun dönem göl yüzey alanı değişimi

Bu değerlendirme kapsamında göl yüzey alanındaki değişim oranı hesaplanmıştır. Landsat Uydu görüntüleri üzerinden 1985-2024 yılları arasında, göl yüzey alanına ait eski ve güncel görüntüler karşılaştırarak değişim oranı hesaplanmıştır. Göl yüzey alanındaki değişim için geçmiş dönem uydu görüntülerinden gölün maksimum olduğu yüzey alanı ve gölün izleme zamanındaki yüzey alanı sayısallaştırılmıştır.

Gölü besleyen nehirler üzerinde bulunan baraj ve göletlerin gölün su bütçesine ve hidrolojik rejimine etkisi

Bu değerlendirme kapsamında baraj ve gölet toplam drenaj alanının (A), gölün toplam drenaj alanına (B) oranı aşağıdaki formülle hesaplanmıştır.

$(A \div B)*100$

Regülatör mevcudiyeti

Göl çıkışındaki regülatör mevcudiyeti kontrol edilmiştir.

Göl kıyı seti ve sahildeki modifikasyon durumu

Bu değerlendirme kapsamında araziden toplanan bilgiler çerçevesinde uydu görüntüleri kullanılarak kıyı seti ve sahil modifikasyonlarına ilişkin sayısallaştırma yapılmıştır. Sayısallaştırma işleminden sonra modifikasyona uğramış bölümlerin toplam uzunluğu bulunmuş ve bu uzunluk göl kıyı çevresi uzunluğuna bölünerek modifikasyona uğrayan bölümün yüzde oranı hesaplanmıştır.

Göl kıyı kenar bölgesi arazi kullanım durumu

Bu değerlendirme kapsamında göl çevresinin tamamı için göl kıyısından karaya doğru 200 metrelik tampon bölge oluşturulmuştur. Daha sonra tampon bölge içerisinde kalan ve CORINE (Coordination of Information on The Environment) arazi verilerine göre doğal olmayan arazi kullanımlarının (CORINE kodları: Kod 1 ve Kod 211, 212, 213, 221, 222, 223, 241, 242) yüzde oranı hesaplanmıştır.

Göl etrafındaki orman durumu

Bu değerlendirme kapsamında göl çevresinin tamamı için göl kıyısından karaya doğru 200 metrelik tampon bölge oluşturulmuş ve CORINE verilerine göre bu tampon bölge içerisinde kalan orman alanı (CORINE Ana Kod 3) yüzde oranı hesaplanmıştır.

Hidromorfolojik durum değerlendirmesi

Mogan Gölü'nün hidromorfolojik durumunun değerlendirilmesi için Türkiye'de standart metot olarak önerilen Göl Hidromorfolojisi Değerlendirme İndeksi (GHDİ) (SYGM, 2024) kullanılmıştır. Arazi formları ile araziden toplanan veriler, araziden toplanan bilgiler çerçevesinde uydu görüntüleri kullanılarak sayısallaştırılan veriler ve CBS verileri kullanılarak GHDİ ile hidromorfolojik değerlendirme yapılmıştır.

GHDİ kapsamında doğal, modifikasyona uğramamış koşulları temsil eden şartlar için 1'den başlayarak doğallığını kaybetmiş tamamen modifikasyona uğramış koşulları temsil eden şartlar için 5 olacak şekilde her bir parametre için skorlama oluşturulmuş, ayrıca her bir parametre için ayrı ayrı ağırlık katsayısı tanımlanmıştır. GHDİ'nin skorlama sistemi çerçevesinde parametre skorlarının parametre ağırlık katsayısı ile çarpılması ile elde edilen sonuçlar dikkate alınarak hem hidrolojik modifikasyon, morfolojik modifikasyon ve habitat kalitesi skorları hem de hidromorfolojik değerlendirme skorları elde edilmiştir.

GHDİ sonuçları ile SÇD'ye göre beş sınıflı değerlendirme yapılmaktadır. Bu kapsamda değerlendirme skorları "çok iyi", "iyi", "orta", "zayıf" ve "kötü" olacak şekilde bir sınıfa atanmakta ve gölün hidromorfolojik durumu bu beş sınıftan biri olarak belirlenmektedir. Çalışmada değerlendirme skorları iki dönem için ayrı ayrı elde edilmiş gölün nihai durumu iki dönemin aritmetik ortalamasına göre belirlenmiştir.

Bulgular ve Tartışma

Arazi çalışması sonuçları

Hidromorfoloji arazi formu çerçevesinde öncelikle Mogan Gölü için temel karakterizasyon bilgileri toplanmıştır. Temel karakterizasyon adımı kapsamında Mogan Gölü'nün derinliği, vadi tipi, göl büyüklüğü ve gölün bulunduğu alanın iklim karakteristiği değerlendirilmiştir. Mogan Gölü'nün, 5 metreden az derinliği ile sığ, 500 hektardan büyük yüzey alanı ile büyük göl olduğu belirlenmiştir. Bunun yanı sıra gölün sığ vadi tipi yapısı içerisinde konumlandığı ve bulunduğu bölge itibarı ile bozkır kuşağı iklim özelliği göstermeyen ormanlık alanların bulunabileceği bir bölgede yer aldığı tespit edilmiştir.

Göl su durumu değerlendirmesi kapsamında göldeki derinlik değişimi ve gölden su çekimi ve deşarjı olup olmadığına ilişkin bilgiler toplanmıştır. Araziye gidilen dönemde kıyı bölgesindeki izler çerçevesinde gölün derinliğinde 1 metre civarında bir derinlik değişimi olduğu tespit edilmiştir. İlk dönem arazi çalışması sırasında kuru dönemde gölden pompalarla tarla sulaması için su çekildiği tespit edilmiş, ikinci dönem ise bu duruma rastlanmamıştır.

Göl kıyı bölgesi ve kıyı seti değerlendirmesi kapsamında; göl kıyı setinde müdahaleler olduğu tespit edilmiş ve özellikle üçüncü izleme istasyonunda yapılan rekreasyon düzenlemesi sebebiyle beton ve kaya dolgu yapmak suretiyle kıyı setinde ağır modifikasyon gerçekleştirildiği tespit edilmiştir.

Litoral bölge değerlendirmesi kapsamında; mevcut dip materyali olarak sazlıklar başta olmak üzere bitkilerle kaplı organik bir dip materyali yapısı tespit edilmiş bunun dışında silt ve kil yapısı gösteren ince dip materyali, litoral bölgedeki göl dibinde gözlemlenmiştir. Su seviyesindeki değişim ile bağlantılı olarak bir ila iki metre arasında değişen genişlikte kumsal yapısının oluştuğu yerler tespit edilmiştir. Habitat çeşitliliği açısından önem arz eden göl içi makrofitler, odunsu kalıntılar ve göle doğru uzanan ağaç köklerinin gölde mevcut olduğu tespit edilmiştir.

Hidromorfolojik müdahale ve baskı unsuru olarak gölde yoğun iskele varlığı belirlenmiştir. Göl etrafında hayvancılık faaliyeti ve özellikle gölün rekreasyon amaçlı düzenleme yapılan bölgesinde motorlu tekne kullanımı ve çeşitli eğlence amaçlı aktiviteler gerçekleştirildiği gözlemlenmiştir. Gölün bir bölümünde dolgu çalışması yapılarak düzenleme yapıldığı tespit edilmiş olup, gölde genel olarak hafif bir renk değişimi olduğu ve gölün bulanık olduğu belirlenmiştir. Ayrıca gölde kuru dönem olan Ekim ayında hafif koku durumu olduğu da gözlemlenmiştir. Gölden arazi formları ile toplanan bilgiler istasyon ve izleme dönemi bazında Tablo 2'de sunulmuştur.

Tablo 2. Mogan Gölünde izleme dönemlerine göre hidromorfolojik parametreler

Hidromorfolojik Parametreler	**1. Dönem İzleme / Ekim 2022**			**2. Dönem İzleme / Mayıs 2023**		
İzleme istasyonları	1. İstasyon	2. İstasyon	3. İstasyon	1. İstasyon	2. İstasyon	3. İstasyon
Göl Derinlik Değişimi	0,5-<2 m	0,5-<2 m	0,5-<2 m	0,5-<2 m	0,5-<2 m	0,5-<2 m
Su Çekimi	Yoğun Olmayan	Yoğun Olmayan	Yoğun Olmayan	Yok	Yok	Yok
Kıyı Seti Modifikasyonları	Var, <%35	Yok	≥%75	Var, <%35	Var, <%35	≥%75
Ağır Kıyı Seti Modifikasyonu	Var, <%35	Yok	≥%35	Var, <%35	Yok	≥%35
Kıyı Orman Bitki Örtüsü Kaplaması	Orman Kaplaması Yok	Orman Kaplaması Yok	Orman Kaplaması Yok	Orman Kaplaması Yok	Orman Kaplaması Yok	Orman Kaplaması Yok
Orman Bağlantı Durumu	Yok	Yok	Yok	Yok	Yok	Yok
Mevcut Dip Materyali Tipleri	Organik, Silt/Kil	Silt/Kil	Organik, Silt/Kil	Organik, Silt/Kil	Silt/Kil	Organik, Silt/Kil
Dip Materyali Tip Sayısı	Tip Sayısı 3	Tip Sayısı ≤2	Tip Sayısı 3	Tip Sayısı 3	Tip Sayısı ≤2	Tip Sayısı 3
Baskın Dip Materyali Kompozisyonu	Baskın organik	Baskın ince materyal mevcut, (ince materyal >%60)	Baskın organik	Balçık/Çamur	Baskın ince materyal mevcut, (ince materyal >%60)	Baskın organik
Sahil Mevcudiyeti	Yok veya <1 m	1-5 m	Yok veya <1 m	Yok veya <1 m	1-5 m	Yok veya <1 m
Heterojenlik Bileşenleri (HB)	HB >1	HB 1 tane	HB >1	HB 1 tane	HB 1 tane	HB >1
	Odunsu kalıntı, Göl içinde makrofit	Göl içinde makrofit	Odunsu kalıntı, Göl içinde makrofit, Göle doğru uzanan ağaç kökleri	Göl içinde makrofit	Göl içinde makrofit	Odunsu kalıntı, Göl içinde makrofit, Göle doğru uzanan ağaç kökleri
Gölde Uygulanan Faaliyetler	Faaliyet >2	Faaliyet >2	Faaliyet >2	Faaliyet >2	1 veya 2 Faaliyet	Faaliyet >2
	Göl içi ve çevresi bitki ve ağaç kesimi var, İskele var, Motorlu tekne kullanımı var	Göl içi ve çevresi bitki ve ağaç kesimi var, Hayvancılık faaliyeti var	Göl içi ve çevresi bitki ve ağaç kesimi var, İskele var, Motorlu tekne kullanımı var, Turistik faaliyet var	Göl içi ve çevresi bitki ve ağaç kesimi var, İskele var, Hayvancılık faaliyeti var	Hayvancılık faaliyeti var	İskele var, Motorlu tekne kullanımı var, Turistik faaliyet var
Göl İçi Ağır Baskı Unsurları	Unsur yok	Unsur yok	Unsur ≥1	Unsur yok	Unsur yok	Unsur ≥1
			Arazi kazanımı amaçlı arazi dolgu yapımı			Arazi kazanımı amaçlı arazi dolgu yapımı
Göl Niteliği Bozulması (GNB)	GNB≥1	GNB≥1	GNB≥1	GNB≥1	GNB≥1	GNB≥1
	Doğal olmayan renk değişimi var, Gölde koku var, Bulanıklık var	Doğal olmayan renk değişimi var, Gölde koku var, Bulanıklık var	Doğal olmayan renk değişimi var, Bulanıklık var	Bulanıklık var	Doğal olmayan renk değişimi var, Bulanıklık var	Doğal olmayan renk değişimi var, Bulanıklık var
Çöp Mevcudiyeti	Göl içi: Birkaç Parça	Göl içi: Birkaç Parça	Göl içi: Birkaç Parça	Göl içi: Birkaç Parça	Göl içi: Birkaç Parça	Göl içi: Birkaç Parça
	Göl dışı: Birkaç Parça	Göl dışı: Birkaç Parça	Göl dışı: Birkaç Parça	Göl dışı: Birkaç Parça	Göl dışı: Birkaç Parça	Göl dışı: Yok
Uzman Değerlendirmesi	Orta	Orta	Orta	Orta	Orta	Orta

CBS analizi sonuçları

CBS analizleri kapsamında öncelikle uydu görüntüleri ve araziden toplanan bilgiler kullanılarak sayısallaştırma işlemi gerçekleştirilmiştir. Uydu görüntüleri kullanılarak gölün geçmiş dönemlerdeki göl yüzey alanı belirlenmiştir. Bu çerçevede 1985-2024 arası dönemde göl yüzeyinin en fazla olduğu alana sahip uydu görüntüsü sayısallaştırılmıştır. Bunun yanı sıra göl yüzey alanındaki değişimin değerlendirilebilmesi için izlemenin gerçekleştiği dönemdeki uydu görüntüsü kullanılarak mevcut göl su yüzeyi haritalandırılmıştır. Göl su yüzey alanının sayısallaştırılmasının ardından göl kıyı seti için araziden toplanan bilgiler de kullanılarak uydu görüntüleri üzerinden göl kıyı setinde gerçekleştirilen modifikasyonlar CBS ortamında aktarılmış ve ilgili verilerin sayısallaştırılmasının ardından CBS analizleri gerçekleştirilmiştir (Şekil 1).

Göl yüzey alanının uzun dönemli değişimi değerlendirmesi kapsamında, uydu görüntüleri kullanılarak yapılan analiz sonucunda 1985-2024 arası dönem için en geniş yüzey alanı 798 ha olarak hesaplanmıştır. İzlemenin gerçekleştiği dönem için uydu görüntüsü üzerinden yüzey alanı 638 ha olarak hesaplanmış ve Mogan Gölü'nün yüzey alanının yaklaşık %20'lik bir değişimle 158 ha azaldığı ortaya konulmuştur.

Gölü besleyen nehirler üzerinde bulunan baraj ve göletlerin etkisinin tespit edilmesi çerçevesinde CBS ile gölün toplam drenaj alanı (B) 885,3 km^2 olarak hesaplanmıştır. Gölü besleyen kollar üzerinde iki adet baraj ve gölet olduğu tespit edilmiştir. Bu baraj ve göletlerin drenaj alanlarının (A); 149,3 ve 11,56 olmak üzere toplamda 160,9 km^2 olduğu belirlenmiştir. Yapılan analiz sonucunda gölü besleyen nehirler üzerindeki baraj ve göletlerin drenaj alanlarının gölün drenaj alanına oranı %18,2 olarak tespit edilmiştir. Mogan Gölü için regülatör varlığı kontrol edilmiş ve Mogan Gölü'nün çıkışında regülatör olduğu belirlenmiştir. Sayısallaştırılan kıyı modifikasyon verisi kullanılarak mevcut durumda göl çevresi uzunluğu 22,010 km olan Mogan Gölü'nün 9,078 km'lik kıyı setinin modifikasyona uğradığı belirlenmiş ve modifikasyona uğrayan kıyı seti yüzdesi yaklaşık %41 olarak hesaplanmıştır. CORINE arazi verileri kullanılarak gerçekleştirilen analizler sonucunda; göl kıyı bölgesinin %80'inin tarım arazisi ve yapılaşma için kullanıldığı, bunun yanı sıra CORINE arazi verilerine göre göl etrafında orman kaplaması bulunmadığı tespit edilmiştir.

Mogan gölü hidromorfolojik durum değerlendirmesi

Gerçekleştirilen analizler sonucunda, Mogan Gölü'nün hidrolojik modifikasyon, morfolojik modifikasyon ve habitat kalitesi bakımından mevcut durumu ortaya konulmuş ve gölün genel hidromorfolojik sınıflandırması yapılmıştır.

GHDİ kapsamında yapılan analiz ve değerlendirmeler sonucunda Mogan Gölü'nün hidromorfolojik durumu tespit edilmiştir. Yapılan analizler sonucunda Mogan Gölü'nde hidrolojik modifikasyonları gösteren parametreler çerçevesinde hidrolojik modifikasyon skoru birinci izleme dönemi için 3,17, ikinci izleme dönemi için 3,00; morfolojik değişimleri gösteren parametreler için morfolojik modifikasyon skoru birinci ve ikinci izleme dönemi için 3,31 ve habitat kalitesini gösteren parametreler için habitat kalitesi ve niteliği skoru birinci izleme dönemi için 2,83, ikinci izleme dönemi için 3,50 olarak belirlenmiştir.

Mogan Gölü için hidromorfolojik durum skoru ise birinci dönem için 3,125, ikinci dönem için 3,275 olarak hesaplanmış, nihai durum için birinci ve ikinci dönemin ortalaması alınarak hidromorfolojik durum skoru 3,2 olarak belirlenmiştir. Elde edilen skorların indeks kapsamında SÇD ile uyumlu olacak şekilde beş sınıflı değerlendirme skalasına göre değerlendirilmesi sonucunda Mogan Gölü'nün Hidromorfolojik durumu "orta" olarak belirlenmiştir. Hidromorfolojik analiz ve değerlendirmeler sonucunda elde edilen skorlara ve skorlar sonucunda elde edilen durumlara ilişkin özet çizelge aşağıda sunulmuştur (Tablo 3).

Tablo 3. Mogan Gölünün hidromorfolojik değerlendirme sonuçları

Hidromorfolojik Değerlendirme	**1. Dönem İzleme**		**2. Dönem İzleme**		**Nihai Durum**	
	Skor	Durum	Skor	Durum	Skor	Durum
Hidrolojik Modifikasyon Durumu	3,17	Orta	3,00	Orta	3,08	Orta
Morfolojik Modifikasyon Durumu	3,31	Orta	3,31	Orta	3,31	Orta
Habitat Kalitesi ve Niteliği Durumu	2,83	Orta	3,50	Zayıf	3,17	Orta
Hidromorfolojik Durum	3,13	Orta	3,28	Orta	3,20	Orta

Göl hidromorfolojisi üzerine yapılan çalışmalar, nehir ortamlarına kıyasla daha sınırlı bir literatüre sahiptir. SÇD'ye göre hidromorfolojik baskı değerlendirmesi zorunlu olmasına rağmen, genel olarak kabul görmüş tek yöntem yoktur. Avrupa'da, göl hidromorfolojik durumunun izlenmesi ve değerlendirilmesine yönelik başlıca yöntemler arasında İskoçya ve Kuzey İrlanda Çevre Araştırmaları Forumu (SINIFFER) tarafından geliştirilen göllerde Göl Habitat Araştırması (LHS: Lake Habitat Survey) (Rowan vd., 2006a, 2006b, Rowan, 2008) ve Göl Hidromorfolojik Değerlendirme Sistemi (Lake-MimAS: Lake Morphological Impact Assessment System) (Rowan, 2008) öne çıkmaktadır. Håkanson (2005) ve Ostendorp vd. (2004) tarafından yapılan göl kıyısı kalite değerlendirmesi üzerine hazırlanan çalışmalardan sonra, arazi veya CBS verilerinden göl morfolojik koşullarının değerlendirilmesine odaklanan bazı çalışmalar yayınlanmıştır (Bragg vd., 2003; Ostendorp ve Ostendorp, 2015; Rowan vd., 2006a, 2012). LHS yöntemi, özellikle göl ve rezervuar morfolojik koşullarının değerlendirilmesinde yaygın olarak kullanılmış (Ciampittiello vd., 2017), göl veya rezervuar morfolojik koşullarını karakterize etmek için uyarlanarak SÇD kapsamında uygulanabilecek bir yöntem olduğu sonucuna varılmıştır (Kutyla vd., 2021; Latinopoulos vd., 2018). Bu yöntem, göl kıyısı modifikasyonlarının biyolojik topluluklar üzerindeki etkisini incelemek için de sıklıkla kullanılmaktadır. Siligardi vd. (2010) tarafından geliştirilen SFI (Lake Shorezone Functionality Index); Peterlin ve Urbanič (2013) tarafından geliştirilen LMI (Lakeshore Modification Index); Amerika'da, USDS (United States Department of Agriculture) Orman Hizmetleri tarafından hazırlanan Göl Örnekleme Formu (NLF: Lake Sampling Form) (Sullivan vd., 2012) ile göl değerlendirme çalışmalarında kullanılan Göl Değerlendirme Formu (LAsF: Lake Assessment Form) (Baker vd., 1997) hidromorfolojik değerlendirme yöntemlerinden bazılarıdır. Polonya gölleri için LHS yöntemi temel alınarak Göl Habitat Araştırması (LHS_PL) geliştirilmiş ve 2016'dan bu yana Polonya'daki yüzey su kütlelerinin ekolojik durumunun değerlendirilmesinde, göllerin hidromorfolojik incelemesinde kullanılan bir yöntem olarak bildirilmiştir (Kutyla vd., 2021). GHDİ ise T.C. Tarım ve Orman Bakanlığı Su Yönetimi Genel Müdürlüğü tarafından yürütülen çalışmalarla ülkemiz koşullarına uygun parametreler çerçevesinde oluşturulan bir indekstir (Anonim, 2021).

Elde edilen bulgular, Mogan Gölü'nün hidrolojik modifikasyon durumunun "orta", morfolojik modifikasyon durumunun "orta", habitat kalitesi ve niteliğinin "orta" kalite sınıfında olduğunu göstermektedir. Göl Hidromorfolojisi Değerlendirme İndeksi (GHDİ) kullanılarak yapılan analizlerde, Su Çerçeve Direktifi (SÇD) kapsamında beş sınıflı değerlendirme sistemi doğrultusunda gölün genel hidromorfolojik durumunun "orta" seviyede olduğu tespit edilmiştir. Bu değerlendirme, iki izleme döneminin sonuçlarının aritmetik ortalaması alınarak elde edilmiştir. Ekolojik durum değerlendirme çalışmaları çokça olmakla birlikte hidromorfoloji özelinde yapılan çalışma sayısı oldukça sınırlıdır. Mogan Gölü, 2006 yılında yapılan bir çalışmada fitoplankton kalite elementine göre "orta" ekolojik durumda olarak tanımlanmıştır (Demir vd., 2014). Gölün sucul makrofitlere göre ekolojik durumu 2003 yılında orta olarak tahmin edilirken 2013'de kötü olarak bildirilmiştir (Şanal vd., 2015). Epifitik diyatomelere ilişkin hesaplanan farklı indekslere göre ise gölün ekolojik durumunun III. sınıf (orta) ekolojik kalite olduğu bildirilmiştir (Şanal ve Demir, 2018). Mogan Gölü, klorofil *a* derişimi, Secchi derinliği ve toplam fosfor derişimine göre ötrofik olarak bildirilmiştir (Manav ve Yerli, 2008; Yerli vd., 2012). Sakarya Nehir Havza Yönetim Planı kapsamında elde edilen sonuçlara göre ise Mogan Gölü'nün makrofitlere göre durumu biyolojik, fizikokimyasal ve belirli kirleticiler yönünden "orta", hidromorfolojik durum açısından ise "çok iyi hidromorfolojik durum" altı, ekolojik ve nihai durum ise "orta" olarak tespit edilmiştir (Anonim, 2023). Mogan Gölü'nde biyolojik kalitenin ötrofikasyon ve restorasyon çalışmaları ile uyumlu zamansal değişimler gösterdiği, hidromorfolojik durumunda gölün trofik durumu ve biyolojik durumu ile benzerlik gösterdiği sonucuna varılmıştır.

Sonuç

Bu çalışmada, Mogan Gölü'nün güncel hidromorfolojik durumunun ortaya konulması için detaylı hidromorfolojik izleme ve değerlendirme çalışması gerçekleştirilmiştir. Çalışma kapsamında, ıslak ve kuru dönemleri temsil edecek şekilde Ekim 2023 ve Mayıs 2024 aylarında iki farklı arazi çalışması yürütülmüştür. Arazi gözlemleri, uydu görüntüleri analizleri ve Coğrafi Bilgi Sistemleri (CBS) uygulamaları ile gerçekleştirilen analizler doğrultusunda göl ekosistemindeki değişimler ve insan etkileri değerlendirilmiştir. Mogan Gölü'nde gerçekleştirilen hidromorfolojik değerlendirmeler, gölün su dengesinin ve fiziksel yapısının ekosistem sağlığı üzerindeki etkilerini ortaya koymuştur.

Çalışmanın sonucuna göre, Mogan Gölü'nün hidromorfolojik açıdan kısmen değiştirilmiş olduğu, su rejimi, kıyı morfolojisi ve habitat özelliklerinin ise insan faaliyetlerin etkisi altında olduğu değerlendirilmiştir. Bu durum, gölün doğal ekosistem işleyişini kısmen sınırlamakta ve ekosistem hizmetlerinin sürdürülebilirliğini tehdit etmektedir. Çalışma kapsamında gerçekleştirilen uydu görüntüleri ve CBS analizleri, göldeki yapay müdahalelerin ve doğal süreçlerdeki değişimlerin sayısallaştırılması açısından katkı sunmuştur.

Gerçekleştirilen çalışma, Mogan Gölü'nün mevcut hidromorfolojik durumunu belirleyen güncel ve kapsamlı bir değerlendirme sağlamaktadır. Çalışma bulgularının, gölde uygulanabilecek ekosistem temelli yönetim stratejilerinin belirlenmesine katkı sağlayacağı düşünülmektedir. Göl ekosisteminin iyileştirilmesi için su rejiminin doğal işleyişine daha yakın hale getirilmesi, kıyı ve bentik habitatların korunması ve restorasyon çalışmaları ile desteklenmesi önerilir. Ayrıca, hidromorfolojik izleme çalışmalarının sürekliliğinin sağlanması, uzun vadeli değişimlerin izlenmesi açısından uygun yönetim stratejilerinin geliştirilmesinde kritik bir gereklilik olarak değerlendirilmektedir.

Çıkar Çatışması

Yazarlar çıkar çatışması olmadığını beyan ederler.

Yazar Katkıları

D. Coşkun ve N. Demir araştırmayı planladı, D. Coşkun, M. Azlak hidromorfolojik arazi çalışmasını gerçekleştirdi, A. Uğurluoğlu CBS değerlendirmesine yardımcı oldu. Tüm yazarlar araştırmanın veri toplanması, verilerin analizleri ve sonuçların değerlendirilmesi ile makalenin yazım sürecinde görev almışlardır.

Etik Onay

Bu çalışma için etik kurul iznine gerek yoktur.

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COMU J Mar Sci Fish 2025 8(1): 75-86
https://doi.org/10.46384/jmsf.1658346
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Sustainable Crab Fishing with Rakkang (Stick Dip Nets): Catch Performance and Socio-Economic Evaluation in Tanah Laut, Indonesia

Ahmadi Ahmadi

Department of Capture Fishery, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarbaru, Indonesia https://orcid.org//0000-0001-9691-9107

Received: 15.03.2025 /Accepted: 23.05.2025 / Published online: 14.07.2025

Keywords:

Crab fishery
Productivity
Sustainability
Indonesia
Blue economy

Abstract: This study aims to evaluate the catch performance and socio-economic relevance of traditional stick dip nets, by comparing two types, *Rakkang*-A in Muara Kintap and *Rakkang*-B in Bawah Layung, Tanah Laut Regency, Indonesia. The fieldworks were conducted between September and October 2024. A total of 580 *Rakkang* were deployed in the mangrove areas, with sample size: *Rakkang*-A (n = 73 lifts, 10.43±0.04) and *Rakkang*-B (n = 71 lifts, 10.14±2.89). The crab catch data on *Scylla serrata* and *S. olivacea* were collected, and statistical analysis was performed to determine significant differences in catch rates. Results showed that the two *Rakkang* types effectively captured both *S. serrata* and *S. olivacea*, but with species-specific preferences. *Rakkang*-A was more effective for *S. olivacea*, while *Rakkang*-B yielded higher catches of *S. serrata*. Catch per unit effort (CPUE) analysis revealed that *Rakkang*-A had a higher CPUE for *S. olivacea* (0.14 ind/trap) compared to *Rakkang*-B (0.05 ind/trap). Conversely, both *Rakkang* types performed equally for *S. serrata* (0.16 ind/trap). Overall, CPUE of *Rakkang*-A (0.30±0.03) was approximately 1.34 times greater than that of *Rakkang*-B (0.22±0.05). The bait selection played a crucial role, with *S. olivacea* being more attracted to fish bait, whereas *S. serrata* preferred crab bait. These findings highlight the importance of selecting the appropriate *Rakkang* type and bait for optimizing crab fishing efficiency. Further research on environmental factors and gear modifications could enhance sustainability and economic viability for local fishers.

Anahtar kelimeler:

Yengeç balıkçılığı
Üretkenlik
Sürdürülebilirlik
Endonezya
Mavi ekonomi

Rakkang (Katlanabilir Sepet) ile Sürdürülebilir Yengeç Balıkçılığı: Endonezya, Tanah Laut'ta Yakalama Performansı ve Sosyo Ekonomik Değerlendirme

Öz: Bu çalışma, Endonezya, Tanah Laut Regency, Muara Kintap'taki Rakkang-A ve Bawah Layung'daki Rakkang-B olmak üzere iki türü karşılaştırarak, geleneksel katlanabilir sepetlerin yakalama performansını ve sosyo-ekonomik önemini değerlendirmeyi amaçlamaktadır. Saha çalışmaları Eylül ve Ekim 2024 arasında yürütülmüştür. Mangrov alanlarına toplam 580 Rakkang konuşlandırılmış olup, örneklem büyüklüğü şu şekildedir: Rakkang-A (n = 73 kaldırma, 10,43 ± 0,04) ve Rakkang-B (n = 71 kaldırma, 10,14 ± 2,89). Scylla serrata ve S. olivacea'da yengeç yakalama verileri toplanmıştır ve yakalama oranlarındaki önemli farklılıkları belirlemek için istatistiksel analiz yapılmıştır. Sonuçlar, iki Rakkang türünün hem *S. serrata*'yı hem de *S. olivacea*'yı etkili bir şekilde yakaladığını, ancak türlere özgü tercihlerin olduğunu göstermiştir. Rakkang-A, *S. olivacea* için daha etkiliyken, Rakkang-B daha fazla *S. Serrata*'yı avlamıştır. Birim çaba başına yakalama (CPUE), Rakkang-A'nın *S. olivacea* için (0,14 ind/tuzak) Rakkang-B'ye (0,05 ind/tuzak) kıyasla daha yüksek bir CPUE'ye sahip olduğunu ortaya koydu. Tersine, her iki Rakkang tipi de *S. serrata* için eşit performans gösterdi (0,16 ind/tuzak). Genel olarak, Rakkang-A'nın CPUE'si (0,30 ± 0,03), Rakkang-B'den (0,22 ± 0,05) yaklaşık 1,34 kat daha fazlaydı. Yem seçimi önemli bir rol oynadı; *S. olivacea* balık yemine daha çok çekilirken, *S. serrata* yengeç yemini tercih etti. Bu bulgular, yengeç avcılığının verimliliğini optimize etmek için uygun Rakkang tipini ve yemini seçmenin önemini vurgulamaktadır. Çevresel faktörler ve ekipman değişiklikleri üzerine daha fazla araştırma yapılması, yerel balıkçılar için sürdürülebilirliği ve ekonomik uygulanabilirliği artırabilir.

Introduction

Rooted in the traditions of a coastal community, *Rakkang* (stick dip net) is a time-honored fishing gear particularly effective for catching crabs. It consists of a circular bamboo frame enclosing a net, anchored to the seabed by a central wooden pole. The *Rakkang* is equipped with bait that releases an odour that stimulates the olfactory senses of crabs, drawing them into the trap. Once inside, the net effectively confines them, enabling fishermen to harvest them without causing physical damage and mortality. It offers a sustainable approach to harvesting the crabs while minimizing environmental impact (Supeni et al., 2020).

*Corresponding author: ahmadi@ulm.ac.id

How to cite this article: Ahmadi, A (2025). Sustainable Crab Fishing with Rakkang (Stick Dip Nets): Catch Performance and Socio-Economic Evaluation in Tanah Laut, Indonesia. COMU J. Mar. Sci. Fish, 8 (1): 75-86. doi:10.46384/jmsf.1658346

The traditional use of *Rakkang* reflects global patterns in small-scale fisheries (SSF), which are vital to food security, livelihoods, and sustainable resource use. According to the Illuminating Hidden Harvests (IHH) report (FAO, Duke University & WorldFish, 2023), SSFs contribute over 40% of global fish catches and support 492 million people, especially in developing countries. Yet, despite their importance, practices like *Rakkang* remain under-researched, particularly in terms of catch performance and socio-economic impact. Integrating such traditional practices into the blue economy framework, which emphasizes inclusive, sustainable, and economically viable use of ocean resources, can enhance policy relevance while promoting equitable benefits for coastal communities (Patil et al., 2016).

To support such integration, a clearer understanding of the technical and functional aspects of *Rakkang* is essential. The gear typically consists of a circular frame, a conical net body, and a central anchoring pole. The frame, made of bamboo, rattan, or metal, provides structural support, while the polyethylene (PE) net functions as an effective trap for the target species. A central bamboo pole stabilizes the gear and positions bait hooks near the entrance, keeping the net properly tensioned (Rosalina and Utami, 2021).

Rakkang is an efficient fishing method that requires minimal effort and simple tools. It can be folded, stacked and carried in large numbers when in use. However, successful *Rakkang* fishing requires a deep understanding of several key factors, including strategic trap placement, precise timing that coincides with the target species' behavior, and the selection of highly effective bait (Wijaya et al., 2010; Kabalmay et al., 2017; Diana et al., 2018).

Fishermen must carefully select locations frequented by the target species to maximize their catch (Afriani et al., 2024). *Rakkang* is specifically designed for operation in shallow waters, where it can be easily deployed and retrieved. The traps are usually spaced 15-30 m apart and soaked overnight to maximize efficiency. This makes it a practical tool for small-scale fishing operations (Hanafi et al., 2019). *Rakkang* is typically deployed in mangrove ecosystems with muddy or sandy substrates. These environments provide ideal habitats for mud crabs of the genus Scylla such as *Scylla Serrata*, *S. tranquebarica*, *S. paramamosain*, and *S. olilvaces* (Fazhan et al., 2022). These crabs are highly valued as a food source and play a significant role in the local economy (Abidin et al., 2022).

Understanding the biological behavior of crabs as a target species is crucial in the *Rakkang* fishery (Tahmid et al., 2015). Timing trap deployments to coincide with peak crab feeding activity is paramount (Rosalina and Utami, 2021). This maximizes encounter rates between crabs and the traps, increasing the likelihood of capture. Moreover, selecting the most attractive bait is crucial, as it directly influences crab foraging behavior and ultimately determines the success of the fishing operation (Haqie et al., 2024).

Rakkang exemplifies environmentally friendly fishing practices by utilizing recycled materials and minimizing its impact on the marine ecosystem. This aligns with the increasing recognition of the importance of environmentally responsible fishing practices (Yulisti et al., 2024). Despite adhering to fundamental principles, *Rakkang* designs and construction methods exhibit variations that often reflect the unique needs and preferences of individual fishing communities (Supeni et al., 2020). This study aims to evaluate the catch performance and socio-economic implications of *Rakkang* use, contributing empirical evidence to both national and global discussions on sustainable small-scale fisheries.

Material and Methods

Study site

Two paralel studies were carried out in the fishing villages of Muara Kintap (3°88'62"S, 115°24'33"E) and Bawah Layung (3°67'40"S, 114°62'87"E), located in Tanah Laut Regency, South Kalimantan, Indonesia (Figure 1). These villages, home to diverse ethnic groups including Banjar, Javanese, and Bugis, primarily rely on fishing and fish processing for their livelihoods. The fieldworks were conducted between September and October 2024, and did not encompass a full lunar or monsoon cycle; therefore, potential temporal bias was acknowledged. This study was supported by the Collective business groups, namely KUB Dermaga Bersama in Muara Kintap and KUB Bina Harapan Bersama in Bawah Layung. During the study period, physical oceanographic conditions included water temperature of 28.5-29 °C, water brightness of 45-60 cm, the current speed of 0.05-0.17 m s^{-1}, and salinity level of 5-10 ppt.

Data collection

Two distinct variations of traditional fishing gears: *Rakkang*-A, as practiced in Muara Kintap Village, and *Rakkang*-B, as employed in Bawah Layung Village, were investigated. A detailed summary of the distinctive characteristics of each *Rakkang* types are presented in Table 1. The performance of *Rakkang* itself was visualized in Figure 2, with the following explanations:

Frame Construction

The frames of both *Rakkang* are constructed from bamboo, a sturdy and lightweight material. The upper and bottom parts of the frames differ in size. In Muara Kintap, both the upper and bottom parts measure 510 mm, providing a consistent design. In Bawah Layung, the upper part measures 340 mm, and the bottom part is slightly larger at 410 mm, indicating a variation in structure likely suited to local fishing conditions.

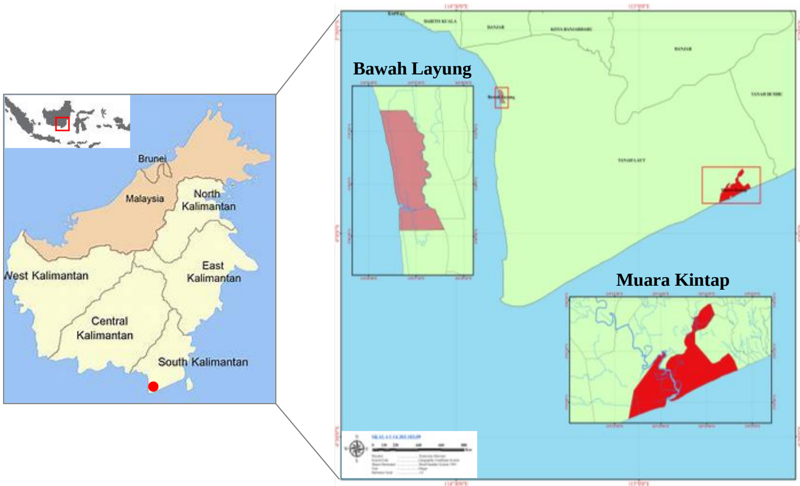

Figure 1. Geographic locations of Muara Kintap and Bawah Layung villages in Tanah Laut Regency, Indonesia, were the *Rakkang* were deployed

Net body

Both *Rakkang* types utilize a PE net body, which is favored for its durability and water resistance. However, they differ in mesh sizes: Muara Kintap uses a 50.8 mm mesh, while Bawah Layung incorporates a larger 63.5 mm mesh.

Entrance

The entrance materials and dimensions differ between the two villages. Muara Kintap uses green mesh with a fine 10 mm mesh size, while Bawah Layung employs PE with a significantly larger 38 mm mesh size. In Muara Kintap, the entrances are wider (240 mm) and taller (115 mm) compared to those in Bawah Layung (220 mm wide and 100 mm high). The slit entrance, which facilitates crab entry, is designed to be slightly narrower in Bawah Layung (70 mm) than in Muara Kintap (80 mm).

Support pole

Both villages utilize bamboo support poles to stabilize the gear and ensure the net opening remains secure. Their lengths and diameters vary slightly. Muara Kintap utilizes longer poles (1550 mm) with a 16 mm diameter, while Bawah Layung employs shorter poles (1450 mm) but with a thicker diameter of 18 mm.

Bait clip

The bait clip in Muara Kintap measures 250 mm in length, while in Bawah Layung, it is slightly shorter at 230 mm. Both locations utilize thin bamboo slices (20 mm) to enhance the bait clip's flexibility and strength.

Binding rope

Binding ropes are crucial for securing the gear components. Both sites use PE ropes, but the lengths vary significantly. Muara Kintap uses 820 mm ropes, while Bawah Layung uses shorter ropes of 540 mm. Both ropes share a consistent diameter of 3 mm, balancing strength and ease of handling.

Support ring

Support rings, made from polyethylene, serve to reinforce the gear structure. In Muara Kintap, the rings measure 25 mm in diameter, whereas those in Bawah Layung are slightly larger at 30 mm.

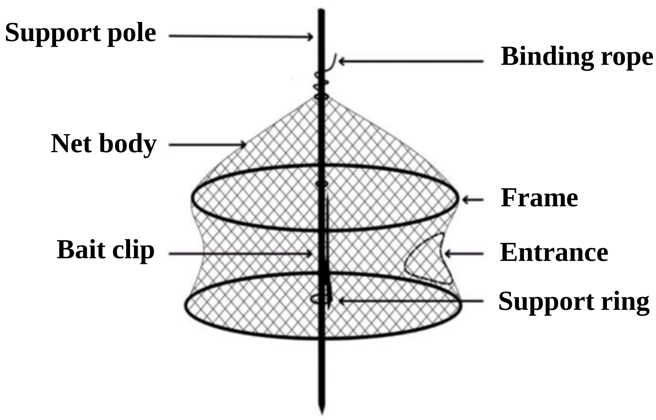

Figure 2. Illustration of the *Rakkang* used in Muara Kintap and Bawah Layung villages

Tabel 1. Technical specification of *Rakkang* used in Muara Kintap and Bawah Layung

No	Gear Construction	Location	
		Muara Kintap	Bawah Layung
1	Frame		
	- Material	Bamboo	Bamboo
	- Upper part (mm)	510	340
	- Bottom part (mm)	510	410
2	Net body		
	- Material	Polyethylene	Polyethylene
	- Mesh size (mm)	50.8	63.5
3	Entrance		
	- Material	Green mesh	Polyethylene
	- Mesh size (mm)	10	38
	- Width (mm)	240	220
	- Height (mm)	115	100
	- Slit entrance (mm)	80	70
4	Support pole		
	- Material	Bamboo	Bamboo
	- Length (mm)	1550	1450
	- Diameter (mm)	16	18
5	Bait clip		
	- Material	Bamboo	Bamboo
	- Length (mm)	250	230
	- Thin bamboo slice (mm)	20	20
6	Binding rope		
	- Material	Polyethylene	Polyethylene
	- Length (mm)	820	540
	- Diamter (mm)	3	3
7	Support ring		
	- Material	Polyethylene	Polyethylene
	- Diameter (mm)	25	30

The research steps for *Rakkang* fishing are include (1) Pre-operation: Preparing the bait, attaching the bait to the clip attached to the pole, preparing the boat and loading the *Rakkang* onto the boat. (2) Operation: Assembling the *Rakkang*, anchoring and soaking them in the water, then lifting the catch and detaching the pole from the *Rakkang*. (3) Post-operation: Removing the catch from the *Rakkang*, measuring their sizes, tying each catch individually, cleaning the *Rakkang*, and transporting the catch to the local collector, as well as maintaining the *Rakkang* properly for reuse. According to fishermen, *Rakkang* typically remains functional for two years.

In Muara Kintap, fishermen operated the *Rakkang* using 1 GT wooden boats (L8×B1.2×D0.6 m) powered by Honda GFK engines (diesel/pertalite). In contrast, in Bawah Layung, fishermen employed larger 1.5 GT vessels (L11.5×B1.52×D0.7 m) propelled by Jiang Fa (JF) 26 HP diesel engines. A total of 580 *Rakkangs* (245 units for Muara Kintap and 335 units for Bawah Layung) were deployed in mangrove areas, with depths between 0.40 and 0.65 m, during the period from 4 pm to 7 am. The *rakkang* were hauled in the morning, starting from the first installed to the last. The process begins by lifting and shaking the support sticks in water to remove mud. The equipment is then brought onto the boat, the binding ropes are loosened, and the support sticks are removed. Any remaining bait is discarded. This process is repeated until all *rakkang* are collected.

The daily operational periods for *Rakkang* are outlined in Table 2. Selecting the most attractive bait is crucial.

Two distinct types of bait were selected for crab fishing, namely: *Plicofollis tonggol*, which was used in Muara Kintap, and *Parathelphusa convexa*, was the favored chosen in Bawah Layung. The locations where Scylla crabs were recorded using GPS coordinates, and each crab was measured for carapace width and length. The crabs were then sold to the local market. Prices were initially recorded in Indonesian Rupiah (IDR) and later converted to US Dollars (USD) using the average exchange rate for October 2024, which was approximately IDR 15,690 per 1 USD. Research activities are visualized in Figure 3.

Table 2. The daily operational time periods for *Rakkang* in Muara Kintap and Bawah Layung

Site	Trip	Setting	Soaking	Hauling	Total time (h)
Muara Kintap	1	07:25 - 07:57	07:57 - 13:54	13:54 - 14.23	8
	2	16:10 - 17:24	17:24 - 06:00	06:00 - 07:15	15
	3	16:21 - 17:03	17:03 - 06:07	06:07 - 07:03	15
	4	17:04 - 18:33	18:33 - 06:54	06:54 - 07:59	15
	5	18:49 - 19:51	19:51 - 06:15	06:15 - 07:48	13
	6	16:28 - 17:08	17:08 - 06:03	06:03 - 06:52	14
	7	16:06 - 16:24	16:28 - 06:13	06:13 - 06:32	14
Bawah Layung	1	17.39 - 19.02	19.02 - 05.45	05.45 - 07.22	14
	2	17.05 - 19.25	19.25 - 05.38	05.38 - 07.38	15
	3	17.10 - 19.57	19.57 - 05.44	05.44 - 07.30	14
	4	17.15 - 20.00	20.00 - 06.01	06.01 - 08.02	15
	5	17.12 - 20.05	20.05 - 05.33	05.33 - 07.45	15
	6	17.05 - 19.48	19.48 - 05.33	05.33 - 08.00	15
	7	17.22 - 19.59	19.59 - 05.23	05.23 - 07.45	14

Data analysis

Subsequent to data collection, the proportion of each crab type, expressed as a percentage, was determined using a standardized formula (Anggreini et al., 2017):

$$Pi = \frac{ni}{N} \times 100\ \%$$

where *Pi* denotes the relative abundance of catch (%), *ni* signifies the number of catches for species i (ind.), and *N* represents the total catch (ind.). The productivity of each *Rakkang* was subsequently estimated using the following formula (Dahle, 1989):

$$P = \frac{c}{t}$$

where *P* is the productivity, *C* is total daily catch (ind.), and *t* is effective fishing time (h)

Statistical analysis

Data analysis was performed using Microsoft Excel or SPSS version 18. Descriptive statistics, including mean ± standard error (SE) and percentages, were computed and presented in both graphical and tabular formats. Prior to inferential testing, the Shapiro–Wilk test was applied to assess the normality of continuous data. When data met normality assumptions, independent sample t-tests were used to compare catch number, CPUE, and productivity rate between crab types, and between the two Rakkang designs. Statistical significance was established at the $p<0.05$ level.

The lagoon, belonging to the Strait of Messina ecosystem (Fig. 1), is subject to various protection regimes, in the framework of the Natura 2000 network (cod. ITA 030008). It includes two connected but differently featured basins, i.e., Lake Faro, known for its notable depth and a peculiar meromictic regime (Leonardi et al., 2009), and the markedly brackish Lake Ganzirri (Azzaro et al., 2005). On September 22th, 2024, in the morning, during an excursion along Lake Ganzirri, Mrs. Lucrezia Pietramala had the opportunity to observe a sepecimen of *L. surinamensis* in a clam farming area (38°15'29" N; 15°36'38"E).

Figure 3. The photographs of the *Rakkang* used in Muara Kintap and Bawah Layung

Results and Discussion

Rakkang has demonstrated significant effectiveness in capturing both giant mud crab (*S. serrata*) and orange mud crab (*S. olivacea*) within the two villages (Figure 4). In Muara Kintap, *S. serrata* displayed a narrower carapace (102±0.4 mm) than *S. olivacea* (89±1.3 mm), although their caparace lengths were similar. The respective weights of the two species were 247 g and 184 g. In contrast, *S. olivacea* in Bawah Layung had a significantly broader carapace (124±0.5 mm) than *S. serrata* (96.5±1.4 mm). While there was some overlap in carapace length between the two species, *S. olivacea* generally exhibited a slightly longer carapace (81±0.2 mm) compared to *S. serrata* (65±1.1 mm). This trend was also reflected in their weights, with *S. olivacea* and *S. serrata* weighing 300 g and 200 g, respectively.

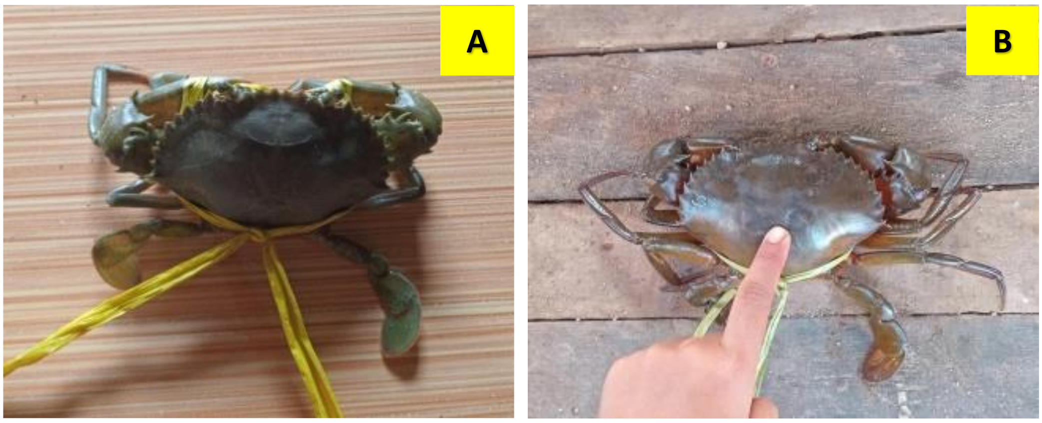

Figure 4. The main catch of *Rakkang*: A. *Scylla serrata*, and B. *Scylla olivacea*
Sample size: *Rakkang*-A (*n* = 73 lifts, 10.43±0.04) and *Rakkang*-B (*n* = 71 lifts, 10.14±2.89)

A more in-depth analysis was performed to investigate the relationship between *Rakkang* usage and daily catch at both sites during the fishing period. While the catch fluctuates, the use of *Rakkang* remains steady in Muara Kintap, unlike in Bawah Layung where it varies (Figure 5). The actual daily catch in both locations consistently falls short of what would be expected based on the number of *Rakkang* employed. This discrepancy suggests that other factors such as environmental conditions or crab abundance, are likely influencing the catch. Moreover, both locations are accessible to both local and outside fishermen. Further investigation is needed to comprehend the dynamics of this relationship fully.

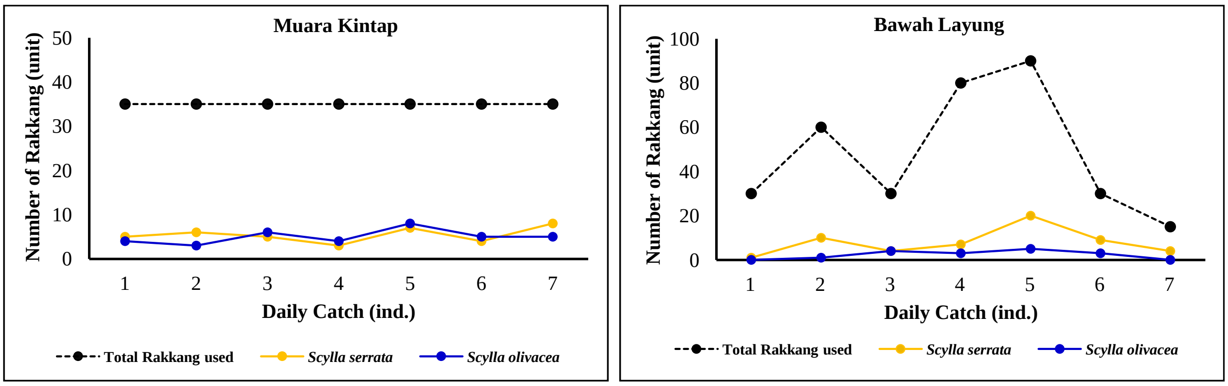

Figure 5. Comparison of *Rakkang* catches in Muara Kintap and Bawah Layung villages

The comparative analysis indicates that both *Rakkang* types exhibit similar effectiveness, with only slight variations in catch numbers, productivity, and fishing duration (Table 3). The findings highlight the adaptability of *Rakkang* to different conditions in Muara Kintap and Bawah Layung, offering insights into optimizing gear use for improved efficiency. Overall, there were no significant differences in the catch number and productivity rate between the two *Rakkang* types within the given time period ($p > 0.05$). The proportion of the total catch attributed to each *Rakkang* type shows a near-equal distribution. *Rakkang*-A contributed 48.9% of the total catch, while *Rakkang*-B accounted for 51.1%. This minor difference suggests that both *Rakkang* types are comparably effective, with *Rakkang*-B (0.755 ind/h) was slightly higher productivity compared to *Rakkang*-A (0.723 ind/h).

Table 3. Comparison of *Rakkang* effectiveness based on the productivity rate

Rakkang	Total catch (ind.)	Duration (h)	Productivity (nd/h)	Proportion (%)
A	73	101	0.723	48.9
B	71	94	0.755	51.1
Total	144	195	1.478	100

The comparative analysis of *Rakkang* CPUE based on crab species caught revealed that *Rakkang*-A was significantly more efficient for *S. olivacea*, with a CPUE of 0.14 ind/trap compared to 0.05 ind/trap for *Rakkang*-B, represent 74% of the total *S. olivacea* catch. Dealing with *S. serrata*, both *Rakkang*-A and *Rakkang*-B performed equally, with a CPUE of 0.16 ind/trap, each contributing 50% to the total *S. serrata* catch (Table 4), possibly due to the species' less selective behavior or broader habitat range compared to *S. olivacea*.

Table 4. Comparison of *Rakkang* CPUE and its proportion based on crab species caught

Scylla serrata	Total catch (ind.)	Σ *Rakkang* (trap)	CPUE (ind/trap)	Proportion (%)
Rakkang-A	38	245	0.16	50
Rakkang-B	55	335	0.16	50
Total	93	580	0.32	100
Scylla olivacea				
Rakkang-A	35	245	0.14	74
Rakkang-B	16	335	0.05	26
Total	51	580	0.19	100

The effectiveness of *Rakkang* in catching Scylla crabs in Muara Kintap (*Rakkang*-A) and Bawah Layung (*Rakkang*-B) was presented in Table 5. A total of 93 specimens of *S. serrata* were caught across both sites, with *Rakkang*-B proved to be better than *Rakkang*-A. The productivity rate of *Rakkang*-B for *S. serrata* (0.585 ind/h) was significantly higher than *Rakkang*-A (0.376 ind/h). *Rakkang*-B made up 61% of the total catch, whereas

Rakkang-A accounted for 39%. This responds with the greater number of *Rakkang* employed. Conversely, *Rakkang*-A was more effective in catching *S. Olivacea* than *Rakkang*-B. The productivity rate of *Rakkang*-A (0.347 ind/h) was twice as high as that of *Rakkang*-B (0.170 ind/h). The proportion of total catch also reflects this trend, with *Rakkang*-A contributing 67% and Rakkang-B only 33%. These findings suggested that different *Rakkang* types perform better for different crab species, likely due to variations in their deployment or the selection of bait used.

Regarding the profitability metrics, confidence intervals for net profit per day could not be directly calculated due to incomplete data on operational costs such as fuel, bait, and labor.

Table 5. Comparison of *Rakkang* productivity based on crab species caught

Scylla serrata	**Total catch (ind.)**	**Duration (h)**	**Productivity (ind/h)**	**Proportion (%)**
Rakkang-A	38	101	0.376	39
Rakkang-B	55	94	0.585	61
Total	93	195	0.961	100
Scylla olivacea				
Rakkang-A	35	101	0.347	67
Rakkang-B	16	94	0.170	33
Total	51	195	0.517	100

Discussion

A key advantage of *Rakkang* trap is its minimal impact on the captured crabs. Being a passive method, it reduces stress and physical damage compared to other fishing methods such as trawl net and trammel net. The active use of the *Rakkang* involves lifting and resetting it, as failure to do so increases the risk of crabs escaping. Several factors need to be considered for the success of *Rakkang* fishing operation. First, select the right installation location. The *Rakkang* should be set near crab holes/burrows, in mild weather with sea-to-land winds. Strong winds frequently lead to overnight high tides, drawing crabs out of their burrows in search of food (Rusmilyansari et al., 2021). A crucial element of *Rakkang* is a central wooden pole that anchors the trap to the muddy soil, keeping it stable and upright against currents. Its conical net guides target species towards the center. *Rakkang* should not be set in areas with strong currents, as the bait may be carried away, and the rakkang could shift slightly due to nighttime tides and the soft, muddy soil in the mangrove area. The loss of bait and the shifting are beyond the fishers' expectations.

Secondly, the timing of trap installation should align with the biological cycle or feeding patterns of the target species. Synchronizing trap deployment with the crabs' active feeding periods increases the chances of a successful catch per unit effort (Rosalina and Utami, 2021). Understanding crab behavior helps fishermen target specific areas and times, minimizing the impact on non-target species and the broader ecosystem (Tahmid et al., 2015). Thirdly, selecting the appropriate bait, including its type and freshness, is critical to enhance crab catch efficiency (Gustiawan et al., 2018; Haqie et al., 2024). It is essential to securely attach the bait from end to end to prevent it from being swept away. Also, regularly check the *Rakkang* traps, particularly the netting, for damage that may allow crabs to escape.

Fourth, there is considerable interest in improving catchability by choosing the right materials particularly for trap entrances. In Muara Kintap, slit entrance is made of green mesh, while in Bawah Layung, it is constructed from polyethylene. For trap entrances, green mesh is generally the better choice due to its natural camouflage, flexibility, and cost-effectiveness compared to polyethylene. It helps attract crabs into the trap without deterring them, resulting in maximizing catch efficiency. Polyethylene, while durable, is better suited for structural parts of the trap rather than the entrance. If environmental sustainability is a priority, consider using biodegradable or recyclable polyethylene materials to minimize ecological impact. However, if cost and ease of use are more important, green mesh is a practical and effective option. Additionally, combining the two materials (e.g., using polyethylene for structural strength and green mesh for the trapping area) could offer a balanced solution. A single-entrance trap featuring green netting at the entrance has also proven effective in the swimming crab fishery, as demonstrated by Tran et al. (2020).

The highest catch proportion of *S. serrata* in Bawah Layung is similarly documented in the Central Tapanuli District, North Sumatra (Larosa et al., 2013). In contrast, *S. olivacea* is identified as the dominant species in Muara Kintap, as well as in Terengganu, Malaysia (Fazhan et al., 2022). The average CPUE for crabs in our study (0.16 ind/trap) is relatively lower than the CPUE for Scylla crabs (0.80 ind/trap) in Terengganu, Malaysia

(Fazhan et al., 2022). Regarding bait usage, the results showed that *Rakkang*-A is more effective for targeting *S. olivacea*, while *Rakkang*-B performs slightly better for *S. serrata*. This suggests that *S. olivacea* is more attracted to fish bait, whereas *S. serrata* shows a preference for crab bait. This aligns with Haqie et al. (2024), who highlighted the importance of bait selection in optimizing catch efficiency and reducing ecological impact. While *S. serrata* is associated with dense mangroves, *S. olivacea*, as an intertidal species, is more easily located in mangrove areas because of its limited movement within a specific home range and its habit of returning to the same area after foraging. This behavior is beneficial for trap deployment, enhancing the chances of a successful catch. The movement of mud crabs in mangrove areas, tracked using telemetry systems, is further detailed in studies by Ikhwanuddin et al. (2012) and Fazhan et al. (2022). According to Putri et al. (2022) *S. olivacea* exhibits a greater adaptation rate compared to *S. serrata* within this mangrove ecosystem. While *S. serrata* is prefered species for mud crab farming (Quinitio & Parado-Estepa, 2017).

The use of *rakkang* traps in this study likely had minimal ecosystem impact due to their lightweight design and passive operation. Although some contact with the seabed occurs, these traps are not actively dragged and thus are unlikely to cause significant benthic disturbance. Moreover, the risk of ghost fishing is considered low, as the traps are typically retrieved daily and are constructed with open entryways that reduce continued entrapment of organisms if lost. Similar findings have been reported for other passive gear types, such as crab pots and fish traps, which show limited impact on benthic habitats and low ghost fishing persistence when properly managed (Rijkure et al., 2024). Nevertheless, routine monitoring and lost gear retrieval programs are recommended to minimize potential long-term ecological risks.

The crab prices in Muara Kintap and Bawah Layung vary significantly according to category, sex, weight, and physical condition as outlined in Table 6. These variations reflect the market's valuation of crabs based on their quality, size, and reproductive status. For instance, the egg-bearing female crabs typically command higher prices due to their desirability for breeding or culinary purposes, while crabs with physical defects, such as missing limbs or soft shells, are priced lower. In both locations, the captured crabs weighed between 184 and 300 g, are classified as standard size. This weight range is considered ideal for commercial purposes, as it balances meat yield and market preferences. Crabs within this range are large enough to provide substantial meat but are not overly mature, which can sometimes affect texture and taste. The consistency in weight across both locations suggests stable environmental conditions and effective fishing practices that target crabs within this preferred size range. The sizes of Scylla crab captured in our study align with the requirements of Indonesia's Ministerial Regulation No. 17/2021, which mandates a minimum legal size for domestic consumption or export is 150 g in weight or 120 mm in carapace width.

The outcomes of this study are also directly relevant to the management of lobster, crab, and swimming crab within Indonesia's territory, as regulated under Ministerial Regulation No. 12/2020. This regulation prohibits the capture and trade of egg-bearing females and crabs below a minimum size threshold (≤100 g) to protect reproductive stocks and promote sustainability. However, market observations from this study indicate that undersized crabs and berried females are still frequently harvested and sold, particularly through informal supply chains. This underscores the need for stronger enforcement mechanisms and local stakeholder engagement to ensure compliance. Aligning community-based practices with national regulations could enhance resource sustainability while maintaining livelihoods.

An understanding of local market conditions and crab biological traits proved crucial in assessing the economic contributions of the crab fishery. The classification of crabs into categories based on weight, sex, and physical condition not only helps fishermen maximize their profits but also ensures that collectors/buyers receive the crabs that meet specific quality standards. Furthermore, the consistent capture of crabs within the 184-300 g range indicates sustainable fishing practices, as it avoids overharvesting juvenile crabs, which are critical for population replenishment, and overly mature crabs, which may have lower market value. The prices of mud crabs fluctuate depending on marketing channels, seasonal variations, and the interplay of supply and demand (Manzano et al., 2023).

While this study provides insights into the economic returns of crab fishing, confidence intervals for daily net profit could not be directly determined due to limitations in collecting comprehensive cost data (e.g., fuel, bait, labor). This limitation should be considered when interpreting the profitability results. Nevertheless, the observed variability in crab selling prices (USD/kg) across different categories and study sites is provided as a proxy to reflect potential fluctuations in revenue streams. After all, maintaining market transparency and ensuring fair pricing in small-scale crab fisheries is essential to prevent conflicts of interest and promote equitable practices.

Although sex-specific catch data were not recorded in this study, price classifications based on sex and reproductive condition (e.g., egg-bearing females) suggest a significant presence of females in the catch. Monitoring sex ratios is essential in crustacean fisheries management, as overharvesting of females, particularly gravid individuals, can negatively impact reproductive output and stock sustainability (FAO, 2015). Future research should incorporate sex ratio assessments to inform more targeted and ecologically sound management interventions.

While this study focused on the income generated by crab harvesting activities, it is important to acknowledge the role of women in the post-harvest segment of the crab value chain. In both Muara Kintap and Bawah Layung, women are actively involved in tasks such as sorting, cleaning, boiling, and selling crabs in local markets. These roles, although often informal and underrecognized, are essential in maintaining product quality and ensuring access to market. Women's participation in these stages contributes to household income and food security, and therefore should be considered in the design of equitable fisheries management and value chain interventions (Harper et al., 2013).

Table 6. Crab marketing prices in Muara Kintap and Bawah Layung at the collector level.

Muara Kintap			Bawah Layung		
Category	**Weight (g)**	**Price (USD/kg)**	**Category**	**Weight (g)**	**Price (USD/kg)**
Egg-bearing female [1,2]	410	14	Egg-bearing female [1]	400	25
	300	11		300	19
	250	10		<300	13
Super	455-500	10	Large male	300-400	6
Large male	300-450	5	Egg-bearing female [2]	400	27
Medium male	190-220	4		300	20
Black large male	300-450	5		<300	14
Black medium male	190-220	3	Large male	300-400	6
Missing 1 claw	190-220	3	Standard large male	>300	2
Missing 2 claws	150-180	2	Standard large female	<300	4
Small crab for			Missing 1 claw	<300	1
seedlings	100-150	1	Missing 2 claws/small	100-150	1

Note: [1] *S. serrata.* [2] *S. olivacea*

To support a sustainable *Rakkang* fishery in Muara Kintap and Bawah Layung, the following recommendations can be implemented: (1) Harvest only crabs that meet the legal size requirements and refrain from capturing the egg-bearing females to protect juvenile crabs and sustain breeding stocks. Restrict fishing access during breeding seasons to allow crab reproduction and population growth. (2) Replace non-biodegradable materials with biodegradable alternatives like green mesh to reduce environmental impact. Modify *Rakkang* to improve selectivity for target species and minimize bycatch rate. (3) Restore degraded mangrove areas to provide essential habitats for crab breeding and growth. Prevent illegal fishing or habitat destruction to ensure compliance with regulations. (4) Engage local fishers in decision-making to encourage collective responsibility for sustainable crab fishery. Establish fair pricing based on crab size, sex, and condition to discourage the capture of undersized or egg-bearing crabs. Incentivize sustainable practices with certifications or eco-labels. (5) Perform regular stock assessment to monitor crab population growth, condition factor, and utilization trends to inform adaptive management strategies.

Conclusion

Rakkang is an eco-friendly and highly selective fishing gear that maintains catch quality, minimize bycatch, and promotes sustainability, making it a favored choice among fishermen. *Rakkang*-A was more effective for catching *S. olivacea*, while *Rakkang*-B proved to be better for catching *S. serrata*. Furthermore, *S. olivacea* is more attracted to fish bait, whereas *S. serrata* shows a preference for crab bait. By optimizing trap placement, timing, and bait selection, *Rakkang* enhances catch efficiency in mangrove environments. This study also provides a basis for future inclusion of local crab fisheries in Fishery Improvement Projects (FIPs), helping to improve sustainability and market access.

Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The author thanks Mr Firnando, Muara Kintap fisherman and Mr. Toni, Bawah Layung fisherman, as well as students of Faculty of Fisheries and Marine Sciences ULM for their assistances and support during fieldworks. Special thanks also go to the anonymous reviewers for their valuable feedback.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

The author conceived, designed, analyzed, and prepared this manuscript.

Ethics Approval

No ethics committee approval is required for this study.

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COMU J Mar Sci Fish 2025 8(1): 87-96
https://doi.org/10.46384/jmsf.1684096 e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Assessment of Antibiotic-Resistant Bacteria in Recreational Coastal Waters of Çanakkale, Türkiye

Belgin Kılıç Çetinkaya[1], Mine Çardak[2]*

[1]Department of Natural Resources Management, School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0002-1553-0712
[2]Department of Fisheries Technology, Çanakkale Faculty of Applied Sciences, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0003-1383-4875

Received: 28.04.2025 /Accepted: 15.05.2025 / Published online: 14.07.2025

Keywords:

Antibiotic resistance
Heterotrophic bacteria
Marine water
Çanakkale Strait

Abstract: This study investigates the prevalence of antibiotic-resistant bacteria in recreational coastal waters along the Çanakkale Strait (Türkiye), a region experiencing increasing anthropogenic pressure from urbanization, maritime activity, and seasonal tourism. A total of 211 bacterial isolates were obtained from surface seawater samples collected at 14 different recreational coastal sites during the summer season of 2024. These isolates were identified using the VITEK® 2 Compact system and tested against 15 antibiotics via the Kirby-Bauer disk diffusion method, following CLSI (2018) guidelines. The results revealed alarmingly high resistance rates, with vancomycin (92.89%), kanamycin (81.04%), and sulphonamides (64.45%) being the most affected. The Multiple Antibiotic Resistance (MAR) index ranged from 0.14 to 0.71, with over 95% of isolates scoring ≥ 0.2, indicating exposure to high-risk environments. *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus intermedius* and *Bacillus cereus* were among the most frequently isolated multidrug-resistant species. Comparative analysis with other marine studies from Türkiye confirms that Çanakkale's coastal waters are significantly impacted by antibiotic contamination. The findings highlight the urgent need for regular surveillance and enhanced wastewater treatment strategies to mitigate the spread of resistance genes in coastal ecosystems. This study contributes essential baseline data to the national inventory on marine antibiotic resistance and underscores the public health risks posed by recreational waterborne exposure to resistant bacteria.

Anahtar kelimeler:

Antibiyotik dirençliliği
Heteretrofik bakteri
Deniz suyu
Çanakkale Boğazı

Çanakkale Rekreasyonel Kıyı Sularında Antibiyotik Dirençli Bakterilerin İncelenmesi

Öz: Bu çalışma, artan kentleşme, denizcilik faaliyetleri ve mevsimsel turizm nedeniyle insan kaynaklı baskı altındaki Türkiye'nin Çanakkale Boğazı kıyılarındaki rekreasyonel sularda antibiyotik dirençli bakterilerin yaygınlığını araştırmaktadır. 2024 yaz döneminde, 14 farklı rekreasyonel kıyı noktasından alınan yüzey deniz suyu örneklerinden toplam 211 bakteri izolatı elde edilmiştir. Bu izolatlar, VITEK® 2 Compact sistemi kullanılarak tanımlanmış ve CLSI (2018) standartlarına uygun şekilde Kirby-Bauer disk difüzyon yöntemi ile 15 farklı antibiyotiğe karşı test edilmiştir.Sonuçlar, en yüksek direnç oranlarının sırasıyla vankomisin (%92,89), kanamisin (%81,04) ve sülfonamidler grubu (%64,45) antibiyotiklere karşı geliştiğini göstermiştir. Multiple Antibiotic Resistance (MAR) indeks değerleri 0.14 ile 0.71 arasında değişmiş; izolatların %95'inden fazlası ≥ 0.2 değerinde bulunarak yüksek riskli çevresel maruziyeti işaret etmiştir. En sık izole edilen çoklu dirençli bakteri türleri arasında *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus intermedius* ve *Bacillus cereus* yer almıştır.Türkiye'de yapılan diğer denizel çalışmalarla karşılaştırmalı analizler, Çanakkale kıyı sularının antibiyotik kontaminasyonundan önemli ölçüde etkilendiğini doğrulamaktadır. Elde edilen bulgular, direnç genlerinin kıyı ekosistemlerinde yayılımını önlemek amacıyla düzenli çevresel izleme ve atık su arıtma stratejilerinin güçlendirilmesi gerekliliğini vurgulamaktadır. Bu çalışma, denizel antibiyotik dirençliliği konusunda ulusal veri tabanı için temel veri sağlamakta ve rekreasyonel su yoluyla dirençli bakterilere maruz kalmanın halk sağlığı açısından oluşturduğu risklerin altını çizmektedir.

Introduction

Antibiotics are widely used pharmaceutical agents for treating infections in both human and veterinary medicine. However, the excessive and uncontrolled use of these substances has led to the emergence of antibiotic-resistant bacterial populations in various environments, including aquatic systems. A significant portion of administered

*Corresponding author: cardakm@hotmail.com

How to cite this article: Kılıç Çetinkaya, B.& Çardak, M. (2025). Assessment of Antibiotic-Resistant Bacteria in Recreational Coastal Waters of Çanakkale, Türkiye. COMU J. Mar. Sci. Fish, 8 (1): 87-96. doi:10.46384/jmsf.1684096

antibiotics is not fully metabolized and is introduced into the environment through domestic wastewater, hospital effluents, and agricultural runoff, eventually reaching coastal and marine waters (Kümmerer, 2009; Larsson, 2014).

The presence of antibiotic-resistant bacteria (ARB) in natural water bodies is not only an ecological concern but also a serious threat to public health. These bacteria can act as reservoirs for antibiotic resistance genes (ARGs), which can be horizontally transferred to pathogenic species, potentially leading to infections that are more difficult to treat (Baquero et al., 2008; Marti et al., 2014).

Numerous studies in Türkiye have demonstrated high rates of antibiotic resistance in marine bacteria. For instance, Matyar et al. (2008) reported ampicillin resistance in 94.4% of bacterial isolates from sediment samples in İskenderun Bay. Similarly, in Güllük Bay, bacteria isolated from seawater and sediment exhibited near-complete resistance to sulfonamides, rifampicin, and oxytetracycline (Altuğ et al., 2020).

The Dardanelles Strait (Çanakkale Boğazı) is a highly dynamic water body with significant ship traffic and coastal recreational activity, particularly during summer months. Despite its ecological and public health relevance, there is a lack of systematic research assessing antibiotic resistance levels in bacteria isolated from recreational coastal waters in this region.

The aim of this study is to investigate the antibiotic resistance profiles of heterotrophic bacteria isolated from seawater samples collected at popular recreational swimming areas along the Çanakkale coast. The findings are intended to contribute to microbial water quality assessment and raise awareness of the potential public health risks associated with antibiotic-resistant bacteria in coastal environments.

Material and Methods

This study was conducted using seawater samples collected from multiple recreational coastal sites along the Dardanelles Strait (Çanakkale, Türkiye), where public swimming is common (Figure 1). A total of 14 sampling stations were selected based on proximity to human settlements, degree of coastal use, and potential pollution sources (Table 1). Sampling took place during the summer season of 2024, covering the months of June, July, and August.

At each site, 500 mL of surface seawater was collected using sterile glass bottles. Samples were transported to the laboratory under cold chain conditions (~4 °C) and processed within six hours of collection.

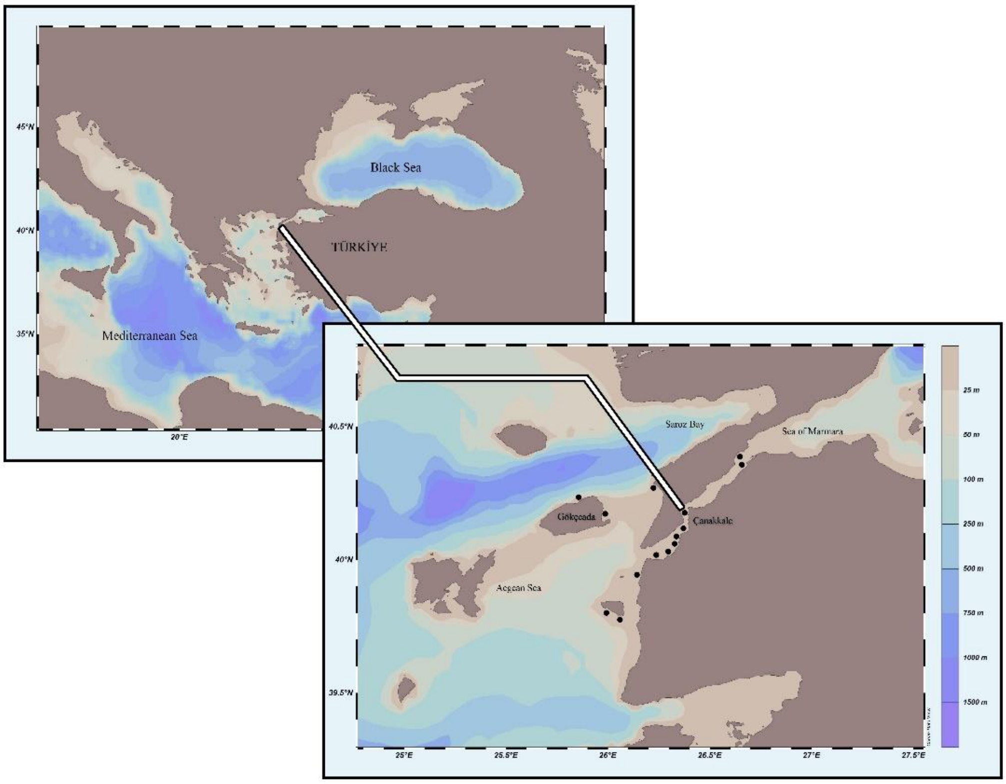

Figure 1. The sampling stations in the Çanakkale Strait

Table 1. Names and coordinates of the stations where sea water samples were collected

Station No	District	Sampling Site Name	Latitude - Longitude
1	Ayvacık	Assos Bathing Water Site	39°29′070″N - 26°20′210″E
2	Ayvacık	Küçükkuyu Inner Harbor Recreational Coastal Water	39°32′680″N - 26°36′380″E
3	Biga	Karabiga Public Beach	40°23′774″N - 27°18′865″E
4	Bozcaada	Ayazma Public Beach	39°48′636″N - 26°00′600″E
5	Bozcaada	Akvaryum Bay Bathing Water Site	39°48′203″N - 26°04′877″E
6	Çanakkale (Central)	Dardanos Municipality Public Beach	40°05′250″N - 26°21′735″E
7	Çanakkale (Central)	Güzelyalı Municipality Public Beach	40°02′950″N - 26°20′578″E
8	Çanakkale (Central)	Yeni Kordon Bathing Water Site	40°08′285″N - 26°23′890″E
9	Eceabat	Eceabat Bathing Water Site	40°10′690″N - 26°21′800″E
10	Eceabat	Kabatepe Recreational Coastal Water	40°11′825″N - 26°15′980″E
11	Ezine	Geyikli Odunluk Pier Recreational Coastal Water	39°46′720″N - 26°09′385″E
12	Gelibolu	Güneyli Bathing Water Site	40°30′610″N - 26°41′640″E
13	Gökçeada	Aydıncık Recreational Coastal Water	40°07′470″N - 25°56′070″E
14	Gökçeada	Yıldız Bay Recreational Coastal Water	40°14′110″N - 25°54′230″E

Bacterial isolation and identification

One milliliter of each seawater sample was serially diluted and spread on Marine Agar (Difco™) using the surface plating method. Plates were incubated at 22 ± 0.1°C for 72 hours, and colony forming was monitored daily.
Based on colony morphology, representative isolates were selected and subjected to Gram staining. Isolates were then identified to the species level using the VITEK® 2 Compact 30 microbial identification system (bioMérieux, France). Pure cultures of all isolates were maintained for subsequent antibiotic susceptibility testing.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method. The following antibiotic disks were used in this study. The antibiotic derivatives and doses are summarized in Table 2.

Bacterial suspensions were adjusted to 0.5 McFarland standard and spread evenly on Mueller-Hinton Agar (Oxoid™). Antibiotic disks were placed at equal distances, and plates were incubated at 37 ± 0.1 °C for 24 hours.

The inhibition zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines. Each isolate was classified as Resistant (R), Intermediate (I), or Susceptible (S). *Escherichia coli* ATCC 25922 was used as a reference strain for quality control.

Calculation of MAR (multiple antibiotic resistance) ındex

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate using the formula defined by Krumperman (1983): MAR index = Number of antibiotics to which the isolate is resistant / Total number of antibiotics tested

Isolates with a MAR index ≥ 0.2 were considered to originate from high-risk environments with frequent exposure to antibiotics.

Table 2. Antibiotic derivatives and doses used in the tests.

Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)
Amoxycillin	AMC	30
Ampicillin	AMP	10
Ceftazidime	CAZ	30
Kanamycin	K	5
Vancomycin	VA	30
Aztreonam	ATM	30
Cefotaxime	CTX	30
Cefuroxime	CXM	30
Gentamycin	CN	120
Imipenem	IPM	10
Ofloxacin	OFX	5
Oxytetracycline	OT	30
Rifampicin	RA	2
Sulphonamides compound	SE	300
Tetracycline	TE	30

Results and Discussion

Antibiotic resistance

A total of 211 bacterial isolates were obtained from the coastal waters of the Dardanelles Strait and evaluated for their resistance profiles against 15 distinct antibiotics. The findings indicated notably high resistance levels, particularly to vancomycin (92.89%), kanamycin (81.04%), sulphonamides (64.45%), and ampicillin (51.18%). In contrast, ceftazidime exhibited the lowest resistance rate (13.27%), suggesting it remains relatively effective in marine bacterial populations. These observations highlight a concerning decline in the effectiveness of widely used antibiotics in coastal aquatic environments.

Resistance frequencies of the isolates against the 15 antibiotics were analyzed (Table 2). The highest percentage values among the categories (R: resistant, S: susceptible, I: intermediate) for each antibiotic are presented in bold. Vancomycin resistance was the most prevalent, reaching 92.89%, while ceftazidime showed the least resistance across all isolates. The highest intermediate response was 36% for rifampicin, whereas the lowest was 4% for ceftazidime. The greatest rate of susceptibility was found to be 81.99% for ceftazidime, while vancomycin, kanamycin, and sulphonamides had the lowest susceptibility levels, each at just 1%.

Several of the bacterial species isolated in this study are well-known environmental or opportunistic pathogens and have been frequently reported in marine ecosystems. For example, *Acinetobacter* spp. (especially *A. baumannii, A. haemolyticus,* and *A. nosocomialis*) are often found in marine waters and even fish viscera. These species are of global concern due to their multidrug resistance, including resistance to carbapenems, a last-resort antibiotic group (Dewi et al., 2021).

A. baumannii is frequently isolated from various marine environments, including seawater, marine sediments, and fish viscera. Studies show that environmental strains of this species can survive in seawater for up to 10 days and are more prevalent in coastal areas exposed to organic pollution (Twala, 2023). Although primarily known as a hospital-associated species, *A. nosocomialis* has also been identified in marine and estuarine environments. Coastal studies from South Korea have isolated this species from seawater, especially in transitional zones between freshwater and saltwater (Adewoyin et al., 2018). These findings suggest that *A. nosocomialis* can adapt to dynamic aquatic ecosystems and may serve as a reservoir for resistance genes (Sanz-García et al., 2021). While less frequently studied, *A. haemolyticus* has occasionally been recovered from coastal waters, particularly in areas impacted by urban wastewater. Its detection in marine samples near outfall zones suggests a possible link to human-derived contamination and points to its ability to persist in saline aquatic environments (Van Assche, 2019).

Table 3. The isolate codes and tested bacteria species

Bacteria species	Isolation Frequency (%)	Station Code
Acinetobacter baumannii complex (Bouvet and Grimont 1986)	1.14	St 4
Acinetobacter haemolyticus	2.27	St 5,St 6
Acinetobacter nosocomialis	2.27	St 1, St 6
Bacillus mycoides Flügge 1886	1.14	St 6, St 8,St 10
Bacillus pumilus Meyer and Gottheil 1901	2.27	St 6, St 7,St 8
Bacillus thuringiensis Berliner 1915	2.27	St 6, St 7,St 8
Bacillus cereus Frankland and Frankland 1887	5.68	St 3, St 6, St 7,St 8
Bacillus megaterium de Bary 1884	2.27	St 6, St 7,St 8
Burkholderia mallei (Zopf 1885) Yabuuchi et al. 1993	9.09	St 6,St 9,St 10,St 14
Citrobacter braakii Brenner et al. 1993	6.82	St 6, St 7,St 8
Citrobacter frenduii (Braak 1928) Werkman and Gillen 1932	5.68	St 10,St 13,St 14,
Enterobacter aerogenes Hormaeche and Edwards 1960	7.95	St 11, St 7,St 8
Enterobacter cloaceae (Jordan 1890) Hormaeche and Edwards 1960	10.23	St 13, St 7,St 8
*Enterobacter sakazakii (*Farmer et al. 1980)	2.27	St 4, St 7,St 8
Enterococcus faecalis (Andrewes and Horder. 1906) Schleifer and Kilpper-Bälz. 1984	10.23	St 10,St 12,St 14,
*Escherichia coli (*Migula 1895) Castellani and Chalmers 1919	28.41	St 5, St 7,St 8
Klebsiella ornrthioytica	6.82	St 4, St 7,St 8
Klebsiella oxytoca (Flügge 1886) Lautrop 1956	4.55	St 1,St 5
Klebsiella pneumoniae (Schroeter 1886) Trevisan 1887	5.68	St 6, St 7,St 8
Kocuira kristinae	1.14	St 2
Pantoe agglomerans (Ewing and Fife 1972) Gavini et al. 1989	5.68	St 2,St 9,St 13,St 13
Proteus mirabilis Hauser. 1885	4.55	St 6, St 7,St 8,St 11
Proteus vulgaris Hauser. 1885	3.41	St 4,St,13
Pseudomonas fluorescens Migula 1895	3.41	St 6, St 7,St 8
Pseudomonas stutzeri	1.14	St 13
Raoultella planticola (Bagley et al. 1982) Drancourt et al. 2001	2.27	St 4,St 12
Staphylococcus intermedius Hajek 1976	11.36	St 6, St 7,St 8
Salmonella enterica Le Minor & Popoff 1987	3.41	St 6, St 7,St 8
Serratia marcescens Bizio 1823	4.55	St 14,St 5
Serratia plymuthica (Lehmann and Neumann 1896) Breed et al. 1948	5.68	St 9,St 13,St
Sphingomonas paucimobilis (Holmes et al. 1977) Yabuuchi et al. 1990	18.18	St 3, St 6, St 7,St 8
Staphylococcus pseudintermedius Devriese et al. 2005	2.27	St 11,St 2
Stenotrophomonas malthophilia Hugh 1981) Palleroni and Bradbury 1993	12.50	St 6, St 7,St 8,St 9,St 11
Streptococcus uberis Diernhofer 1932 (Approved Lists 1980)	1.14	St 13
Vibrio alginolyticus (Miyamoto et al. 1961) Sakazaki 1968	1.14	St 10

Bacillus species such as *B. cereus*, *B. pumilus*, *B. thuringiensis*, and *B. mycoides* are prevalent in marine sediments and contribute to organic matter turnover through extracellular enzyme activity. However, *B. cereus*, in particular, is known for its toxigenic potential and resistance traits, making it relevant for both food safety and environmental monitoring (Das et al., 2006).

Pseudomonas spp. including *P. fluorescens* and *P. stutzeri* are metabolically versatile and widely distributed in seawater and coastal sediments. These bacteria play key roles in nitrogen cycling and hydrocarbon degradation and often harbor plasmid-mediated resistance genes (Das et al., 2024).

Vibrio alginolyticus, a species common in warm, saline waters, acts both as a free-living marine bacterium and as a fish pathogen. It has been found to exhibit resistance to antibiotics like tetracycline and ampicillin in estuarine and polluted coastal areas (Toraskar, et al., 2022).

Members of the Enterobacteriaceae family such as *Enterobacter*, *Citrobacter, Klebsiella*, and *Escherichia* are commonly detected in coastal waters influenced by wastewater discharge. These genera often indicate fecal contamination and show high rates of extended-spectrum beta-lactamase (ESBL) production and multidrug resistance (Dewi et al., 2020).

Other less frequently reported but environmentally significant bacteria like *Stenotrophomonas maltophilia*, *Sphingomonas paucimobilis,* and *Raoultella planticola* were also detected in this study. These organisms, although isolated in low abundance, are known to carry resistance determinants and adapt to polluted marine niches. Similarly, *Enterococcus faecalis* and *Staphylococcus pseudointermedius* are indicators of human or animal waste contamination and may persist in shoreline environments.

Overall, the bacterial community identified from the coastal waters of the Çanakkale Strait includes numerous species previously associated with antibiotic resistance in marine settings. This highlights the importance of continuous environmental surveillance and reinforces the role of coastal waters as reservoirs of resistance. The widespread occurrence of isolates with MAR index ≥ 0.2 strongly supports the hypothesis that these environments are heavily impacted by anthropogenic antibiotic inputs and serve as active reservoirs for resistant bacteria.

Table 4. The antibiotic resistance frequencies of the bacterial isolates

	Number of isolates			**Frequency (%)**		
	R	**S**	**I**	**R**	**S**	**I**
Amoxycillin (AMC 30 µg)	75	80	56	35.55	37.91	26.54
Ampicillin (AMP 10 µg)	108	57	46	51.18	27.01	21.80
Ceftazidime (CAZ 30 µg)	28	173	10	13.27	81.99	4.74
Kanamycin (K 5 µg)	171	16	24	**81.04**	7.58	11.37
Vancomycin (VA 30 µg)	196	3	12	**92.89**	1.42	5.69
Aztreonam (ATM 30 µg)	63	127	21	29.86	60.19	9.95
Cefotaxime (CTX 30 µg)	35	152	24	16.59	**72.04**	11.37
Cefuroxime (CXM 30 µg)	97	67	47	45.97	31.75	22.27
Gentamycin (CN 120 µg)	44	107	60	20.85	50.71	28.44
Imipenem (IPM10 µg)	32	148	31	15.17	**70.14**	14.69
Ofloxacin (OFX 5 µg)	47	145	19	22.27	68.72	9.00
Oxytetracycline (OT 30 µg)	34	108	69	16.11	51.18	32.70
Rifampicin (RA 2 µg)	92	41	78	43.60	19.43	**36.97**
Sulphonamides compound (S3 300 µg)	136	36	39	64.45	17.06	18.48
Tetracycline (TE 30 µg)	100	91	20	47.39	43.13	9.48

R: Resistance S: Susceptible I: Intermediate

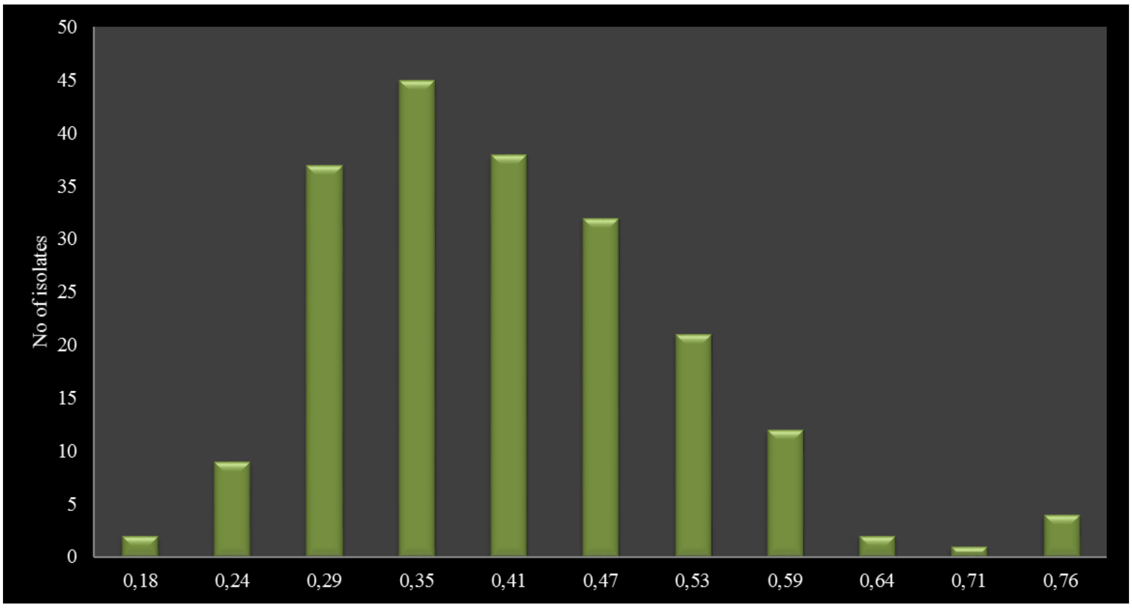

Figure 2. Distribution of multiresistant bacterial isolates according to stations

Multiple antibiotic resistance

Figure 2 summarizes bacterial isolates multiple antibiotic resistance (MAR). The multiple antibiotic resistance (MAR) index values of the bacterial isolates were recorded as 0.35 (%95), 0.29 (88%), 0.64 (853) and 0.71 (47%).

Investigating bacterial diversity within marine ecosystems is essential for understanding environmental processes and determining sources of pollution. Due to the fluctuating nature of marine environments—unlike the relative stability of terrestrial habitats—bacterial communities evolve resistance strategies to cope with ongoing environmental pressures. The detection of resistant strains in these systems points to continuous exposure to antibiotic and chemical pollutants (Zeglin, 2015; Delgado-Baquerizo, 2016).

The inappropriate use of antibiotics continues to be a major risk factor for both human health and ecological integrity. Aquatic systems, which are significantly influenced by human activity, act as hotspots for the development and spread of antimicrobial resistance. While there is growing interest in the discovery of novel antibiotic compounds, pharmaceutical research and development in this field is often limited by economic constraints. As such, the implementation of strong policies to curb the unnecessary use of antibiotics has become increasingly critical. Antibiotics reach marine systems through domestic, industrial, and healthcare-related discharges, and facilitate the horizontal transmission of resistance genes among microbial populations. Since bacteria readily adhere to surfaces, coastal zones offer ideal conditions for exchanging resistance determinants developed in response to environmental stressors like antibiotics and heavy metals (Sabatino et al., 2020; Zhang et al., 2020; Marti et al., 2014).

Numerous studies have confirmed antibiotic and heavy metal resistance among bacterial isolates from Turkish marine sediments. For instance, Matyar et al. (2008) documented that isolates from Iskenderun Bay sediment displayed high resistance to ampicillin (94.4%) and lower resistance to imipenem (4.4%). In Güllük Bay, Altuğ et al. (2020) observed that sediment-derived bacteria were entirely resistant (100%) to sulfonamide, rifampicin, tetracycline, and ampicillin, and showed near-complete resistance to nitrofurantoin (98%) and oxytetracycline (98%). Similarly, Kacar and Kocyigit (2013) reported gentamicin and tobramycin resistance among bacteria from sediments collected at the Aliağa shipbreaking zone in the Eastern Aegean. Çardak et al. (2016) identified high resistance levels in isolates from the Marmara Sea and Turkish Straits—specifically to kanamycin (82%), vancomycin (78%), and ampicillin (60%).

The findings of the current research also demonstrate considerable resistance in marine bacteria isolated from Çanakkale's coastal waters, especially against vancomycin (93.3%), kanamycin (81.04%), sulphonamides (64.45%), ampicillin (51.1%), tetracycline (47.39%), cefuroxime (45.97%), and ceftazidime (13.27%). These outcomes suggest that even locations perceived as relatively clean, such as the Çanakkale Strait, may harbor higher-than-expected levels of antibiotic contamination. Consequently, environmental monitoring, particularly in areas with heavy maritime traffic and recreational use, is strongly advised.

The MAR (Multiple Antibiotic Resistance) index values ranged from 0.14 to 0.71. Notably, over 95% of the isolates had a MAR index $\geq$ 0.2. According to Krumperman's criteria, MAR values above 0.2 are indicative of high-risk contamination sources, likely from anthropogenic activities. The widespread nature of these elevated MAR values along the Çanakkale coast underscores the persistent impact of untreated or partially treated urban wastewater, maritime discharges, and seasonal tourism activities.

Average antibiotic resistance across all isolates was calculated at 95.46%. This is consistent with findings in urban-impacted regions. Heterotrophic aerobic bacteria exhibited 100% resistance, followed by enterococci and coliform groups. These bacteria, typically indicators of fecal pollution, are known to acquire resistance genes rapidly in polluted environments.

Comparable studies further validate these observations. For instance, in Gökçeada, sulphonamide resistance reached 93.3%, and cefotaxime resistance 78.9%, mirroring the high resistance seen in the current data. Research in Istanbul's Golden Horn reported ampicillin (71.5%), sulphonamides (43.8%), and rifampin (24.3%) resistance among 144 isolates, again indicating strong antibiotic pressure in urban estuaries. Interestingly, gentamicin and imipenem had 0% resistance there, a trend similar to the low resistance.

In another investigation conducted in Kınalı Island, nearly all isolates (95.46%) were resistant to antibiotics, with oxytetracycline and tetracycline showing the highest resistance (98.7%). Even nitrofurantoin, typically considered a last-resort antibiotic, showed 87.3% resistance (Türetken et al., 2024), raising concerns about limited therapeutic options.

Aegean Sea sediment isolates exhibited relatively lower resistance to amikacin (36%), cefotaxime (20%), ampicillin (16%), and kanamycin (16%). The elevated resistance rates in Çanakkale likely reflect the difference between enclosed coastal waters affected by urban runoff and the more open, less polluted marine sediments.

Of particular note is the frequent identification of *Bacillus cereus* in the current samples, a bacterium recognized in both Aegean and Gökçeada datasets as one of the most multidrug-resistant. Its recurrence across studies confirms the need for targeted monitoring of key resistant species in marine surveillance programs.

The role of marine environments as reservoirs for resistance genes has been repeatedly emphasized. While aquaculture sites are often scrutinized for their role in antibiotic release, recreational zones like Çanakkale pose direct threats to public health. The detection of multidrug-resistant bacteria in waters used for swimming and fishing increases the likelihood of exposure for humans, especially immunocompromised individuals (Al-Bahry et al., 2009; Di Cesare et al., 2012; Altuğ et al., 2020).

Furthermore, the high MAR values in this study surpass those reported in other Turkish regions. For example, bacteria isolated from the Sea of Marmara had MAR indices ranging from 0.30 to 0.34 (Çardak et al., 2016), whereas in this study, MAR values reached up to 0.71. This points to a greater cumulative impact of urbanization and shipping traffic in Çanakkale, emphasizing the urgent need for stricter discharge regulations and continuous microbial water quality assessments.

In terms of aminoglycoside resistance patterns, Çanakkale isolates exhibited higher resistance to kanamycin compared to amikacin. This suggests possible selective antibiotic exposure, where local medical waste or domestic discharges may contain specific compounds that promote kanamycin resistance. In contrast, amikacin resistance dominates in sediment samples from aquaculture zones, hinting at differences in antibiotic usage practices.

Overall, the findings of this study highlight a serious concern: coastal ecosystems, particularly those near urban centers, are increasingly burdened with antibiotic-resistant bacteria. The elevated resistance frequencies, high MAR indices, and cross-regional comparisons all reinforce the necessity for integrated marine management policies, enhanced wastewater treatment infrastructure, and routine antimicrobial surveillance programs.

In this study, it was observed that bacterial resistance to antibiotics in the coastal waters of the Çanakkale Strait likely developed through spontaneous mutations or via the horizontal transfer of resistance genes from other bacterial populations. Exposure to antibiotic residues in the aquatic environment has created a selective pressure that favors the survival and proliferation of resistant strains, allowing them to dominate local microbial communities. This pattern reinforces the understanding that environmental antibiotic pollution plays a central role in the emergence and spread of antimicrobial resistance within aquatic ecosystems.

Although the "One Health" approach has increased interdisciplinary awareness regarding the connections between environmental, human, and animal health, antibiotic resistance remains a serious and growing concern. Marine inventory studies, particularly in urban and tourism-affected regions like Çanakkale, are vital to track the spread of resistance genes and better understand the dynamics of contamination.

The distribution and density of antibiotic-resistant bacteria vary depending on geographic exposure levels, yet aquatic environments consistently contribute to the persistence and transmission of resistance. The findings from Çanakkale support the need for ongoing monitoring programs in coastal zones that face multiple anthropogenic pressures.

The elevated levels of resistance detected for the first time in this region, particularly along the Çanakkale coastline, underscore the necessity of regular and long-term microbial assessments. These results not only reflect current contamination patterns but also highlight potential risks to both marine ecosystems and public health, especially in the context of climate change and seasonal urban activity.

Conclusion

The present study provides the first detailed account of antibiotic resistance in bacterial populations isolated from the coastal waters of the Çanakkale Strait. The high frequency of resistance, coupled with widespread multidrug resistance patterns and elevated MAR index values, clearly demonstrates the anthropogenic pressures affecting the region. These findings confirm that the Çanakkale coastline has become a local reservoir for antibiotic-resistant bacteria, likely driven by urban discharge.

This study contributes valuable baseline data to the national marine antibiotic resistance inventory. However, to fully understand the evolving threat and its ecological implications, continuous and expanded monitoring efforts are required. Future research should integrate environmental variables such as seasonal changes and pollution sources, and adopt multidisciplinary frameworks that can inform policy and guide sustainable coastal management practices in Turkiye and beyond.

Acknowledgement

The authors acknowledge the financial support provided by the Çanakkale Onsekiz Mart University Scientific Research Projects Unit (COMU BAP Project No: FYL-4336). The authors would also like to express their sincere thanks to Assoc. Prof. Dr. Oğuz Tunç and Mehmet Can from the Faculty of Fine Arts for their valuable contributions to the improvement of the maps and geographical coordinates used in this study. We are also grateful to Assist. Prof. Dr. Didem Hekimoğlu Tunç from the Department of Health Management, Çanakkale Onsekiz Mart University, for her valuable technical support.

Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article. No funding was received for conducting this study. The authors declare they have no financial interests.

Author Contributions

The conception and design of the study were carried out by the first author, Belgin Kılıç Çetinkaya. The manuscript was written by Belgin Kılıç Çetinkaya and revised with contributions and feedback from the second author, Mine Çardak. Both authors read and approved the final version of the manuscript.

Ethics Approval

The authors declare that this study did not include any experiments with human or animal subjects.

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COMU J Mar Sci Fish 2025 8(1): 97-102
https://doi.org/10.46384/jmsf.1719494
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Zooplankton Composition in the Southwestern Sea of Marmara During the Summer Season

Dalida Bedikoğlu*[1], Sandra Jerinić[2], Nazlı Demirel[1]

[1]Institute of Marine Sciences and Management, Istanbul University, Istanbul, 34134, Türkiye https://orcid.org//0000-0001-5088-7373
[2]University of Split, Department of Marine Studies, 2100 Split, Croatia https://orcid.org//0009-0007-2714-1419
[1]Institute of Marine Sciences and Management, Istanbul University, Istanbul, 34134, Türkiye https://orcid.org//0000-0003-4542-9276

Received: 13.06.2025 /Accepted: 07.07.2025 / Published online: 14.07.2025

Keywords:

Mesozooplankton
Diversity indices
Abundance
Erdek Bay

Abstract: The Sea of Marmara (SoM) is a highly sensitive semi-enclosed marine ecosystem that has been undergoing considerable ecological changes in the recent decades. This study aimed to evaluate the summer zooplankton community structure via vertical hauls at 0-20 m depth in Erdek Bay, located in the southwestern part of the SoM, during two consecutive years, 2019 and 2020. The results revealed marked year-to-year shifts. In 2020, total zooplankton abundance declined markedly compared to 2019, primarily due to a sharp decrease in the abundance of *Penilia avirostris*, which was dominant in the community during 2019. Despite the higher abundance observed in 2019, diversity indices were lower, indicating that the community structure was largely shaped by the dominance of a single species. In contrast, unlike the Cladocera group, Copepoda abundance increased in 2020 compared to 2019. As the most recent data for this region date back to the early 2010s, this research provides an updated contribution to the understanding of summer zooplankton composition in Erdek Bay. The findings highlight the necessity for long-term zooplankton monitoring with high temporal resolution to better understand zooplankton dynamics in response to environmental changes in the southwestern part of the Sea of Marmara.

Anahtar kelimeler:

Mesozooplankton
Çeşitlilik indeksleri
Bolluk
Erdek Körfezi

Marmara Denizi'nin Güneybatısında Yaz Dönemi Zooplankton Kompozisyonu

Öz: Marmara Denizi, son yıllarda önemli ekolojik değişimlerin yaşandığı, oldukça hassas yarı kapalı bir denizel ekosistemdir. Bu çalışma, Marmara Denizi'nin güneybatısında yer alan Erdek Körfezi'nde yaz dönemi zooplankton topluluk yapısını, iki farklı yıl olan 2019 ve 2020'de 0-20 m derinlikte yapılan dikey çekimler ile değerlendirmeyi amaçlamıştır. Elde edilen sonuçlar yıllar arası belirgin farklılıkları ortaya koymuştur. 2020 yılında, zooplanktonun toplam bolluğu 2019 yılına kıyasla önemli ölçüde azalmıştır. Bu düşüşün başlıca nedeni, 2019 yılında toplulukta baskın olan *Penilia avirostris* türünün bolluğundaki keskin azalmadır. 2019 yılında yüksek bolluk gözlemlenmesine rağmen, çeşitlilik indekslerinin daha düşük olması, topluluk yapısının büyük ölçüde tek bir türün baskınlığıyla şekillendiğini göstermektedir. Buna karşılık, Cladocera grubunun aksine, Copepoda bolluğu 2020 yılında 2019'a göre artış göstermiştir. Bölgeye ait en güncel veriler 2010'lu yılların başına dayandığından, bu çalışma Erdek Körfezi'ndeki yaz dönemi zooplankton bileşimine ilişkin güncel bir katkı sunmaktadır. Elde edilen bulgular, Marmara Denizi'nin güneybatı kesiminde çevresel değişimlere bağlı olarak zooplankton dinamiklerini daha iyi anlayabilmek için yüksek zaman çözünürlüğüne sahip uzun dönemli zooplankton izleme çalışmalarının gerekliliğini vurgulamaktadır.

Introduction

Zooplankton occupy a central position in marine food webs. Through their feeding on phytoplankton, they modulate primary production dynamics while simultaneously serving as the key prey for planktivorous fishes such as anchovy and sardine within the upper pelagic system. This dual role positions zooplankton as trophic intermediaries that actively mediate the flow of energy through pelagic ecosystems (Steinberg and Landry, 2017). The short generation times of zooplankton and their diverse reproductive strategies enable rapid responses to both favourable and adverse environmental changes. Consequently, they serve as sensitive indicators in marine ecosystem monitoring (Shiganova, 2005; Büyükateş and İnanmaz, 2007; İşinibilir *et al.*, 2008; Bedikoğlu *et al.*, 2020). Shifts in zooplankton abundance and community composition provide key signals of ecosystem change, with cascading effects on all biological components of the

*Corresponding author: dalidabedikoglu@gmail.com

How to cite this article: Bedikoğlu, D., Jerinić, S. & Demirel, N (2025). Zooplankton composition in the southwestern Sea of Marmara during the summer season. COMU J. Mar. Sci. Fish, 8 (1): 97-102. doi:10.46384/jmsf.1719494

upper pelagic food web, beginning with primary producers (Cushing, 1990; Clark, 1992).

The Sea of Marmara (SoM) is a semi-enclosed marine system that serves as a transition zone between the Black Se and Mediterranean Sea via the narrow Istanbul and Çanakkale Straits. Topographically, the SoM features three deep basins exceeding 1000 m in depth and an extensive shallow shelf area less than 100 m deep in its southern half. The SoM is characterized by distinct stratification: less saline Black Sea waters flow in through the Istanbul Strait, while denser, more saline waters from the Aegean enter through the Çanakkale Strait (Beşiktepe *et al.*, 1994). The southwestern part of the SoM, encompassing Erdek Bay, the Southern Marmara Islands, and adjacent shelf areas, constitutes a distinctive ecological and oceanographic sub-region within the basin. This region is shaped by complex interactions among physical dynamics, biogeochemical gradients, and biological communities, and has long supported rich marine biodiversity (Altuğ *et al.*, 2011). It also serves as a key spawning and nursery ground for several fish species (Daban *et al.*, 2024) and supports regional small-scale fisheries.

Zooplankton studies conducted in the SoM remain limited and have generally focused on fisheries-related objectives (Yılmaz and İşinibilir, 2016). While several studies have evaluated zooplankton communities in the northeastern parts of the SoM (Yılmaz, 2002; Yılmaz, 2015; Bedikoğlu *et al.*, 2020), particularly in the eastern İzmit Bay (İşinibilir *et al.*, 2008; İşinibilir Okyar *et al.*, 2015), comparable data for the southwestern part, including Erdek Bay, are lacking (İşinibilir, 2010; Toklu-Alıçlı *et al.*, 2014). Despite its high biodiversity (Altuğ *et al.*, 2011) and relatively low anthropogenic pressure (Toklu-Alıçlı and Balkıs-Özdelice, 2014), zooplankton studies in Erdek Bay remain scarce, and no recent data are available.

This study aims to evaluate zooplankton composition, diversity, abundance, and associated environmental parameters in the inner and outer parts of Erdek Bay during two consecutive summer periods (2019 and 2020), to provide an updated dataset and baseline information before the mucilage crisis in 2021 for this ecologically valuable region.

Material and Methods

Zooplankton samples were collected during the summer periods of 2019 and 2020 from two stations located in the inner and outer parts of Erdek Bay (Figure 1). Sampling was conducted from the upper 20 meters of the water column using a WP2 - net (0.57 m mouth diameter, 200 µm mesh size). All samples were fixed in a borax-buffered formaldehyde solution at a final concentration of 4%. Mesozooplankton were identified to the lowest possible taxonomic level using Alden *et al.* (1982). Abundances were expressed as individuals per cubic meter (ind. m^{-3}). Replicate subsamples (1–10 mL) were taken using Stempel pipettes, with volumes ranging from 1/50 to 1/500 of the total sample, depending on organism density and composition. Taxonomic identifications were performed under a LEICA MZ125 stereo microscope. Temperature, salinity and,dissolved oxygen (DO) of the water column were measured at the surface and at depths of 5, 10, 15, 20, and 30 meters using a multi-parameter probe (YSI Professional Pro Plus). In addition, water samples were collected from these depths using 5 L Niskin bottles for the analysis of chlorophyll-a (Chl-a) and dissolved oxygen. Chlorophyll-a concentrations were determined using the acetone extraction method (Parsons *et al.*, 1984), and DO was measured according to the Winkler titration method, following the guidelines of APHA (2012). For the interpretation of environmental parameters, values were averaged across the zooplankton sampling depth (upper 20 meters) to ensure consistency with the biological data.

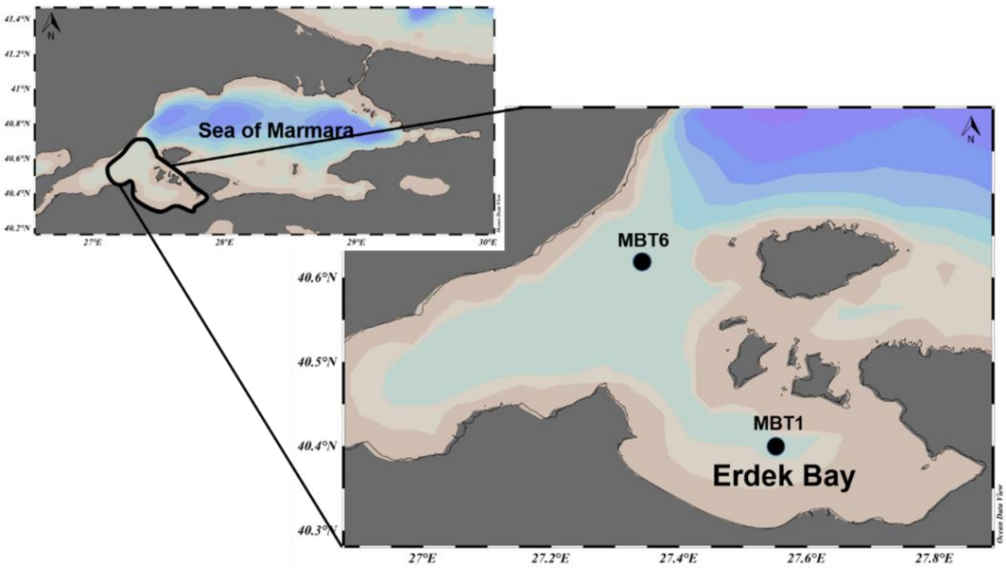

Figure 1. Sampling stations (indicated by black dots) and the location of the Sea of Marmara
Coordinates for MBT1 27.536E 40.422N, and MBT6 27.373E 40.623N)

Data were examined per sampling stations and years. Zooplankton abundance data were log(x+1) transformed to stabilize variance and reduce the influence of dominant taxa in multivariate analyses. The resulting resemblance matrix was used to conduct hierarchical cluster analysis using the group-average linkage method, producing a dendrogram to visualize patterns of community similarity among samples. To compare zooplankton community diversity and evenness between the two sampling periods (summers of 2019 and 2020), the Shannon-Wiener diversity index (H') and Pielou's evenness index (J') were calculated based on abundance data. All statistical analyses were performed using PRIMER7 + PERMANOVA software (v.7.0; PRIMER-E).

Results and Discussion

Upper layer temperature in Erdek Bay ranged between 24.9 and 25.2 °C during the summers of 2019 and 2020, while salinity varied between 23.5 and 27.8 psu. Surface chlorophyll-a (Chl-a) concentrations were higher in 2019 compared to 2020. Although Chl-a values remained consistently below 1 µg L^{-1} in both years, concentrations at MBT1 (hereafter, inner bay) were lower than those recorded at MBT6 (hereafter, outer bay). In contrast, an opposite pattern was observed for dissolved oxygen (DO) concentrations, with surface DO levels lower in 2019 than in 2020. No consistent spatial pattern was observed between stations: DO levels were higher in the surface waters of the inner bay in 2019, whereas in 2020, higher values were recorded in the outer bay (Fig. 2).

In the zooplankton community, species number varied between 10 and 15. The lowest number of species (10) was recorded at the outer bay station in 2019, while the highest species richness was observed at the inner bay station in 2020. In both years, species number in the outer bay remained lower than in the inner bay. The Shannon-Wiener and Pielou evenness indices were higher in 2020 than in 2019. While diversity indices were higher in the outer bay in 2019, they were lower in 2020. The most diverse and compositionally even zooplankton community was recorded at the inner bay station in 2020 (Fig. 2).

Zooplankton abundance in both the inner and outer bays decreased by approximately three- and four-fold, respectively, in 2020 compared to 2019. In both years, zooplankton abundance at the outer bay station was higher than that at the inner bay. The highest abundance was recorded at the outer bay in 2019 (19,681 ind. m^{-3}), while the lowest value was observed at the inner bay in 2020 (4,869 ind. m^{-3}) (Fig. 2).

At the group level, Copepoda abundance remained lower than that of Cladocera throughout the study period. Previous studies also reported Cladocera dominance in the summer zooplankton community of the region in 2006, as well as in the eastern SoM between 2004 and 2008 (İşinibilir, 2010; Yılmaz, 2015; Bedikoğlu *et al.*, 2022). The high zooplankton abundance observed in 2019 coincided with elevated Chl-a concentrations, while salinity and dissolved oxygen levels were lower compared to 2020.

Throughout the study, a total of 20 distinct taxa/groups were identified. These included 4 Copepoda species, 5 Cladocera species, 4 holoplanktonic groups, 7 meroplanktonic groups, and 1 jellyfish group (Table 1). In a previous study conducted in 2006–2007, which used vertical hauls through the entire water column in the region, 27 copepod species were reported (Altuğ *et al.*, 2011). Another study carried out between 2006 and 2008, based on vertical hauls from 30 m to the surface, reported 12 copepod species in the zooplankton assemblage (Toklu-Alıçlı *et al.*, 2014). Differences in copepod species number observed among studies conducted during similar periods but using different sampling depths were attributed to the contribution of Mediterranean-origin species.

In 2019, findings from the present study indicated a decline in copepod species richness in the region, while the number of Cladocera species remained unchanged. The dominant copepod species was *Paracalanus parvus*, while *Penilia avirostris* was the most prominent among Cladocera (Table 1). In both years, the copepod species *P. parvus* and *Acartia clausi* were among the most abundant. Within Cladocera, *P. avirostris* and *Pseudevadne tergestina* stood out. Among holoplankton, *Oikopleura dioica* was prominent, while Gastropoda larvae were the leading group among meroplankton. In another study conducted at similar sampling depths (vertical hauls from 30 m) during 2006–2008, the dominant zooplankton species were again reported as *A. clausi* and *P. parvus* (Toklu-Alıçlı *et al.*, 2014).

The increase in zooplankton abundance observed in 2019 was primarily driven by the high dominance of *P. avirostris*, followed by *P. parvus* as the second most abundant species. As a result, a similarity rate exceeding 70% was determined between the study years and stations. In 2019, due to the increase in a single dominant species and lower diversity index values, inter-station similarity was approximately 75%. In contrast, in 2020, which was characterized by relatively lower abundance and higher diversity index values, inter-station similarity increased to approximately 83% (Fig. 3).

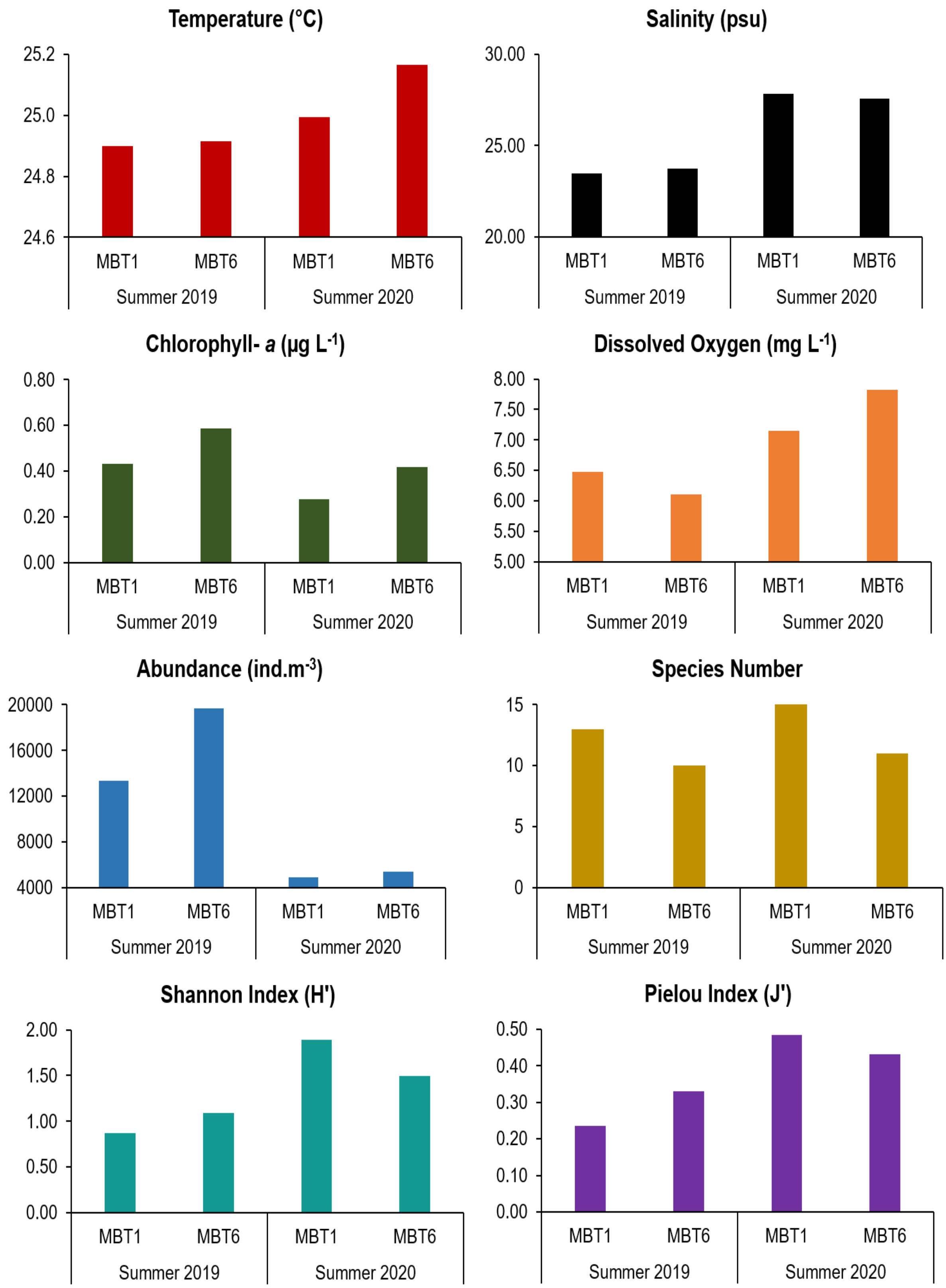

Figure 2. Changes in environmental parameters, abundance, species number and diversity index' at MBT1 and MBT2 stations in summer 2019 and 2020

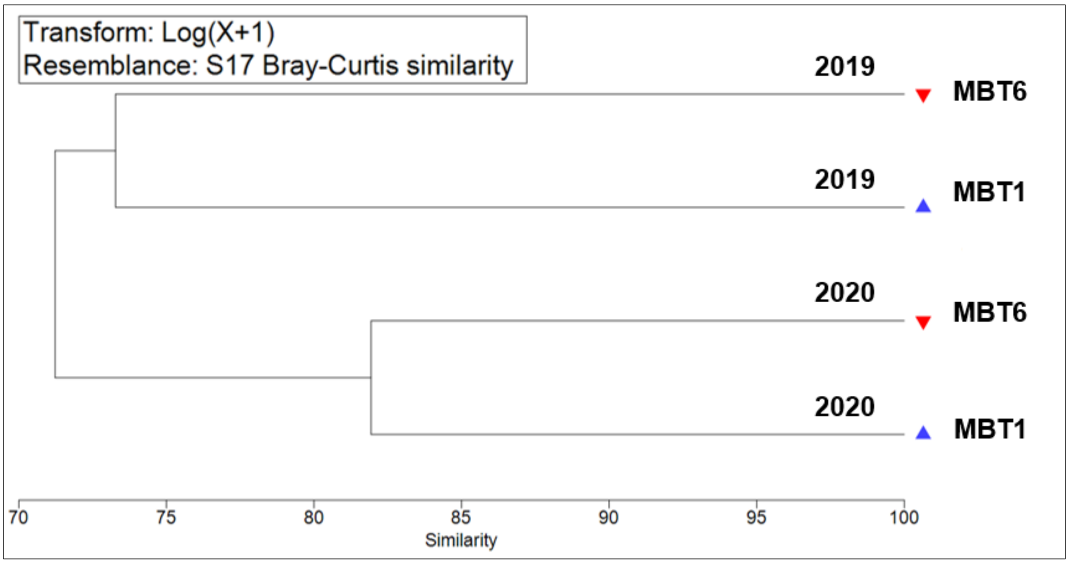

Figure 3. Hierarchical cluster dendrogram (Bray-Curtis similarity, log(x+1) transformed abundance data) of zooplankton communities at MBT1 and MBT2 stations in 2019 and 2020.

Conclusion

The Sea of Marmara (SoM) is a highly vulnerable semi-enclosed marine ecosystem that has undergone significant ecological changes over the past three decades (Demirel *et al.*, 2023). This study, conducted in the relatively understudied southwestern region of Erdek Bay, provides an updated assessment of the summer zooplankton community composition for the years 2019 and 2020. In 2020, zooplankton abundance sharply decreased compared to 2019, coinciding with a decline in chlorophyll-a concentrations. Despite this reduction in abundance, diversity indices were higher in 2020, indicating a shift in community composition. As the most recent previous study in this region dates back to the early 2010s, the present research helps fill a decade-long data gap. Although zooplankton communities are generally known to respond strongly to environmental variability, this study did not reveal consistent relationships with the measured environmental parameters. This is likely due to the limited temporal resolution of the dataset, which was restricted to two discrete summer periods. Therefore, long-term monitoring with higher temporal resolution is needed to more accurately assess temporal dynamics in zooplankton community structure.

Conflict of Interest

The authors declare no conflict of interest

Author Contributions

Dalida Bedikoğlu: Study design, sample, collection, species identification, data analysis, writing original draft preparation. Nazlı Demirel: Supervision, funding, study design, sample collection, writing reviewing, editing.

Ethics Approval

Ethics committee approval is not necessary for this study.

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COMU J Mar Sci Fish 2025 8(1): 103-120
https://doi.org/10.46384/jmsf.1723617

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Determination of Quality Changes in Traditional and Liquid Smoked Fish Pastirma Prepared from Meagre (*Argyrosomus regius* Asso, 1801)

Buminhan Burkay Selçuk[1]* Fikret Çakır[2]

[1*]School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0003-4650-5592
[2]Department of Fisheries and Fish Processing, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye https://orcid.org//0000-0001-5261-2365

Received: 20.06.2025 /Accepted: 11.07.2025 / Published online: 14.07.2025

Keywords:

Meagre
Fish Pastirma
Liquid smoke
Nutritional value
Quality change

Abstract: In this study, the nutritional composition and the physicochemical, microbiological, and sensory quality parameters of traditional and liquid smoke-treated meagre pastirma were investigated under refrigerated storage conditions at +4 °C. For this purpose, analyses were conducted over a 120-day storage period on four different sample groups: traditional pastirma with fenugreek paste (FMP), traditional pastirma without fenugreek paste (MP), liquid smoke-treated with fenugreek paste (LSFMP), and liquid smoke-treated without fenugreek paste (LSMP). Moisture content in fresh fish, originally 71.63%, decreased to approximately 42–44% in all pastirma groups. Despite this notable reduction, statistical analysis revealed no significant differences in moisture levels among the processed groups ($p > 0.05$). Protein content increased to 30–33% following pastirma processing, and significant differences were observed between traditionally and smoke-treated samples ($p < 0.05$). Crude fat content, initially 4.07% in fresh fish, ranged between 11% and 17.99% in the final products. Significant differences were found between fenugreek-coated and non-coated groups in terms of lipid content ($p < 0.05$). Ash content increased significantly in all processed groups ($p < 0.05$). pH values exhibited irregular fluctuations over time; the most stable pH trend was observed in the LSFMP group. Lower pH levels were associated with enhanced microbial and chemical stability. Salt content was initially around 10% in all groups and varied between 9% and 11% throughout storage. Thiobarbituric acid (TBA) values, indicative of lipid oxidation, increased over time in all groups and exceeded the 5 mg MDA/kg threshold in some samples (MP, FMP, LSMP). Trimethylamine nitrogen (TMA-N) values fluctuated but remained below the 4 mg/100 g acceptability limit, while total volatile basic nitrogen (TVB-N) levels increased throughout storage yet stayed well below the 25 mg/100 g threshold considered indicative of high quality. The findings demonstrated that both liquid smoke and fenugreek paste contributed positively to quality stabilization. Total viable counts increased significantly over the storage period across all groups ($p < 0.05$), but the lowest microbial load was observed in the LSFMP group, processed with both liquid smoke and fenugreek paste. No growth of yeasts, molds, coliforms, or *Staphylococcus spp.* was detected in any group. Sensory evaluation scores (appearance, color, odor, taste, texture, and overall acceptability) declined gradually in all groups during storage; however, all samples remained within the "acceptable quality" range by day 120. Notably, liquid smoke treatment provided improvements in aromatic profile, microbial stability, and taste characteristics. In contrast, fenugreek paste negatively affected sensory quality in some cases. In conclusion, the combined use of liquid smoke and fenugreek paste was found to be effective in extending the shelf life and preserving the quality of *meagre pastirma* during cold storage.

Anahtar kelimeler:

Kaya levreği
Balık pastirması
Sıvı duman
Besin değeri
Kalite değişimi

Kaya Levreği (*Agyrosomus regius* Asso, 1801) Kullanılarak Hazırlanan Geleneksel ve Sıvı Dumanlı Balık Pastırmalarının Kalite Değişimlerinin Belirlenmesi

Öz: Bu çalışmada, geleneksel ve sıvı duman ilaveli kaya levreği pastırmalarının besin değerleri ve +4Cº'deki depolama koşullarında meydana gelen fiziko-kimyasal, mikrobiyolojik ve duyusal kalite parametreleri araştırılmıştır. Bu amaçla geleneksel çemenli (FMP), geleneksel çemeni sıyrılmış (MP), sıvı duman ekstraktı ilaveli çemenli (LSFMP) ve sıvı duman ekstraktı ilaveli çemeni sıyrılmış (LSMP) 4 grup üzerinden 120 günlük depolama süresi boyunca analizler yapılmıştır. Taze balıkta %71,63 olan nem oranı tüm pastırma gruplarında %42–%44 değerlerine düşmüş ve bu düşme sonrasında pastırma gruplarındaki nem oranları değişimi istatistiksel olarak anlamlı bulunmamıştır (p>0,05). Protein oranı pastırma işlemi sonrasında tüm gruplarda %30–%33 seviyelerine ulaşmış, dumanlı ve geleneksel yöntemler arasında anlamlı farklar tespit edilmiştir (p<0,05). Yağ oranı ise taze balıkta %4,07 iken pastırmalarda %11–%17,99 arasında değişmiştir; çemenli ve çemeni sıyrılmış gruplar arasında farklar önemli bulunmuştur (p<0,05). Kül oranı tüm pastirma gruplarında anlamlı şekilde artmıştır (p<0,05). pH değerleri zamanla düzensiz dalgalanmalar göstermiş olup en stabil grup LSFMP olmuştur. Düşük pH seviyeleri, ürünün mikrobiyal ve kimyasal stabilitesiyle ilişkilendirilmiştir. Tuz oranı başlangıçta tüm gruplarda %10 civarında olup depolama süresince %9–11 aralığında değişmiştir. Lipid oksidasyonunu gösteren TBA değerleri tüm gruplarda zamanla artmış ve bazı gruplarda (MP, FMP, LSMP) 5 mg MA/kg sınırını aşmıştır. TMA-N değerleri dalgalı seyir göstermiş, ancak kalite sınırlarının altında kalmıştır (<4 mg/100g). TVB-N değerleri de depolama boyunca artmış ancak tüm gruplarda "çok iyi" kabul edilen 25 mg/100g sınırının oldukça altında kalmıştır. Bulgular, sıvı duman ve çemenin kalite stabilitesini olumlu etkilediğini göstermektedir. Tüm gruplarda depolama süresince toplam bakteri sayısında istatistiksel olarak anlamlı artışlar gözlenmiş (p<0,05), en düşük mikrobiyal yük sıvı duman ve çemen kombinasyonuyla işlenen LSFMP grubunda tespit edilmiştir. Maya-küf, koliform ve *Staphylococcus spp*. tespit edilmemiştir. Duyusal analizlerde tüm gruplarda görünüş, renk, koku, tat, kıvam ve genel beğeni skorları zamanla azalmış olsa da 120. gün sonunda tüm gruplar hâlâ "iyi kalite" sınırında kalmıştır. Özellikle sıvı duman uygulamasının aromatik yapı, mikrobiyal stabilite ve tat parametrelerine olumlu etkiler sağladığı belirlenmiştir. Çemenin bazı durumlarda duyusal kaliteyi olumsuz etkileyebileceği gözlemlenmiştir. Sonuç olarak, sıvı duman ve çemen uygulamalarının balık pastirmalarının raf ömrünü uzatmada etkili olduğu ortaya konmuştur.

*Corresponding author: buminhanselcuk@gmail.com

How to cite this article: Selçuk, B.B. & Çakır, F (2025). Determination of Quality Changes in Traditional and Liquid Smoked Fish Pastirma Prepared from Meagre (*Argyrosomus regius*, Asso, 1801). COMU J. Mar. Sci. Fish, 8 (1): 103-120. doi:10.46384/jmsf.1723617

Introduction

In contemporary times, significant changes have been observed in traditional dietary habits as a result of socio-economic transformations, urbanization, and shifts in individual lifestyles. The intensification of work schedules, time constraints associated with urban living, and the prevalence of individualized modes of life have led to increased consumption of convenience foods. Consequently, the food industry has been compelled to develop products that are more practical, have extended shelf life, and are perceived as healthier (Şimşek, 2011). The health implications of dietary patterns are now better understood, and due to the association of red meat consumption with cardiovascular diseases, there is a growing preference for more balanced protein sources rich in unsaturated fatty acids. In this context, seafood—particularly fish—has gained prominence owing to its high nutritional value, high-quality protein content, low fat levels, and richness in omega-3 fatty acids (Ormancı, 2005; Kaban & Kaya, 2006; Dursun et al., 2010; Babur, 2017). Processing fish into various forms for consumption is significant both for extending shelf life and for meeting diverse consumer demands. Among the processed seafood products, one of the notable items in recent years is fish pastirma. This product is manufactured using fish meat by adapting traditional pastirma production techniques originally developed for red meat. Traditional pastirma is a meat product typically made from beef or water buffalo, involving salting, drying, and fenugreek paste application processes, and is defined under the TS 1071 standard (Anonymous, 2002). Fish pastirma, on the other hand, represents a modified version of this traditional method, tailored to the delicate structure of fish. Historically, pastirma production was transferred from Central Asia to Anatolia and has since become a significant element of Turkish food culture. Traditionally produced in many cities, particularly in Kayseri, pastirma today shows similarities to products made using comparable techniques in other countries (Ekmekçi, 2012).

Liquid smoke is a natural derivative of smoke condensate, commonly utilized to impart a characteristic smoky flavor without the need for conventional smoking methods. It offers several advantages, including ease of application, cost-effectiveness, precise dosage control, and the ability to minimize the presence of undesirable compounds. Most notably, it presents a significantly lower environmental impact compared to traditional smoking systems. However, its antimicrobial and preservative efficacy remains relatively limited. Liquid smoke applications are generally performed by immersing the product in liquid smoke, spraying it onto the surface, or exposing it to its vapor. In this way, only the smoke flavor and aroma are imparted to the product (Ayvaz vd., 2010; Racioppo vd., 2023).

In conclusion, fish pastirma production constitutes an important alternative food product that not only meets the increasing demand for healthy food but also preserves traditional flavors (Baygar et al., 2002; Babur, 2017). Studies in this field contribute to the valorization of Turkey’s rich aquatic resources and offer significant opportunities for the development of new products within the food industry.

In this study, in addition to the use of a different fish species (meagre, *Argyrosomus regius*) to contribute to fish pastirma production, the effects of incorporating liquid smoke treatment alongside the traditional fish pastirma process were investigated in terms of sensory properties and other quality parameters.

Material and Methods

Material

In this study, meagre (*Argyrosomus regius*, Asso, 1801) was used as the raw material. The fish were obtained from a private aquaculture facility located in İzmir, Türkiye. All specimens were transported to the processing technology laboratory under cold chain conditions to ensure freshness and minimize quality degradation.

The water-based liquid smoke extract used in the study was procured from a local food additive supplier.

Preparation of meagre (Argyrosomus regius) pastirma samples

The average weight and length of the meagre fish brought to the laboratory ranged from 1.5 to 2.2 kg and 42 to 64 cm, respectively. After removal of the heads and internal organs, the fish were thoroughly washed and drained. The fillets were then separated and skinned. Skinless fillets were rinsed again, patted dry, and subjected to the salting process. For salting, coarse commercial salt with a particle size of 1–2 mm was used, and the process was carried out over a period of three days. Following salting, the samples were washed to remove excess salt and subsequently dried.

The dried samples were then pressed under weights for two days. After pressing, the samples proceeded to the fenugreek paste coating stage. The fenugreek paste was prepared by proportionally mixing 450 g of *Trigonella foenum-graecum* (fenugreek) flour, 300 g of ground red pepper, and 225 g of dried garlic (processed in a blender). These dry ingredients were homogenized in a container, and water was gradually added to form a paste with the desired consistency.

Two types of fenugreek paste were prepared for coating the pastirma:
(1) Traditional fenugreek paste, composed of fenugreek flour, red pepper powder, blended dried garlic and water.

(2) Liquid smoke-enriched fenugreek paste, which included the same ingredients as the traditional formulation, with the addition of 5 mL of liquid smoke extract (diluted at a 1:2 ratio) per 1 kg of fenugreek paste.

During the coating phase, the fish fillets were divided into two groups. Half of the fillets were coated with the traditional fenugreek paste and the other half with the liquid smoke-enriched fenugreek paste, with a uniform thickness of 1–2 mm. All coated samples were then subjected to a 15-day ripening process.

After ripening, the pastirma samples were vacuum-packed and stored at refrigerated conditions of +4 ± 2 °C. The storage period lasted for 120 days, and sampling was conducted at specific intervals beginning on day 0.

The samples were categorized into four analytical groups:

1. MP (Meagre Pastirma): Pastirma samples from which the fenugreek paste was removed before analysis; only the fish meat was evaluated.

2. FMP (Fenugreek paste-Coated Meagre Pastirma): Pastirma samples analyzed together with the fenugreek paste, without separation.

3. LSMP (Liquid Smoke-Treated Meagre Pastirma): Samples coated with liquid smoke-enriched fenugreek paste, with the paste removed prior to analysis; only the fish meat was analyzed.

4. LSFMP (Liquid Smoke-Treated Fenugreek paste-Coated Meagre Pastirma): Samples coated with liquid smoke-enriched fenugreek paste, analyzed together with both fish meat and coating material.

Methods

Moisture analysis

The moisture content of both fresh fish and pastirma samples was determined in accordance with the oven-drying method outlined by AOAC (2000). For each analysis, 5 grams of homogenized sample were accurately weighed into pre-dried and tared petri dishes. The samples were then dried in a convection oven maintained at 105 °C for a period of 16 to 18 hours to ensure complete removal of moisture. Upon completion of drying, the samples were cooled in a desiccator and reweighed. Moisture content was calculated based on the weight loss of the samples and expressed as a percentage of the initial sample weight.

Protein analysis

Protein content was determined using the Kjeldahl method in accordance with AOAC (2000). Approximately 1 gram of homogenized sample was transferred into Kjeldahl digestion tubes, to which 20 mL of concentrated sulfuric acid (H_2SO_4) and a Kjeldahl catalyst tablet were added. The samples were subjected to digestion for 2.5 hours until a clear solution was obtained. Following digestion, 50 mL of distilled water was added to each tube, and the samples were transferred to a distillation unit. During distillation, the liberated ammonia was captured in a receiving flask containing 25 mL of boric acid solution and 3–5 drops of Toshiro indicator. The distillate was then titrated with 0.1 N hydrochloric acid (HCl), and protein content was calculated based on the volume of acid consumed during titration.

Fat analysis

The lipid content of fresh and pastirma samples was determined according to the method described by Bligh and Dyer (1959). For each analysis, 10 grams of homogenized sample were mixed with 50 mL of a methanol/chloroform solution (1:2, v/v). The resulting homogenate was rinsed with an additional 10 mL of the same solvent mixture and filtered through filter paper into a pre-weighed round-bottom flask. The filtrate was allowed to stand in a dark environment for 24 hours to ensure complete extraction. Subsequently, the flask was placed in a rotary evaporator (IKA, RV 10 basic) to remove the solvent under reduced pressure. After the evaporation process and separation of lipids, the flask was transferred to an oven at 105 °C and dried for 1 hour. It was then cooled in a desiccator to room temperature and reweighed to determine the lipid content gravimetrically.

Ash analysis

Ash content of the samples was determined using the dry ashing method in accordance with AOAC (2000). Approximately 2 grams of each sample were weighed into pre-ashed crucibles and incinerated in a muffle furnace at 550 °C until a uniform light gray (cigarette ash-like) residue was obtained. After ashing, the crucibles were cooled in a desiccator to room temperature and reweighed. The ash content was calculated based on the weight of the inorganic residue remaining after combustion.

pH analysis

The pH values were determined by mixing the homogenized samples with an equal volume (1:1) of distilled water. The pH was then measured using a calibrated pH meter (Hanna Instruments, HI2211 Basic).

Salt

Salt content was determined using the Mohr titration method. For each analysis, 10 grams of sample were mixed with 90 mL of distilled water and thoroughly homogenized. The mixture was then filtered into a volumetric flask using filter paper. A 10 mL aliquot of the filtrate was taken, and 1 mL of potassium chromate (K_2CrO_4) indicator was added. The solution was titrated with 0.1 N silver nitrate ($AgNO_3$) until a persistent color change was observed. The amount of titrant consumed was recorded and used to calculate the salt content (Vural & Öztan, 1996).

TVB-N analysis

The determination of total volatile basic nitrogen (TVB-N) was carried out in accordance with the European Commission (2005) method. For each analysis, 10 grams of sample were homogenized with 90 mL of 6% perchloric acid solution and then filtered into a volumetric flask. A 50 mL aliquot of the filtrate was transferred into a distillation tube, followed by the addition of 3 drops of antifoaming agent and 3 drops of phenolphthalein indicator. The sample was then subjected to distillation. The distillate was collected in 25 mL of boric acid solution containing 8 drops of Toshiro indicator. The resulting distillate was titrated with 0.01 N hydrochloric acid (HCl), and the volume of acid consumed was used to calculate the TVB-N content (Maghraby et al., 2013).

TMA-N analysis

Trimethylamine nitrogen (TMA-N) analysis was performed according to the method described by Schörmüller (1968). For each analysis, 10 grams of sample were homogenized with 7.5% trichloroacetic acid (TCA) and then filtered. From the filtrate, 4 mL was transferred into clean glass tubes. Subsequently, 1 mL of 20% formaldehyde, 10 mL of toluene, and 3 mL of 50% potassium hydroxide (KOH) were added. The mixture was vortexed for 2–3 minutes to allow for phase separation. After phase separation, 5 mL of the upper organic phase was collected and mixed with 0.02% picric acid solution. The absorbance of the samples was then measured against a blank at a wavelength of 410 nm using a spectrophotomete.

TBA analysis

TBA analysis was conducted according to the method described by Tarladgis et al. (1960). For each analysis, 10 grams of homogenized sample were combined with 50 mL of distilled water. The mixture was homogenized using an Ultra-Turrax and transferred into a Kjeldahl flask. An additional 47.5 mL of distilled water, 2.5 mL of hydrochloric acid (HCl), and a boiling chip were added. The sample was then distilled until 50 mL of distillate was collected in a round-bottom flask. From the distillate, 5 mL of the sample was mixed with 5 mL of a 0.02 N 2-thiobarbituric acid solution prepared in 90% glacial acetic acid. The mixture was then heated in a water bath at 100 °C for 40 minutes. After cooling, the absorbance was measured at 538 nm against a blank sample.

Microbiological analysis

Total viable count (TVC)

From the prepared serial dilutions, 1 mL of each sample was transferred using an automatic pipette onto sterile glass petri dishes containing Plate Count Agar. The samples were spread using the spread plate technique. For the enumeration of total psychrotrophic bacteria, the plates were incubated at 37 °C for 24–48 hours. After incubation, the formed colonies were counted, and the results were expressed as colony-forming units per gram (cfu/g) (FDA/BAM, 2009)

Total yeast and mold count

For the enumeration of total yeast and mold, 0.1 mL of the prepared dilution was transferred using an automatic pipette onto sterile glass petri dishes containing Plate Dextrose Agar (PDA). The samples were inoculated using the spread plate method. The inoculated plates were incubated at 25 ± 2 °C for 5 days. After incubation, the number of colonies formed was counted and recorded (FDA/BAM, 2009).

Total coliform bacteria count

For the determination of total coliform bacteria, appropriate dilutions of the samples were spread onto Violet Red Bile Agar (VRBA) plates using the spread plate method. The inoculated plates were incubated at 37 ± 1 °C for 24–48 hours. After incubation, the resulting colonies were counted, and the total coliform bacteria count was calculated (FDA/BAM, 2009).

Halophilic bacteria count

Halophilic bacteria enumeration was carried out according to the method described by Anonymous (2018). From the prepared serial dilutions, 1 mL of each sample was transferred using an automatic pipette onto glass petri dishes containing Plate Count Agar supplemented with 7% NaCl. The samples were inoculated using the spread plate method. The plates were incubated at 37 °C for 24–48 hours, after which the resulting colonies were counted to determine the total halophilic bacteria count (Ormancı, 2013).

Total Staphylococcus sp. count

For the enumeration of total *Staphylococcus spp.*, 0.1 mL of the prepared dilutions was spread-plated onto Baird-Parker (BP) agar. The plates were incubated at 35 ± 1 °C for 48 hours. During incubation, colonies exhibiting circular, smooth, convex, and moist characteristics with opaque zones were counted, and the total number of *Staphylococcus spp.* was calculated (FDA, 1998).

Sensory analyses

Sensory evaluations were conducted using a modified version of the Torry Scheme scale. The sensory analyses were performed by a trained panel of five experienced assessors. The products were evaluated based on color, appearance, odor, taste, texture, and overall acceptability, using a 5-point scale. According to Martinsdottir et al. (2009), the products were classified based on the given scores as follows: "5" = very good, "4" = good, "3" = moderate, "2" = poor, and "1" = very poor.

Statistical analyses

The data obtained were transferred to Microsoft Excel, where means and standard deviations were calculated. To evaluate differences between storage days and treatment groups, one-way ANOVA was performed using the SPSS version 21 statistical software package. Following the ANOVA, Duncan's and Tukey's multiple comparison post hoc tests were employed to determine statistically significant differences among the groups.

Results and Discussion

Proximate composition

Table 1 shows the proximate composition of meagre-based pastirma samples.

Table 1. Proximate composition values of meagre pastirma

Proximate	Fresh	MP	FMP	LSMP	LSFMP
Moisture (%)	71.63±1.60^{B}	43.09±1.20^{A}	44.51±1.72^{A}	42.20±0.83^{A}	43.58±3.09^{A}
Protein (%)	23.55±0.11^{A}	30.79±0.46^{B}	30.66±1.40^{B}	33.56±0.89^{C}	31.68±0.62B^{C}
Fat (%)	4.07±0.93^{A}	17.99±0.26^{D}	11.64±1.73^{BC}	15.00±2.38^{CD}	11.10±0.97^{B}
Ash (%)	1.22±0.03^{A}	9.49±0.27^{B}	9.03±0.92^{B}	9.32±0.80^{B}	9.26±1.00^{B}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

As a result of the proximate composition analyses, moisture content in the fresh material was determined as 71.63%. Following salting and fenugreek paste application processes, the moisture contents in the respective groups MP, FMP, LSMP, and LSFMP were found to be 43.09%, 44.51%, 42.20%, and 43.58%, respectively. Although a substantial decrease in moisture was observed compared to the fresh material, the moisture levels among all groups did not differ significantly (p> 0.05). The protein content of the fresh material was recorded as 23.55%. Due to water loss following the salting, pressing, and fenugreek paste treatments, protein contents increased in the respective groups to 30.79% (MP), 30.66% (FMP), 33.56% (LSMP), and 31.68% (LSFMP). While the protein levels of the liquid smoke-treated and traditionally prepared groups were relatively close, statistically significant differences were detected among them (p <0.05). Regarding crude fat content, fresh material had a fat ratio of 4.07%, whereas the values in MP, FMP, LSMP, and LSFMP groups were 17.99%, 11.64%, 15.00%, and 11.10%, respectively. The lowest fat content was found in the fresh material, and the highest in the MP group. Statistically significant differences were observed in fat content between groups with and without fenugreek paste (p <0.05), whereas no significant difference was found between smoked and unsmoked groups (p>0.05). Ash content in the fresh material was 1.22%. This value increased following salting, pressing, and paste application, and was determined to be 9.46% (MP), 9.03% (FMP), 9.32% (LSMP), and 9.26% (LSFMP). Although the ash content showed significant differences between the fresh material and pastirma groups (p <0.05), there were no statistically significant differences among the pastirma groups themselves (p>0.05). Studies on fish-based pastirma products remain scarce and are limited in number. The proximate composition of fish meat is known to vary substantially depending on a range of biological and environmental factors, including the geographical origin of the fish, season of capture, dietary habits, reproductive cycle, and environmental conditions, as well as post-harvest factors such as transportation, storage, and processing methods (Kök & Arslan, 2001; Arslan et al., 1997; Nizamlıoğlu et al., 1998). Babur (2017) reported the proximate composition of fresh seabass (*Dicentrarchus labrax*), with moisture, lipid, ash, and protein contents measured as 72.82%, 5.85%, 1.29%, and 18.42%, respectively. In the resulting pastirma products, these values shifted to 24.22% (moisture), 9.54% (lipid), 36.83% (ash), and 16.64% (protein). At the end of the storage period, protein contents in groups A (pastirma with fenugreek paste) and B (pastirma without fenugreek paste), C (extra-spiced pastirma with fenugreek paste), and D (extra-spiced pastirma without fenugreek paste) were determined as 36.42%, 36.10%, 35.29%, and 32.68%, respectively, while lipid levels were 10.20%, 9.74%, 9.00%, and 10.18%, and ash contents were 15.97%, 16.80%, 18.14%, and 19.74%, respectively. Arslan et al. (1997a) investigated vacuum-packaged mirror carp pastirma stored under refrigerated conditions and reported protein levels of 55.24–62.32%, fat contents of 4.55–10.64%, ash contents of 5.29–8.65%, and moisture levels of 23.06–30.98%. In non-vacuum-packaged samples, these ranges were 39.53–61.11% for protein, 6.48–21.59% for fat, 5.05–12.87% for ash, and 15.19–39.17% for moisture. In a subsequent study, Arslan et al. (1997b) evaluated mirror carp pastirma stored at 20 °C and recorded fat contents of 6.33–8.42% (vacuum-packed) and 7.77–14.88% (non-vacuum), ash contents of 9.75–11.94% and 5.51–15.60%, and moisture levels of 32.48–36.81% and 15.96–38.13%, respectively. Yapar (1993), in his study employing four distinct fenugreek paste formulations in pastirma production, found protein levels ranging from 23.24% to 55.99%, fat levels from 13.12% to 18.22%, and ash levels from 8.07% to 11.01%. Arslan and Kök (2001) investigated sliced, vacuum-packed barbel pastirma stored under refrigeration and observed fat contents of 36.81–38.87%, protein levels of 21.18–24.63%, and moisture contents of 36.31–37.86%. According to Yapar (1993), a proportional decrease in moisture content was observed during processing, while protein, fat, and ash contents increased accordingly. Gürbüz (1994) stated that salting, drying, and other processing methods applied during the conversion of raw meat into final products led to the partial removal of free water in the tissue, thereby significantly lowering the moisture levels compared to fresh meat. In a study conducted by Yapar (1993) on trout *pastirma* prepared using various fenugreek paste formulations, the moisture content decreased gradually throughout the ripening period, ranging between 14.71% and 50.62%. In line with these findings, the current study demonstrated that moisture content decreased in all treatment groups following *pastirma* processing, whereas the contents of protein, fat, and ash increased proportionally due to water loss. Although some variations in protein and fat values were recorded in the liquid smoke-treated groups, these differences are thought to result from the natural variability among the fish fillets used. Overall, the results obtained in this study are consistent with those reported by previous researchers.

Physicochemical analysis

pH

The pH values of pastirma produced from meagre (*Argyrosomus regius*) are presented in Table 2.

Table 2. pH values of meagre pastirma

pH	MP	FMP	LSMP	LSFMP
Fresh		$6.49±0.01^{f}$		
Day 0	$5.90±0.50^{Ae}$	$5.78±0.01^{Bb}$	$5.79±0.01^{Bbc}$	$5.70±0.02^{Ca}$
Day 15	$5.91±0.05^{Ae}$	$6.00±0.05^{Ad}$	$5.93±0.20^{Bg}$	$5.99±0.01^{Be}$
Day 30	$5.89±0.05^{Ade}$	$5.97±0.03^{Ac}$	$5.87±0.02^{Bfg}$	$5.97±0.01^{Bde}$
Day 45	$5.85±0.05^{Acd}$	$5.93±0.01^{Bc}$	$5.87±0.01^{Cf}$	$5.96±0.01^{Dde}$
Day 60	$5.85±0.45^{Acd}$	$5.84±0.05^{Ac}$	$5.85±0.01^{Ade}$	$5.94±0.01^{Bd}$
Day 75	$5.79±0.01^{Ab}$	$5.87±0.25^{Bc}$	$5.86±0.06^{Be}$	$5.89±0.01^{Bc}$
Day 90	$5.87±0.05^{Bcde}$	$5.56±0.20^{Bc}$	$5.85±0.02^{Aa}$	$5.98±0.03^{Cde}$
Day 105	$5.73±0.10^{Ba}$	$5.75±0.30^{Aa}$	$5.63±0.03^{Cb}$	$5.66±0.04^{ABa}$
Day 120	$5.83±0.25^{Bbc}$	$5.82±0.20^{ABb}$	$5.80±0.02^{Bcd}$	$5.76±0.02^{Ab}$

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

In the present study, the pH value of the fresh product was determined to be 6.49 ± 0.01. Irregular increases and decreases in pH values were observed in all pastirma groups throughout the storage period. These fluctuations between the beginning and end of storage were found to be statistically significant in all groups ($p<0.05$). At the beginning of storage, the pH values of the pastirma groups were recorded as 5.90 (MP), 5.78 (FMP), 5.79 (LSMP), and 5.70 (LSFMP), while on the 120th day of storage, these values were 5.83, 5.81, 5.80, and 5.76, respectively. Statistically significant differences were observed between fenugreek paste-coated and non-coated groups on certain days ($p<0.05$). Similarly, significant differences in pH values were also observed between traditionally processed and liquid smoke-treated groups ($p<0.05$). Among the groups, LSFMP was the most stable in terms of pH, while MP exhibited the greatest decrease in pH values over time.

According to Gram and Dalgaard (2002), pH variation in fish meat is an indirect indicator of several biochemical processes, including protein degradation, amino acid deamination, microbial fermentation, and organic acid production. The statistically significant differences observed on certain days between groups may be related to the effects of processing methods on quality parameters such as microbial activity, enzymatic reactions, and lipid oxidation. The findings of this study indicate that the combined use of smoke and fenugreek paste plays a crucial role in maintaining product quality and stability during storage. In a study investigating the effects of different fenugreek paste mixtures on pastirma quality, Nizamlıoğlu et al. (1998) reported that these mixtures influenced microbial growth and led to a gradual decrease in pH over time. Similarly, Kök and Arslan (2003) studied the effect of different fenugreek paste coating durations on pastirma made from Barbus esocinus and found that the pH values ranged between 5.70 and 5.96 over a 90-day storage period. They concluded that longer exposure to fenugreek paste helped maintain pH stability. Doğruer et al. (1998) emphasized the bacteriostatic effect of garlic in fenugreek paste, stating that it inhibited mold and yeast growth, thereby indirectly contributing to pH stability. These researchers collectively suggest that microbial growth, amino acid deamination, and the accumulation of organic acids over time are key contributors to pH variation. Kılınç and Sürengil (2016) reported that the pH values in a pastirma-like product made from whiting (Merlangius merlangus) fluctuated between 6.1 and 6.4 during a 15-day dry salting process. Following the coating phase of the product, the pH was recorded at 6.2 at the beginning of storage at 15–20 °C, subsequently decreasing to 5.6 by the fifth day of storage. The authors also indicated that lactic acid bacteria counts showed a strong correlation with pH levels throughout processing, and that increases in lactic acid bacteria were associated with corresponding decreases in pH values. Furthermore, the decrease in pH values is generally considered an indicator of bacterial fermentation and the onset of spoilage (Gram & Dalgaard, 2002). The pH findings of the current study are consistent with the observations reported by these researchers. Although fluctuations in pH values were observed across all groups during storage, the magnitude of change remained relatively small (around 0.30). The low pH values in the products are likely attributed to the positive microbial and chemical effects of fenugreek paste and the added liquid smoke used in the coating process.

Salt: The salt contents of pastirma produced from meagre (*Argyrosomus regius*) are presented in Table 3.

Table 3. Salt content of meagre pastirma

Salt	MP	FMP	LSMP	LSFMP
Fresh		1.01±0.04^{a}		
Day 0	10.06±0.12^{Ae}	10.16±0.06^{ABe}	10.00±0.06^{Ag}	10.40±0.06^{Cf}
Day 15	9.75±0.08^{Bc}	10.21±0.02^{Ce}	9.55±0.04^{Ae}	10.50±0.14^{Df}
Day 30	9.42±0.03^{Cb}	9.24±0.03^{Ab}	9.23±0.05^{Ac}	9.33±0.06^{Bb}
Day 45	9.93±0.04^{Cd}	9.43±0.05^{Bc}	9.08±0.05^{Ab}	9.42±0.06^{Bb}
Day 60	9.81±0.02^{Cc}	9.64±0.05^{Bd}	9.36±0.06^{Ad}	9.35±0.08^{Ab}
Day 75	10.34±0.07^{Cf}	10.90±0.08^{Df}	9.89±0.03^{Ag}	10.19±0.05^{Be}
Day 90	11.61±0.03^{Bı}	11.51±0.10^{Bg}	10.34±0.16^{Ah}	10.34±0.25^{Aef}
Day 105	11.23±0.09^{Ch}	10.85±0.06^{Bf}	9.99±0.04^{Ag}	9.93±0.07^{Ad}
Day 120	10.85±0.06^{Bg}	10.85±0.06^{Bf}	9.70±0.08^{Af}	9.61±0.05^{Ac}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

The salt content of the fresh material was determined as 1.01%. Following the salting and fenugreek paste treatments, the initial salt values in the pastirma groups at the beginning of storage were found to be 10.06% (MP), 10.16% (FMP), 10.00% (LSMP), and 10.40% (LSFMP), respectively. Throughout the storage period, salt contents in all pastirma groups showed statistically significant variations (p <0.05). During storage, salt levels ranged between 9.42–11.61% in the MP group, 9.24–11.51% in the FMP group, 9.08–10.34% in the LSMP group, and 9.33–10.50% in the LSFMP group. In a study conducted by Yapar (1993) on trout pastirma, the salt content at the beginning of maturation was reported as 10.03%, while on the 30th day it ranged between 10.44% and 11.34% among groups. The researcher noted that salt changes over time were significant (p <0.05), whereas differences among formulations were not statistically significant (p> 0.05). Similarly, Babur (2017) reported that the salt content in sea bass pastirma was 10.01% in groups A (pastirma with fenugreek paste) and B (pastirma without fenugreek paste), and 9.08% in groups C (extra-spiced pastirma with fenugreek paste) and D (extra-spiced pastirma without fenugreek paste) at the beginning of storage, while at the end of storage, values were 9.91%, 9.51%, 9.02%, and 9.19%, respectively. Arslan et al. (1997a) reported that salt values in mirror carp pastirma ranged from 6.92% to 10.50% in vacuum-packed samples, and from 7.25% to 12.31% in non-vacuum samples. In another study, Arslan et al. (1997b) found that during storage at market temperature, the salt content ranged between 8.33–12.91% in vacuum-packed and 7.75–14.68% in unpacked samples. Arslan et al. (2001), in their study on pastirma made from *Barbus esocinus*, reported salt contents between 6.59% and 9.30%. Gürbüz (1994) indicated that salt content in pastirma may vary over time depending on the type and amount of salt used, the processing method, degree and duration of drying, environmental temperature, and storage conditions. According to the Turkish Food Codex, the maximum permitted salt content is 8.5%, while for meat pastirma specifically, it should not exceed 6%. In the current study, these values were exceeded, which is attributed to the use of higher salt concentrations aimed at better microbiological preservation of the product. Nevertheless, the salt contents observed in most fish-based pastirma studies are consistent with the findings of this study.

Thiobarbituric Acid (TBA)

The findings related to the TBA values of pastirma produced from meagre (*Argyrosomus regius*) are presented in Table 4.

Table 4. TBA (Malondialdehyde/kg) values of meagre pastirma

TBA MA/kg	MP	FMP	LSMP	LSFMP
Fresh		1.60±0.06^{a}		
Day 0	1.64±0.03^{Ba}	1.80±0.16^{Bb}	2.31±0.02^{Cb}	1.48±0.05^{Aa}
Day 15	2.03±0.03^{Ab}	2.46±0.11^{Bd}	3.88±0.03^{Cd}	2.35±0.12^{Bb}
Day 30	2.60±0.29^{Bd}	2.18±0.13^{Ac}	3.41±0.09^{Cc}	2.49±0.07^{ABbc}
Day 45	3.00±0.02^{Cf}	2.84±0.08^{Be}	3.99±0.04^{Dde}	2.73±0.06^{Ad}
Day 60	3.03±0.04^{Af}	2.89±0.03^{Be}	4.76±0.08^{Bg}	2.69±0.26^{Ccd}
Day 75	2.26±0.03^{Bc}	2.81±0.06^{Ae}	4.10±0.10^{De}	2.43±0.11^{Cb}
Day 90	2.80±0.10^{Ae}	3.19±0.06^{Bf}	4.79±0.06^{Cg}	2.76±0.14^{Dd}
Day 105	3.74±0.05^{Ag}	4.72±0.11^{Dg}	4.42±0.20^{Cf}	3.98±0.07^{Be}
Day 120	6.18±0.02^{Dh}	5.51±0.02^{Bh}	5.83±0.02^{Ch}	4.06±0.03^{Ae}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

TBA (Thiobarbituric Acid) values were used in this study as an indicator of lipid oxidation occurring during the storage of pastirma products and to assess the level of deterioration in terms of product quality. According to the results obtained, TBA values showed statistically significant increases in all pastirma groups throughout the storage period ($p < 0.05$). At the beginning of storage, TBA values in the MP, FMP, LSMP, and LSFMP groups were recorded as 1.64, 1.80, 2.31, and 1.48 mg MA/kg, respectively, and increased by day 120 to 6.18, 5.51, 5.83, and 4.06 mg MA/kg, respectively. Statistical analyses performed on the same days across different groups revealed that the applied processing methods and varying formulations had a significant influence on the TBA values ($p < 0.05$). According to Schormüller (1968), TBA values exceeding 5 mg MA/kg indicate the onset of oxidative spoilage, while values above 7–8 mg MA/kg are considered beyond the threshold for consumption (Çorapçı, 2013). Based on these limits, it was observed that by the end of storage, all groups except LSFMP approached the critical threshold, with the MP group exceeding it. Babur (2017), in her study on sea bass pastirma, reported TBA values at the end of a 90-day storage period as 6.28, 3.29, 5.04, and 3.02 mg MA/kg for groups A (pastirma with fenugreek paste), B (pastirma without fenugreek paste), C (extra-spiced pastirma with fenugreek paste), and D (extra-spiced pastirma without fenugreek paste) respectively. The highest TBA value was found in the fenugreek paste-coated group. These findings indicate that the combination of liquid smoke and fenugreek paste exhibits a limiting effect on TBA formation and thus contributes positively to oxidative stability.

Trimethylamine Nitrogen (TMA-N)

The TMA-N values of pastirma produced from meagre are presented in Table 5**.**

According to the data obtained from the analyses, TMA-N values exhibited a fluctuating pattern across all pastirma groups from the beginning to the end of the storage period. The differences between the TMA-N values measured at the beginning and end of storage were found to be statistically significant ($p < 0.05$). While statistically significant differences were observed between traditional and smoked groups at the beginning of storage ($p < 0.05$), similar values were detected across all groups on days 45, 60, and 75 ($p > 0.05$). Compared to fresh fish meat, a significant increase in TMA-N values was observed in all pastirma groups starting from day 0 and continuing throughout the storage period ($p < 0.05$). At the beginning of storage, TMA-N values in the MP, FMP, LSMP, and LSFMP groups were recorded as 1.93, 2.05, 2.73, and 2.48 mg/100 g, respectively, while by the end of storage these values had changed to 2.78, 2.67, 2.43, and 2.69 mg/100 g. Although irregular changes were noted during storage, an overall increase in TMA-N values was observed at the end of the storage period. Babur (2017), in his study on sea bass pastirma, reported fluctuating TMA-N values across all groups during storage. He identified a very low initial TMA-N value of 0.07 mg/100 g in fresh material, with all groups showing increases until day 15, followed by a decline. On day 90, TMA-N values in groups A (pastirma with fenugreek paste), B (pastirma without fenugreek paste), C (extra-spiced pastirma with fenugreek paste), and D (extra-spiced pastirma without fenugreek paste) were 0.52, 0.88, 0.61, and 0.76 mg/100 g, respectively. The differences between the fenugreek paste-coated and extra-spiced non-coated groups were statistically significant ($p < 0.05$), while the differences between non-coated and extra-spiced fenugreek paste-coated groups were not statistically significant (p> 0.05). According to international standards, acceptable TMA-N values range between 10 and 15 mg/100 g. In fish, TMA-N values of 12 mg/100 g and above indicate spoilage, values between 4–10 mg/100 g are considered marketable, and values of 4 mg/100 g or less are regarded as high quality (Gökoğlu, 1994). Although the TMA-N values obtained in the current study were higher than those reported by Babur (2017) (0.52–0.88 mg/100 g), they still fell within the "high quality" classification for all pastirma groups even on the 120th day of storage, as indicated by Gökoğlu's (1994) criteria (Table 5). It was observed that the presence or absence of fenugreek paste had no significant effect on TMA-N values in both traditional and liquid smoke-enriched groups. However, the groups treated with liquid smoke extract exhibited slightly lower TMA-N levels compared to their traditionally processed counterparts. The TMA-N values obtained in all groups remained below the consumption threshold reported by Gökoğlu (1994), indicating that the products fall within the good quality category.

Table 5. TMA-N (mg/100 g) values of pastirma produced from meagre

TMA-N mg/100g	MP	FMP	LSMP	LSFMP
Fresh		1.21±0.16^{a}		
Day 0	1.93±0.12^{Abc}	2.05±0.17^{Ac}	2.73±0.24^{Be}	2.48±0.50^{ABcd}
Day 15	2.59±0.12^{Bde}	2.71±0.17^{Bf}	2.34±0.06^{Acd}	2.49±0.13^{ABcd}
Day 30	2.10±0.66^{ABbc}	1.75±0.09^{Ab}	2.87±0.08^{Be}	2.10±0.68^{ABbc}
Day 45	1.88±0.03^{Ab}	1.95±0.07^{Ac}	1.83±0.08^{Ab}	1.84±0.06^{Ab}
Day 60	2.01±0.06^{Abc}	2.04±0.04^{Ac}	1.99±0.07^{Ab}	2.00±0.02^{Abc}
Day 75	2.23±0.06^{Abcd}	2.25±0.06^{Ad}	2.18±0.04^{Ac}	2.19±0.03^{Abcd}
Day 90	2.34±0.05^{Acd}	2.47±0.06^{Be}	2.27±0.04^{Acd}	2.33±0.03^{Abcd}
Day 105	2.63±0.07^{Bde}	2.59±0.06^{Bef}	2.40±0.06^{Ad}	2.38±0.09^{Acd}
Day 120	2.78±0.10^{Be}	2.67±0.09^{Bf}	2.43±0.11^{Ad}	2.69±0.06^{Bd}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (*p* <0.05).

Additionally, the TMA-N levels determined in this study were higher than those reported by Babur (2017). This difference is presumed to be associated with the variation in fish species and the differences in processing methods applied.

Total volatile basic nitrogen (TVB-N)

The TVB-N values of pastirma produced from meagre (*Argyrosomus regius*) are presented in Table 6.

In our study, a significant increase in TVB-N values was observed in all groups from the beginning of storage throughout the entire storage period (p <0.05). At the beginning of storage, TVB-N values were determined as 1.90 mg/100 g (MP), 2.09 mg/100 g (FMP), 2.06 mg/100 g (LSMP), and 2.29 mg/100 g (LSFMP). By the end of storage, these values had increased to 6.34, 5.64, 5.48, and 5.82 mg/100 g, respectively. In general, differences between fenugreek paste-coated and non-coated groups suggest that fenugreek paste has an effect on TVB-N levels. Regarding statistical differences between groups, significant differences were observed between MP and FMP, as well as between LSMP and LSFMP at the beginning of storage (p <0.05), while TVB-N values in FMP and LSMP groups were found to be statistically similar (p> 0.05). At the end of storage, significant differences were detected between the MP group and the other three groups (FMP, LSMP, LSFMP) (p <0.05). The increase in TVB-N values in the LSMP and LSFMP groups appeared to slow down after day 60. This trend is thought to be related to the delaying effect of liquid smoke and fenugreek paste on the accumulation of volatile nitrogen compounds. Babur (2017), in her study on sea bass pastirma, reported a TVB-N value of 15.36 mg/100 g in fresh material and values ranging from 19.93 to 22.02 mg/100 g at the beginning of storage in pastirma groups. Toward the end of storage, TVB-N values increased in all pastirma groups, reaching the consumption threshold on day 45 for group A (pastirma with fenugreek paste), day 30 for groups B (pastirma wihout fenugreek paste) and D (extra spiced pastirma without fenugreek paste), and day 60 for group C (extra spiced pastirma with fenugreek paste). According to Gökoğlu (1994), in fish and fishery products, a TVB-N value of up to 25 mg/100 g is considered "very good," up to 30 mg/100 g as "good," up to 35 mg/100 g as "marketable," and values above 35 mg/100 g are classified as "spoiled" (Kietzmann et al., 1969; Ludorff & Meyer, 1973; Lang, 1979). The TVB-N values obtained in this study were significantly lower than those reported in the literature, and even at the end of storage, all values remained below the spoilage limit of 35 mg/100 g, falling within the "very good" quality category. This is thought to be due to the high freshness of the fish used in the study as well as species-specific differences.

Table 6. TVB-N (mg/100 g) values of pastirma produced from meagre

TVB-N mg/100g	MP	FMP	LSMP	LSFMP
Fresh		1.84±0.04^{a}		
Day 0	1.90±0.02^{Aa}	2.09±0.03^{Bb}	2.06±0.03^{Bb}	2.29±0.02^{Cb}
Day 15	2.60±0.02^{Ab}	2.84±0.03^{Cc}	2.74±0.02^{Bc}	2.86±0.02^{Cc}
Day 30	3.49±0.02^{Ac}	3.70±0.02^{Cd}	3.61±0.02^{Bd}	3.68±0.03^{Cd}
Day 45	4.55±0.10^{Ad}	4.43±0.08^{Ae}	4.42±0.02^{Ae}	4.49±0.05^{Bea}
Day 60	4.66±0.03^{Ae}	4.77±0.02^{Cf}	4.63±0.02^{Af}	4.71±0.04^{Bf}
Day 75	4.87±0.04^{Bf}	5.12±0.02^{Cg}	4.79±0.02^{Af}	4.87±0.04^{Bf}
Day 90	5.09±0.05^{Bg}	6.22±0.02^{Dj}	4.98±0.07^{Ag}	5.29±0.03^{Cg}
Day 105	6.43±0.03^{Cı}	5.89±0.04^{Bı}	5.57±0.04^{Ah}	5.70±0.29^{ABh}
Day 120	6.34±0.03^{Bh}	5.64±0.29^{Ah}	5.48±0.33^{Ah}	5.82±0.01^{Ah}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

Microbiological Analysis

Total viable count

The total viable count (TVC log cfu/g) cvalues of pastirma produced from meagre are presented in Table 7.

Halophilic Microorganism Count

The halophilic microorganism (HBC log cfu/g) counts of pastirma produced from meagre are presented in Table 8.

Table 7. Total viable count (TVC log cfu/g) values of meagre pastirma

TVC (log cfu/g)	MP	FMP	LSMP	LSFMP
Fresh		2.30±0.07^{a}		
Day 0	2.70±0.04^{Bb}	2.48±0.04^{Ab}	2.69±0.09^{Bb}	2.53±0.13^{ABb}
Day 15	2.73±0.17^{Ab}	2.60±0.04^{Ac}	2.77±0.07^{Ab}	2.65±.0.04^{Ac}
Day 30	3.74±0.01^{Cc}	3.03±0.10^{Ad}	3.30±0.04^{Bc}	3.02±0.08^{Ad}
Day 45	4.07±0.09^{Cd}	3.34±0.05^{Ae}	3.74±0.02^{Bd}	3.32±0.05^{Ae}
Day 60	4.64±0.03^{Ce}	4.62±0.02^{Cf}	3.99±0.09^{Be}	3.48±0.04^{Af}
Day 75	4.74±0.02^{Cef}	4.73±0.02^{Cg}	4.18±0.03^{Bf}	3.63±0.03^{Ag}
Day 90	4.80±0.01^{Cf}	4.79±0.02^{Cgh}	4.27±0.01^{Bfg}	4.21±0.02^{Ag}
Day 105	4.84±0.02^{Cf}	4.86±0.02^{Ch}	4.68±0.05^{Bg}	4.60±0.02^{Ag}
Day 120	5.10±0.01^{Cg}	5.06±0.04^{Cj}	4.97±0.03^{Bh}	4.85±0.04^{Ah}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

PCA (Plate Count Agar) values serve as indicators of chemical spoilage or microbial activity increases during the shelf life of meagre pastirma. According to the data obtained, all pastirma groups exhibited statistically significant increases in total viable counts (TVC) throughout the storage period ($p < 0.05$). At the beginning of storage, total bacterial counts ranged from 2.48 to 2.70 log cfu/g, reaching 4.85 to 5.10 log cfu/g by day 120. Statistical comparisons performed between groups on the same days revealed that product formulation and processing techniques had a clear impact on microbial spoilage ($p < 0.05$). Notably, the liquid smoke–fenugreek paste combination (LSFMP) group had the lowest total bacterial count throughout the 120-day storage period. These findings demonstrate the effectiveness of both traditional and modern preservation methods in delaying microbial spoilage in delicatessen products derived from fish. The total bacterial count values observed under 7% salt concentration showed statistically significant increases across all groups depending on storage time ($p < 0.05$).

Table 8. Halophilic microorganism counts of meagre pastirma

HBC (log cfu/g)	MP	FMP	LSMP	LSFMP
Fresh		0.41± 0.01^{a}		
Day 0	0.66±0.24^{Cb}	0.44±0.24^{Ab}	0.90±0.05^{Db}	0.51±0.20^{Bb}
Day 15	0.69±0.21^{Ab}	0.49±0.20^{Ab}	0.89±0.11^{Ab}	0.48±0.44^{Ab}
Day 30	1.25±0.06^{Ccd}	0.86±0.12^{Ac}	0.99±0.13^{Bb}	0.89±0.14^{Ac}
Day 45	1.54±0.08^{Dc}	1.39±0.10^{Ccd}	1.28±0.17^{Bc}	1.04±0.08^{Acd}
Day 60	2.25±0.02^{Ccd}	2.18±0.08^{Cde}	1.72±0.07^{Bcd}	1.33±0.04^{Ade}
Day 75	2.32±0.02^{Dd}	2.22±0.04^{Cdf}	1.74±0.06^{Bcd}	1.52±0.05^{Aef}
Day 90	2.46±0.02^{Dd}	2.36±0.03^{Cdf}	1.81±0.03^{Bde}	1.59±0.02^{Aef}
Day 105	2.63±0.03^{Dd}	2.41±0.03^{Cdf}	2.24±0.09^{Bde}	1.63±0.02^{Aef}
Day 120	3.48±0.01^{Dd}	3.44±0.01^{Cf}	2.99±0.09^{Be}	2.73±0.02^{Af}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods ($p < 0.05$).

Generally, the highest microbial loads were observed in the MP and FMP groups, whereas the groups treated with liquid smoke showed lower microbial levels compared to traditional pastirma groups. At the end of the 120-day storage period, the group with the lowest PCA value was LSFMP (2.73 ± 0.02 log cfu/g), which was statistically significantly different from the other groups. These results highlight the protective effect of the liquid smoke–fenugreek paste combination and suggest that it could be a potential solution for extending the shelf life of meagre pastirma. In this study, total yeast–mold, total coliforms, and *Staphylococcus spp*. were not detected in any of the fresh or pastirma samples throughout the storage period. Özdemir et al. (1999) reported total aerobic mesophilic bacterial counts in meat pastirma between 10^5 and 10^7 cfu/g. Doğruer et al. (1995) recorded microbial loads between 2.8×10^7 and 7.0×10^7 cfu /g on day 1, and between 2.2×10^6 and 3.4×10^6 cfu /g on day 60. Arslan and Kök (2001) found aerobic counts between 4.0 and 7.7×10^4 cfu /g in vacuum-packed Barbus esocinus pastirma. Babur (2017) reported fluctuating total bacterial counts in pastirma with fenugreek paste, pastirma without fenugreek paste, extra-spiced pastirma with fenugreek paste, and extra-spiced pastirma without fenugreek paste sea bass pastirma groups, recording final values of 3.6, 6.7, 6.5, and 3.2 log cfu /g, respectively, after 90 days. Yeast and mold counts were reported as 2.3, 4.1, 4.5, and 0 log cfu/g, respectively. In another study, Arslan et al. (1997a) reported yeast–mold values in vacuum-packed mirror carp pastirma between 5.2×10^2 and 2.5×10^3 cfu /g. Yapar (1993), in a study testing different fenugreek paste formulations in trout pastirma, found yeast–mold counts between 10 and 2.2×10^3 cfu /g. Kılınç and Sürengil (2016) reported that in their study on a pastirma-like product prepared from whiting (*Merlangius merlangus*), the aerobic mesophilic bacterial count was 5.08 log CFU/g at the beginning of the drying process, which significantly decreased to 3.24 log CFU/g after 15 days of dry salting and drying. However, during the second phase—storage at 20 °C—they observed a rapid increase in total aerobic mesophilic bacterial counts. A similar trend was also noted for *Enterobacteriaceae*, coliforms, and lactic acid bacteria. Moreover, the authors stated that *Staphylococcus aureus*, *Escherichia coli*, and yeasts and molds were not detected in any of the samples throughout the study. Arslan and Kök (2001) also reported no yeast–mold growth throughout storage in their study. According to the Turkish Food Codex, the acceptable yeast–mold limit in pastirma is 10^2 cfu /g. Some studies have also reported the presence of coliform bacteria during the pastirma production stages (Yapar, 1993; Babur, 2017). In addition to enhancing sensory attributes such as flavor, aroma, and color, the smoking process plays a crucial role in extending the shelf life of the product (Ledesma et al., 2016; Dışhan et al., 2021). Gürbüz et al. (1997) reported that the application of smoking before and after fenugreek paste coating significantly affected total aerobic mesophilic bacteria, *Staphylococcus–Micrococcus*, and yeast–mold populations in pastirma products. The total viable counts obtained in this study were notably lower than those found in meat pastirma studies and were similar to those in other fish pastirma studies. Although the general microbiological limit for consumption in food products is accepted as 10^6 cfu /g (6 log cfu/g), due to some values reaching 10^5 cfu /g by day 120, consumption beyond this point may pose a risk to human health. All pastirma groups were found to comply with criteria regarding yeast–mold, total coliforms, and *Staphylococcus spp*. Moreover, the application of liquid smoke was shown to have a significant effect on microbial inhibition.

Sensory analysis

Appearance

The appearance scores from the sensory evaluation of pastirma produced from meagre are presented in Table 9.

Table 9. Appearance scores of meagre pastirma

Appearance	MP	FMP	LSMP	LSFMP
Day 0	5.00±0.10^{Af}	4.80±0.45^{Af}	4.80±0.45^{Ae}	4.80±0.45^{Af}
Day 15	5.00±0.11^{Af}	4.60±0.55^{Aef}	4.60±0.55^{Ade}	4.60±0.55^{Aef}
Day 30	4.60±0.55e^{Af}	4.40±0.55^{Adef}	4.40±0.55^{Acde}	4.40±0.55^{Adef}
Day 45	4.40±0.55^{Ade}	4.20±0.45^{Acdef}	4.20±0.45^{Abcde}	4.20±0.45^{Acdef}
Day 60	4.20±0.45^{Acde}	4.00±0.10^{Abcde}	4.00±0.10^{Aabcd}	4.00±0.15^{Abcde}
Day 75	4.00±0.12^{Abcd}	3.80±.45^{Aabcd}	3.80±0.45^{Aabc}	3.80±0.45^{Aabcd}
Day 90	3.80±0.45^{Aabc}	3.60±0.55^{Aabc}	3.60±0.55^{Aab}	3.60±0.55^{Aabc}
Day 105	3.60±0.55^{Aab}	3.40±0.55^{Aab}	3.60±0.55^{Aab}	3.40±0.55^{Aab}
Day 120	3.40±0.55^{Aa}	3.20±0.45^{Aa}	3.40±0.55^{Aa}	3.20±.45^{Aa}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

Appearance, one of the key sensory quality parameters, plays a crucial role in forming the first impression for consumers. In meat products, appearance encompasses multiple dimensions such as color intensity, brightness, surface integrity, fat–membrane structure, moisture–dryness balance, and the uniformity of the fenugreek paste or surface coating. It also serves as a visual indicator of microbiological stability, signs of oxidation, and the effectiveness of processing conditions. In our study, appearance scores showed a statistically significant decline over time in all pastirma groups ($p<0.05$). Although numerical differences in scores were observed among the groups, no statistically significant difference was found between them ($p> 0.05$). The MP group maintained a "very good quality" rating up to day 75, while the FMP, LSMP, and LSFMP groups remained in this category up to day 60. By day 120, appearance scores in all pastirma groups had decreased to between 3.20 and 3.40, which is still considered to reflect "good quality." Toward the end of storage, discoloration in the fenugreek paste and whitening on the meat surface were noted in all groups, contributing to the decline in appearance scores.

Color

The color scores from the sensory evaluation of pastirma produced from meagre are presented in Table 10. Color parameter scores showed a statistically significant decrease over time in all groups ($p<0.05$). However, despite numerical differences in scores, no statistically significant differences were observed among the groups at the same time points ($p> 0.05$). At the beginning of storage, scores reached up to 5.00 (particularly in the LSMP group), but by day 120, they had declined to a range between 3.20 and 3.60.

Table 10. Odor scores of meagre pastirma

Color	MP	FMP	LSMP	LSFMP
Day 0	4.80±0.45^{Ad}	4.60±0.55^{Ae}	5.00±0.15^{Af}	4.80±0.45^{Af}
Day 15	4.60±0.55^{Acd}	4.40±0.55^{Ade}	4.80±0.45^{Aef}	4.60±0.55^{Aaf}
Day 30	4.40±0.55^{Abcd}	4.20±.45^{Acde}	4.60±0.55^{Adef}	4.40±0.55^{Adef}
Day 45	4.40±0.55^{Abcd}	4.00±0.10^{Abcde}	4.40±0.55^{Acdef}	4.20±0.45^{Acdef}
Day 60	4.20±0.45^{Aabc}	4.00±.15^{Abcde}	4.20±0.45^{Abcde}	4.00±0.10^{Abcde}
Day 75	4.00±0.10^{Aabc}	3.80±0.45^{Aabcd}	4.00±0.15^{Aabcd}	3.80±0.45^{Aabcd}
Day 90	4.00±0.15^{Aabc}	3.60±0.55^{Aabc}	3.80±0.45^{Aabc}	3.60±.0.55^{Aabc}
Day 105	3.80±0.45^{Aab}	3.40±0.55^{Aab}	3.60±0.55^{Aab}	3.40±0.55^{Aab}
Day 120	3.60±0.55^{Aa}	3.20±0.45^{Aa}	3.40±0.55^{Aa}	3.20±0.45^{Aa}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

Odor

The odor scores from the sensory evaluation of pastirma produced from meagre are presented in Table 11.

At the beginning of storage, odor scores in all pastirma groups ranged between 4.8 and 5.0, indicating that the products were classified as “very good quality.” Odor scores declined to the “good quality” level by day 75 in the MP, FMP, and LSMP groups, and by day 45 in the LSFMP group. By day 120, only the LSFMP group was rated as “moderate quality” in terms of odor. A statistically significant decrease in odor scores was observed in all groups from day 0 to day 120 ($p < 0.05$). However, although numerical differences were noted among the pastirma groups on the same days, these differences were not statistically significant ($p > 0.05$). The observed sensory changes in odor are thought to result from several factors, including the reduction of aromatic compounds during storage, the formation of undesirable odors due to secondary products from lipid oxidation, and a decline in aroma quality caused by microbial activity.

Table 11. Odor scores of meagre pastirma

Odor	MP	FMP	LSMP	LSFMP
Day 0	5.00±0.01^{Af}	5.00±0.01^{Af}	5.00±0.10^{Af}	4.80±0.45^{Af}
Day 15	4.80±0.45^{Aef}	4.80±0.45^{Aef}	4.80±0.45^{Aef}	4.40±0.55^{Aef}
Day 30	4.60±0.55^{Adef}	4.60±0.55^{Adef}	4.60±0.55^{Adef}	4.20±0.45^{Ade}
Day 45	4.40±0.55^{Acdef}	4.40±0.55^{Acdef}	4.40±0.55^{Acdef}	4.00±0.10^{Acde}
Day 60	4.20±0.45^{Abcde}	4.20±0.45^{Acde}	4.20±0.45^{Abcde}	3.80±0.45^{Abcde}
Day 75	4.00±0.01^{Babcd}	4.00±0.10^{Bcd}	4.00±0.10^{Aabcd}	3.60±0.55^{Babcd}
Day 90	3.80±045^{Aabc}	3.80±0.45^{Abc}	3.80±0.45^{Aabc}	3.40±0.55^{Aabc}
Day 105	3.60±0.55^{Aab}	3.40±0.55^{Aab}	3.60±0.55^{Aab}	3.20±0.45^{Aab}
Day 120	3.40±0.55^{Aa}	3.20±.045^{Aa}	3.40±0.55^{Aa}	3.00±0.10^{Aa}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (p <0.05).

Flavor

The flavor scores from the sensory evaluation of pastirma produced from meagre are presented in Table 12.

According to the results of the sensory analysis, flavor scores decreased significantly over time in all groups ($p < 0.05$). Initially, the MP and LSMP groups received the highest scores (5). Although the liquid smoke-added groups (LSMP and LSFMP) received higher flavor scores than the MP and FMP groups throughout the storage period, these differences were not found to be statistically significant ($p > 0.05$). The MP and FMP groups were classified as "very good quality" up to days 30 and 45, respectively. Between days 45 and 105, they were rated as "good quality," with the FMP group dropping to "moderate quality" on day 105, and the MP group on day 120. In contrast, the "very good quality" classification for the liquid smoke groups lasted until day 75 in the LSMP group and day 60 in the LSFMP group. By day 120, both groups were still rated as "good quality." This trend reflects the expected sensory deterioration in product quality during storage. The decline in flavor scores is likely due to factors such as increased microbial load, lipid oxidation, protein degradation, and the breakdown of aromatic compounds. The application of liquid smoke had a positive effect on flavor, helping to preserve taste quality for a longer period. This effect was observed in both the traditional liquid smoke group (LSMP) and the fenugreek paste-coated version (LSFMP). Additionally, fenugreek paste appeared to have a negative impact on flavor scores over time. The FMP group, which had the lowest flavor scores, suggests that fenugreek paste may contribute to sensory deterioration either through oxidative or microbial mechanisms.

Table 12. Flavor scores of meagre pastirma

Flavor	MP	FMP	LSMP	LSFMP
Day 0	$5.00±0.01^{Bg}$	$4.40±0.55^{Af}$	$5.00±0.01^{Bf}$	$4.80±0.45^{ABf}$
Day 15	$4.40±0.55^{Af}$	$4.20±0.45^{Aef}$	$4.80±0.45^{Aef}$	$4.60±0.55^{Aef}$
Day 30	$4.20±0.45^{Aef}$	$4.00±0.01^{Adef}$	$4.60±0.55^{Adef}$	$4.40±0.55^{Adef}$
Day 45	$4.00±0.01^{Adef}$	$3.80±0.45^{Acdef}$	$4.40±0.55^{Acdef}$	$4.20±0.45^{Acdef}$
Day 60	$3.80±0.45^{Acde}$	$3.60±0.55^{Abcde}$	$4.20±0.45^{Abcde}$	$4.00±0.01^{Abcde}$
Day 75	$3.60±0.55^{Abcd}$	$3.40±0.55^{Aabcd}$	$4.00±.01^{Aabcd}$	$3.80±0.45^{Aabcd}$
Day 90	$3.40±0.55^{Aabc}$	$3.20±0.45^{Aabc}$	$3.80±0.45^{Aabc}$	$3.60±0.55^{Aabca}$
Day 105	$3.20±0.45^{Aab}$	$3.00±0.01^{Aab}$	$3.60±0.55^{Aab}$	$3.40±0.55^{Aab}$
Day 120	$3.00±0.15^{Aa}$	$2.80±0.45^{Aa}$	$3.40±0.55^{Aa}$	$3.20±0.55^{Aa}$

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods ($p < 0.05$).

Texture: The texture scores from the sensory evaluation of pastirma produced from meagre are presented in Table 13.

At the beginning of storage, texture scores in all groups ranged between 4.40 and 4.80, classifying the products as "very good quality." Throughout the storage period, a decrease in texture scores was observed in all pastirma groups over time. These time-dependent changes were found to be statistically significant ($p < 0.05$). The MP group was rated as having the most favorable texture throughout the storage period. By day 120, the FMP, LSMP, and LSFMP groups had declined to the "moderate quality" level. Texture differences between the MP group and the other groups on days 105 and 120 were statistically significant ($p < 0.05$). Texture defines the chew resistance, mouthfeel, and structural integrity of meat products. It is a critical parameter in terms of product acceptance, perceived quality, and shelf life. Particularly, moisture loss during storage can cause notable changes in texture characteristics.

Table 13. Texture scores of meagre pastirma

Texture	MP	FMP	LSMP	LSFMP
Day 0	4.60±0.55^{Aa}	4.40±0.55^{Af}	4.80±0.45^{Af}	4.60±0.55^{Af}
Day 15	4.40±0.55^{Aa}	4.20±0.45^{Aef}	4.60±0.55^{Aef}	4.40±0.55^{Aef}
Day 30	4.20±.45^{Aa}	4.00±0.10^{Adef}	4.40±0.55^{Adef}	4.20±0.45^{Adef}
Day 45	4.00±0.10^{Aa}	3.80±0.45^{Acdef}	4.20±0.45^{Acdef}	4.00±0.10^{Acdef}
Day 60	4.40±0.55^{Ba}	3.60±.55^{Abcde}	4.00±0.10^{ABbcde}	3.80±0.45^{ABbcde}
Day 75	4.00±0.71^{Aa}	3.40±0.55^{Aabcd}	3.80±0.45^{Aabcd}	3.60±0.55^{Aabcd}
Day 90	4.00±0.71^{Aa}	3.20±0.45^{Aabc}	3.60±0.55^{Aabc}	3.40±0.55^{Aabc}
Day 105	4.20±0.45^{Ba}	3.00±0.10^{Aab}	3.40±0.55^{Aab}	3.20±0.45^{Aab}
Day 120	4.20±0.45^{Ba}	2.80±0.45^{Aa}	3.20±0.45^{Aa}	3.00±0.10^{Aa}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (*p* <0.05).

Overall Acceptability: The overall acceptability scores from the sensory evaluation of pastirma produced from meagre are presented in Table 14.

As storage progressed, a significant decrease ($p < 0.05$) in overall acceptability scores was observed in all groups. While the initial scores (day 0) ranged between 4.60 and 4.80, by day 120 they had declined to between 2.80 and 3.20. The LSMP group maintained the highest overall acceptability scores. Particularly between days 0 and 60, the scores remained between 4.80 and 4.00, indicating a high level of consumer acceptance. It is believed that the application of liquid smoke enhanced the aromatic profile of the pastirma, contributing to greater consumer preference. Although the MP group showed a similar trend to the LSMP group, a noticeable quality decline was observed after day 75, with scores dropping to 3.20 by day 120—still within the sensory acceptability range. The LSFMP group remained at acceptable levels during the early storage period (0–60 days), but a decline in acceptability began after day 75, reaching 3.00 by day 120. Among all groups, the GPC group had the lowest overall acceptability score on day 120 (2.80 ± 0.45). Sensory characteristics are among the most critical factors determining the consumer appeal of a product. Particularly for highly perishable products such as seafood, determining freshness and optimizing sensory qualities are among the most reliable parameters for product marketing and quality assurance.

Table 14. Overall acceptability scores of meagre pastirma

Overall Acceptability	MP	FMP	LSMP	LSFMP
Day 0	4.80±0.45^{Af}	4.60±0.55^{Ae}	4.80±0.45^{Af}	4.60±0.55^{Ae}
Day 15	4.60±0.55^{Aef}	4.40±0.55^{Ade}	4.60±0.55^{Aef}	4.40±0.55^{Ade}
Day 30	4.40±0.55^{Adef}	4.20±0.45^{Ade}	4.40±0.55^{Adef}	4.20±0.45^{Ade}
Day 45	4.20±0.45^{Acdef}	4.00±0.10^{Acde}	4.20±0.45^{Acdef}	4.00±0.15^{Acde}
Day 60	4.00±0.10^{Abcde}	3.80±0.45^{Abcd}	4.00±0.12^{Abcde}	3.80±0.45^{Abcd}
Day 75	3.80±0.45^{Aabcd}	3.40±0.55^{Aabc}	3.80±0.45^{Aabcd}	3.80±0.45^{Abcd}
Day 90	3.60±0.55^{Aabc}	3.20±.45^{Aab}	3.60±0.55^{Aabc}	3.40±0.55^{Aabc}
Day 105	3.40±.0.55^{Aab}	3.20±0.45^{Aab}	3.40±0.55^{Aab}	3.20±0.45^{Aab}
Day 120	3.20±0.45^{Aa}	2.80±0.45^{Aa}	3.20±0.45^{Aa}	3.00±0.10^{Aa}

Groups: MP (Traditional pastirma without fenugreek paste), FMP (Traditional pastirma with fenugreek paste), LSMP (liquid smoke-treated without fenugreek paste), LSFMP (Liquid smoke-treated pastirma with fenugreek paste). Results are expressed as mean ± standard deviation. Statistically significant differences among treatment groups are indicated by uppercase letters, while lowercase letters represent significant differences across storage periods (*p* <0.05).

Results from sensory analyses indicate that when sensory quality is insufficient, the marketing and consumption of such products can pose significant risks. Although sensory evaluation is a key criterion for consumption, it is more effective when supported by physical and chemical analyses (Baygar et al., 2002). Babur (2017), in his study involving different formulations of sea bass pastirma, reported that although all groups A (pastirma with fenugreek paste), B (pastirma without fenugreek paste), C (extra-spiced pastirma with fenugreek paste), D (extra-spiced pastirma without fenugreek paste) initially displayed "very good quality" in terms of taste, odor, color, appearance, crispness, and overall acceptability, all groups experienced significant sensory quality declines after day 45. She noted that the extra-spiced pastirma with fenugreek paste group was the most favored in terms of taste and color, while fenugreek paste-coated samples (A, C) developed white spots due to salt crystallization by day 60, and non-coated samples (B, D) showed surface yellowing. Anıl (1988) reported that the pastirma he prepared had excellent sensory qualities in terms of appearance, color, taste, and texture, and that vacuum packaging and slicing allowed the product to retain its quality for three months at 20°C. Doğruer (1992) emphasized that both salting duration and pressing had significant effects on texture, appearance, taste, and color. Yapar (1993) reported that fish pastirma prepared with different fenugreek paste formulations retained their sensory quality for at least 30 days. Arslan et al. (1997a) stated that in non-vacuum-stored samples, excessive water loss caused the texture to become firmer and chewability to decline, suggesting that vacuum packaging prolongs the sensory acceptability of pastirma. Arslan and Kök (2001) concluded that fish pastirma prepared with high-quality raw material and stored under refrigeration in vacuum packaging could retain its quality for 90 days or more. Although the sensory quality parameters obtained in the present study align with findings from the literature, the products in this study retained their sensory quality within acceptable limits for a longer period up to 120 days. It is suggested that both fenugreek paste and liquid smoke contributed significantly to attributes such as flavor, aroma, and taste, while vacuum packaging, as recommended by previous researchers, had a substantial effect on maintaining color, texture, and appearance.

Conclusion

In this study, changes in the physicochemical, microbiological, and sensory properties of pastirma products produced from meagre (*Argyrosomus regius*) using traditional and liquid smoke-assisted methods were evaluated throughout their shelf life. The results demonstrated that the applications of liquid smoke and fenugreek paste were effective in preserving product quality. In particular, the LSFMP group, which was treated with both liquid smoke and fenugreek paste, stood out with the lowest microbial load and the highest sensory scores over the 120-day storage period. Furthermore, the fact that TVB-N, TMA-N, and TBA values in all pastirma groups remained within legal limits supports the products' suitability for consumption. Sensory evaluations also revealed that liquid smoke–treated groups exhibited superior performance in terms of taste, odor, and overall acceptability. It can be concluded that the application of liquid smoke may serve as an effective preservative and flavor-enhancing agent in the production of fish pastirma, contributing both to microbial stability and sensory quality. In addition, fenugreek paste provided positive contributions to taste and odor when used in certain combinations, although its potential to negatively affect sensory quality in some cases suggests that formulation optimization is necessary. The broader application of liquid smoke technology in fish pastirma production offers not only the potential to extend shelf life but also provides an alternative means of enhancing sensory attributes such as flavor, odor, and aroma.

Acknowledgements

This study includes a section from the doctoral dissertation of Buminhan Burkay Selçuk. The research was not supported by any institution. The authors would like to express their gratitude to Group Sagun for providing the materials. Special thanks are extended to Dr. Nilüfer Çiriğ-Selçuk for her financial and moral support; to Assoc. Prof. Dr. İsmail Burak Daban, Prof. Dr. Nermin Berik, Prof. Dr. Mehmet Seçkin Aday, Research Assistant Selin Özge Dinç, Assoc. Prof. Dr. Muhammet Yüceer, Assoc. Prof. Dr. Alkan Öztekin, Lecturer Dr. Gençtan Erman Uğur, and Şeyma Nur Bacaksız for their valuable assistance and dedication during the laboratory studies.

Conflict of Interest

The authors affirm that they do not have any conflicts of interest.

Ethics Approval

This study does not require ethical committee approval.

Author Contributions

Buminhan Burkay Selçuk: Sample collection, analyses, data gathering, laboratory work, writing, and data analysis. Fikret Çakır: Analyses, study design, statistical analysis and calculations, and manuscript editing.

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COMU J Mar Sci Fish 2025 8(1): 121-126
https://doi.org/10.46384/jmsf.1623071

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

SHORT COMMUNICATION

Occurrence of the Rare Fish *Lobotes surinamensis* (Bloch, 1790) in the Capo Peloro Lagoon (Central Mediterranean Sea): Some Implications

Mauro Cavallaro*, Sergio De Matteo, Salvatore Giacobbe

University of Messina, Italy https://orcid.org//0000-0001-6054-0867
University of Messina, Italy https://orcid.org//0009-0005-7924-954X
University of Messina, Italy https://orcid.org//0000-0002-4619-4862

Received: 29.01.2025 /Accepted: 07.03.2025 / Published online: 14.07.2025

Keywords:

Warm water fishes
Spreading
First record
Brackish waters

Abstract: As part of fish fauna monitoring within the “Capo Peloro Lagoon” Natural Reserve, a juvenile specimen of the rare warm-water fish *Lobotes surinamensis* (Bloch, 1790) was detected through a collaborative citizen science effort. This represents the first recorded occurrence of the species in the Strait of Messina area. The finding is particularly noteworthy, as the Strait functions as a climatic cold barrier between the warmer Tyrrhenian and Ionian basins, playing a crucial role in regulating the northward spread of thermophilic species. Furthermore, this record reinforces the importance of the Capo Peloro Lagoon as both a nursery and refuge area, as well as a potential steppingstone in the dispersal of both exotic and native thermophilic species.

Anahtar kelimeler:

Sıcak su balıkları
Yayılım
İlk kayıt
Acısu

Capo Peloro Lagünü’nde (Orta Akdeniz) Nadir Balık *Lobotes surinamensis* (Bloch, 1790)’in Görünüşü: Bazı Önermeler

Öz: “Capo Peloro Lagünü” Doğal Rezervi’nde yürütülen balık faunası izleme çalışmaları kapsamında, nadir görülen bir sıcak su balığı türü olan *Lobotes surinamensis*’in (Bloch, 1790) juvenil bir bireyi, vatandaş bilimi iş birliğine dayalı gözlemler sayesinde tespit edilmiştir. Bu, Messina Boğazı bölgesinden bildirilen ilk kayıttır. Bu bulgu oldukça önemlidir, çünkü boğaz, daha sıcak olan Tiren ve İyon denizleri arasında iklimsel bir soğuk bariyer görevi görmekte ve termofilik türlerin kuzeye doğru yayılımını düzenlemede kilit bir rol oynamaktadır. Ayrıca, bu kayıt Capo Peloro Lagünü’nün bir kuluçka ve sığınma alanı olarak önemini vurgularken, aynı zamanda hem egzotik hem de yerli termofilik türlerin yayılımında potansiyel bir sıçrama noktası işlevi gördüğünü ortaya koymaktadır.

Introduction

Among marine thermophilic fish species, the Atlantic tripletail, *Lobotes surinamensis* (Bloch, 1790), is one of the most widely distributed species, occurring in tropical and subtropical waters worldwide (Riede, 2004). As a euryhaline species, it occurs both in estuarine and marine environments, including open sea, but preferentially in shallow waters (Myers, 1999; Kuiter e Tonozuka, 2001). Despite its broad distribution and wide habitat range, records of *L. surinamensis* are infrequent, and therefore it is considered a rare species in some countries, and “first records” are often highlighted (Wirtz et al., 2013; Parmar et al., 2023). Moreover, due to the low number of catches, its ecology is poorly known, including its native range (Ushakow et al., 2024). In the eastern Atlantic, the south-west coast of the Iberian Peninsula is considered the northerly limit of this thermophilic species, but rare records have been reported from higher latitudes, such as in the Bay of Biscay (Iglésias et al., 2020) and Bristol Channel (Ellis et al., 2024. Such occurrences, which are consistent with the known periodic northern shift of warm-water pelagic assemblages (Beaugrand, 2009) as well as with trans-Atlantic “rafting” of Caribbean organisms (Holmes et al., 2015; Garzia et al., 2022), have probably increased their frequency as a result of global warming. The areal expansion driven by climatic factors has been particularly evident in the Mediterranean Sea, (Bilge et al., 2017), where *L. surinamensis* was first reported in Palermo, by Doderlein (1875), who considered this species indigenous to the Western Atlantic, This contrasts with the recent opinion of Guidetti (2020) who considers the Mediterranean as “part of the native range of the tripletail”. Another earlier report from the Mediterranean Sea was from the Island of Rhodes (Tortonese, 1946), providing evidence for the long-standing occurrence of

*Corresponding author: mcavallaro@unime.it

How to cite this article: Cavallaro, M., De Matteo, S., & Giacobbe, S. (2025). Occurrence of the rare fish *Lobotes surinamensis* (Bloch, 1790) in the Capo Peloro Lagoon (Central Mediterranean Sea): some implications. COMU J. Mar. Sci. Fish, 8 (1): 121-126. doi:10.46384/jmsf.1623071

this thermophilic species in the Eastern basin. The northward spreading of this species in Mediterranean was particularly evident in the Adriatic (Dulčić & Dragičević, 2011; Dulčić et al., 2014a, 2014b) where it reached the maximum possible limit range in the Gulf of Trieste (Bettoso et al., 2016), before descending down along the Italian coasts (Tiralongo, 2018; Licchelli & Denitto, 2020). Such trend, which agrees with recently proposed models of non-indigenous fish species (Perzia et al., 2022), has been related to the Adriatic ingressions phenomenon (Pallaoro, 1988), and more recently to the Adriatic-Ionian Bimodal Oscillating System (BiOS) (Civitarese et al., 2010), with a possible role of anticyclonic circulation of North Ionian gyre in determine the spreading of *L. surinamensis* in all Adriatic Sea (Dulčić et al., 2014a). Similarly, the presence of an adult specimen from France (Guidetti, 2020) represented the northernmost possible record in the North-western Mediterranean Sea. Ergüden et al. (2020), reporting the record of five specimens, four of which were juveniles, agree with Bilge et al. (2017) in considering *L. surinamensis* as common in Eastern and Southern Mediterranean, although based on sporadic reports, as the above cited Tortonese (1946) or, more recently, (Akyol & Kara, 2012; Artüz & Fricke, 2019). We rather agree with Minasidis et al. (2020) which suggested an increasing occurrence of this species in the region. In our opinion, both areal expansion and increasing frequency need to be considered, as early suggested by Deidun et al. (2010) for Maltes coastal waters and proved by some evidence of stable populations of *L. surinamensis* in south Tyrrhenian and, maybe, in Ionian waters (Tiralongo et al., 2018), with the occasional report of a single specimen from the Strait of Messina (Montesanto et al., 2022). In such a contest, the Strait of Messina is not a simple connection between such two basins, as representing a peculiar “micro-sector” (marine biogeographical Sector 4 for the Italian Fauna) with a proper complex ecology (Bianchi et al., 2012; Giacobbe & Oliverio, 2024).

Due to above described reasons, the record of a young *L. surinamensis* specimen in the Strait of Messina, namely in the Capo Peloro Lagoon, described here, is a remarkable update on the current spreading of this rare species in the Mediterranean Sea.

Material and Methods

In the framework of a national program aimed to implicate a Driving Forces-Pressure-State-Impact-Response (DPSIR) model to Sicily transitional areas, the monitoring of local ichthyofauna has been carried out in the Capo Peloro Lagoon since March 2022 (Gatì et al., 2024). Standard visual census techniques were employed, coupled with a citizen science approach.

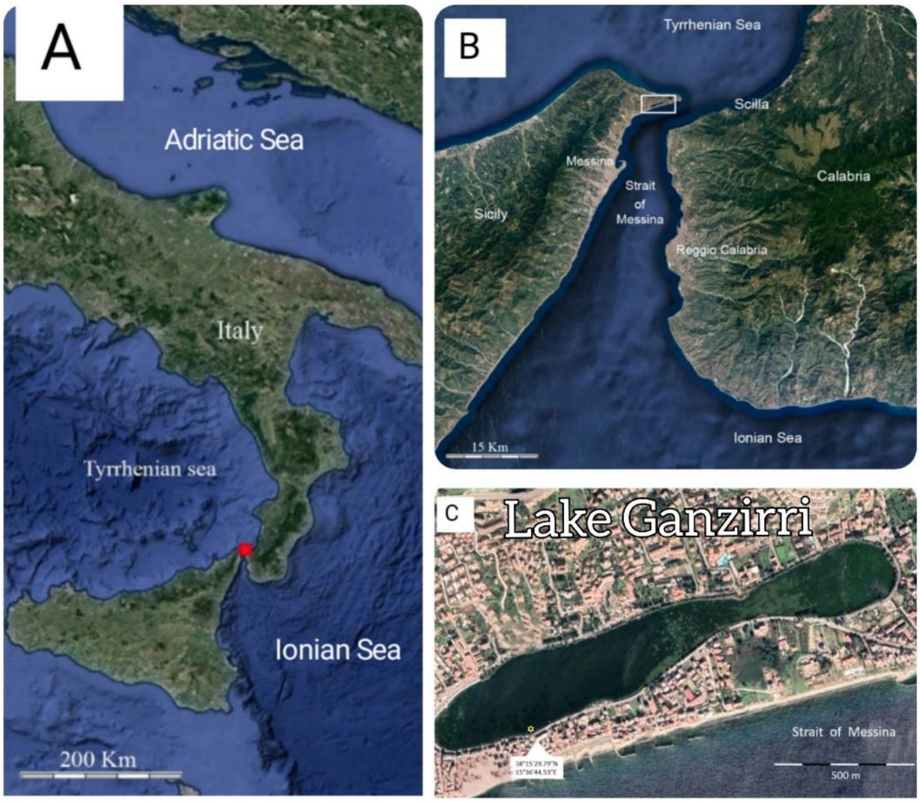

Figure 1. Locations of the occurrences of juvenile specimens of *Lobotes surinamensis* in Ganzirri Lake. The yellow asterisk indicates the point of observation.

The lagoon, belonging to the Strait of Messina ecosystem (Fig. 1), is subject to various protection regimes, in the framework of the Natura 2000 network (cod. ITA 030008). It includes two connected but differently featured basins, i.e., Lake Faro, known for its notable depth and a peculiar meromictic regime (Leonardi et al., 2009), and the markedly brackish Lake Ganzirri (Azzaro et al., 2005). On September 22th, 2024, in the morning, during an excursion along Lake Ganzirri, Mrs. Lucrezia Pietramala had the opportunity to observe a sepecimen of *L. surinamensis* in a clam farming area (38°15'29” N; 15°36'38"E).

Results and Discussion

L. surinamensis is a fish characterized by a bright yellowish color, and unusual behavior. The specimen floating on its side, near a piling, made short dives along the artefact, re-emerging at regular intervals and resuming its position lying on a body side. During the observations, some photos taken with a smartphone were subsequently brought to our attention. The photos, despite the distance of the subject from the lake shore and the turbidity of the lake water, clearly showed a juvenile specimen of *L. surinamensis*, being in accordance with the main morphological features and chromatic pattern described in Heemstra et al. (1986) and Tortonese (1990). The specimen, whose length was estimated to be almost 20 cm, was not captured due to the restrictions imposed by the protection regime of the Capo Peloro Natural Reserve and was no longer observable in the afternoon hours (Fig. 2).

The increasing northward records of *L. surinamensis* throughout the Mediterranean Sea, together with reportedly stable populations in both the Tyrrhenian and Ionian waters of Sicily (Tiralongo et al., 2018, might misleadingly suggest that the presence of this species in the Strait of Messina is a foregone conclusion.

Although since the 20th century the northern shift of the main biogeographic divide, defined by the 15 °C sea-surface February isotherm- has shifted from the north Ionian Sea to the south Tyrrhenian (Bianchi et. al., 2012), the upwelling-related cold waters of the Messina Strait maintain their role as a thermal barrier between the two basins (Azzaro et al., 2004). This means that warm water species can reach this stretch of the sea throughout the Mediterranean surface circulation, but their settlement and northward area expansion are significantly hampered. In this respect, the exceptionally high surface water temperatures recorded in the years 2023 and 2024 in the Strait of Messina (https://arpa.sicilia.it) may have a facilitating role. The record of a specimen in September, further supports previous findings of De Pirro et al. (1997) and Zava et al. (2007) who indicated that all reported captures of *L. surinamensis* in Italian waters have occured in Autumn.

The curious habit of *L. surinamensis*, characterizd by staying on the sea surface, lying on one side, often near floating objects, suggests a preferential association with rafting communities (Thiel & Gutow, 2005), where sessile organisms provide a food resource (Franks et al., 2003) and offer opportunities for ambushing fast swimming prey such as Carangidae (Massuti & Renones, 1996; Zava et al., 2007; Tiralongo et al., 2018). Such behavior, although also observed in bentho-pelagic adults, is peculiar to the epipelagic juveniles, , mimicking a floating leaf that acts as a camouflage against predators (Franks et al., 2003). Moreover, while *L. surinamensis* larvae exclusively occur offshore in the Atlantic , adults and juveniles inhabit estuaries, where they combine wind-driven passive movements with active selection of polyhaline habitats (Ushakow et al., 2024): Such a pattern adequately explains the modality of adult and juvenile records in the Mediterranean, and their prevalent occurrence in transitional environments (Bettoso et al., 2016).All these considerations give to the occurrence of *L. surinamensis* in Lake Ganzirri a precise meaning.

Figure 2. The juvenile *Lobotes surinamensis* specimen recorded.

This record, in fact, increases the number of juveniles found in the Mediterranean, strengthening the hypothesis of effective reproduction in and suggesting that larvae might occur in areas close to Tyrrhenian and Ionian seas, where stable populations have been identified (Tiralongo et al., 2018). From such breeding areas (most probably from the Ionian Sea), juveniles can enter the Peloro Lagoon, both passively and actively, as reported for other non-native pelagic fishes (Karachle et al., 2016). The lagoon, being the only brackish habitat within some hundred kilometers of the coast, might represent an available nursery area for this species and a refuge considering the proximity of colder marine waters. Moreover, a resident population in the lagoon, being interposed between the north-western and south-eastern Mediterranean basins, could favor genetic fluxes between the respective *L. surinamensis* stocks.

Lastly, according to the opinion of Minasidis et al. (2020 and all references within), that the increasing occurrences of *L. surinamensis* in the Mediterranean may have been influenced by the increasing number of citizen science projects and the use of social media. In this context we emphasize that the present record resulted from the report of a collaborative citizen.

The present report of a juvenile *L. surinamensis* in the Capo Peloro Lagoon not only covers a Mediterranean biogeographic sector, i.e., the Strait of Messina, that is not yet reported for the occurrence of this species, but also involves some important implications. The Strait of Messina, in fact, directly connects the western and eastern Mediterranean basins, as acts as a climatic barrier, playing a key role in regulating the northward spreading of thermophilic species. In this context, the Capo Peloro brackish system plays a significant role as both a nursery and refuge area. This is particularly important for euryhaline species, especially those whose life cycles span both marine and brackish environments. In this regard, the potential confirmation of *L. surinamensis* settling in the lagoon could serve as a valuable case study, highlighting the need to monitor the Capo Peloro system as a possible steppingstone in the spread of both exotic and native thermophilic species.

Acknowledgments

The authors are grateful to Mrs. Lucrezia Pietramala, whose observations make possible the present report. A special acknowledgement is due to dr. Maria Molino (director of the oriented reserve of Cape Peloro, Metropolitan city of Messina) for permission and facilities during the DPSIR project.

Conflict of Interest

There is no conflict of interest in this study.

Author Contributions

The authors contributed equally to the drafting of this paper.

Ethics Approval

No ethics committee approval is required for this study.

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COMU J Mar Sci Fish 2025 8(1): 127-130
https://doi.org/10.46384/jmsf.1628210

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

SHORT COMMUNICATION

First Record from the Libyan Coast of *Parophidion vassali* Risso, 1810 (Ophidiiformes: Ophidiidae)

Eman S. Alfergani* [1], Mohammad El-Mabrok[2], Sara A.A Almabruk[3]

[1]Department of Marine Science, Faculty of Science, Omar Al-Mukhtar University, Libya https://orcid.org//0000-0002-5602-1535
[2]Department of Zoology, Faculty of Science, Omar Al-Mukhtar University, Libya https://orcid.org//0000-0002-7163-8795
[3]Higher Institute of Science and Technology, Libya https://orcid.org//0000-0003-0754-4951

Received: 28.01.2025 /Accepted: 07.04.2025 / Published online: 14.07.2025

Keywords:

Parophidion vassali
Libya
Otolith,
Rare species

Abstract: This research presents the first recording of a rare species of fish from Libya. A specimen of *Parophidion vassali* approximately 108.1 mm in length was identified using the characteristics of the otoliths. The specimen was recovered from the contents of the stomach of a lizard fish (*Synodus saurus*) collected from the coast of Benghazi, eastern Libya, during November 2021.

Anahtar kelimeler:

Parophidion vassali
Libya
Otolit
Nadir tür

Parophidion vassali Risso, 1810 (Ophidiiformes: Ophidiidae)'nin Libya Kıyılarından İlk Kaydı

Öz: Bu çalışmada nadir görülen bir balık ilk defa Libya'dan bildirilmiştir. Yaklaşık 108.1 mm uzunluğundaki bir *Parophidion vassali* otolit özelliklerine bakılarak tayin edilmiştir. Bu örnek, Kasım 2021 tarihinde Libya'nın doğusundaki Bingazi kıyılarında yakalanan bir kertenkele balığının (*Synodus saurus*) mide muhteviyatından elde edilmiştir.

Introduction

Ophidiidae are benthopelagic fish found in all oceans' tropical and subtropical regions. They inhabit soft substrates in shallow seas to depths of over 1,000 m below the surface, there are approximately 200 species found all over the world. (Nielsen *et al*., 1999; Golani *et al*., 2006). Subfamily Ophidinae, which includes three species: O*phidion barbatum* (L. 1758), *Ophidion rochei* (Muller, 1845), and *Parophidion vassali* (Risso, 1810), recorded in the Mediterranean Sea (Capape *et al*., 2016; Bradai, 2000; Othman *et al*., 2020).

Parophidion vassali is a species that resembles an eel. It can be found in coastal Posidonia oceanica meadows and shallow rocky-sandy habitats (Capape *et al*., 2016; Pergent *et al*., 2012). However, it is thought to reside in deeper waters up to 600 m (Matallanas and Casadevall, 1990). It is one of the fish that depends on vocal communication, and there is a difference in the sound-producing apparatus between males and females (Eric *et al*., 2022). It has an elongated body, a brown backside, and a white belly, with pelvic fin rays that extend below the eye and reach the base of the pectoral fin, and fins that lack spines (Nielsen *et al*., 1999; Capape *et al*., 2016).

Material and Methods

On November 13, 2021, one specimen of *P. vassali* was found in the stomach contents of an Atlantic lizardfish (*Synodus saurus*; TL: 23.6 cm, TW: 88.1 g) collected by fishermen from the port of Benghazi city (32° 36' N, 20° 03' E). The head region was dissected, and the right and left otoliths were removed from their capsules, cleaned, dried, and the photographs were captured using a digital camera (Olympus, model NO. C-7070) attached to a dissecting microscope (OPTECH, model SZ). Only the left otolith was used for measurements and weighed to the nearest 0.0001 g (OW, g) by using a sensitive balance (Ohaus Adventurer SL, model Adventurer Pro AS214).Otolith length (OL, mm), height (OH, mm), and area (OA, mm^2) were measured to the nearest 0.1 mm using image processing (Digimizer software, version 4). The percentage of otolith length out of the standard length of the fish (OL/SL %) was calculated. Four indices of otolith shape were calculated as follows: ellipticity, roundness, aspect ratio, and rectangularity (Tuest *et al*., 2008). The equation $O_R = 1000 * OA * SL^{-2}$ was used to determine the relative size of otoliths, according to Lombarte and Cruz (2007).

*Corresponding author: *eman.salem@omu.edu.ly*

How to cite this article: Alfergani, E.S, El-Mabrok, M. & Almabruk, S.A.A (2025). First record from the Libyan coast of the *Parophidion vassali* Risso, 1810 (Ophidiiformes: Ophidiidae). COMU J. Mar. Sci. Fish, 8 (1): 127-130. doi:10.46384/jmsf.1628210

Figure 1. Sample of *Parophidion vassali* (Risso, 1810) with otolith

Results and Discussion

There are many traditional methods used to identify and separate fish species, including external body characteristics such as body shape, scale indicators, and color patterns (Strauss & Bond, 1990). Gills: number of gills and length of the arc (Azab *et al*., 2019). In addition to vertebrae; size, shape, and orientation of the dorsal and ventral zygapophysis, etc., show very large differences between species (Granadero and Silva, 2000). In this study, we relied on otoliths, or ear stones, which are calcareous structures in the inner ear of fish that aid in hearing and balance. These structures are widely used in species differentiation (Wakefield *et al.,* 2014) and are most commonly used to identify prey, determine their size, and obtain data on paleodiversity (Pierce and Boyle, 1991; Nolf, 1985). There are several identification keys for otoliths that can be used to separate species (Reichenbacher *et al*., 2017; Tuest *et al*., 2008; Smale *et al*., 1995; Nolf, 1985), and the Analysis of Fish Otolith Shape (AFORO) website also provides an open catalog of otolith images and allows species identification from otolith images (Lombarte *et al*., 2006).

In this research, as a result of the sample being affected by digestive materials, the otolith was relied upon to confirm the classification of this species. The standard length of the sample is about 108.1 mm. The *P.vassali* otolith is oval in shape and thickness, 3.210 mm in length, 2.317 mm in height, and 5.430 mm^2 in area (Table 1). The sulcus was shallow and heterosulcoid. The ostium is oval and large compared to the shorter cauda. According to the value of the relative size of the otolith, 0.464, it is classified as medium-sized. The ratio of otolith length to standard length is about 2.96%. Schwarzhans and Aguilera (2016) showed that there is a high degree of similarity in otoliths between *P. vassali* and *P. schmidti* in several aspects, including the thick oval shape of the otoliths and the long, shallow sulcus. With the difference that the sulcus in *P. vassali* is slightly wider**.** Four indicators of shape were calculated, as shown in the table. The highest values for Roundness >Aspect ratio>Rectangularity > Ellipticity; were 71.288, 1.3854, 0.7302, and 0.1615, respectively.

Table 1. Otolith parameter and shape index of *Parophidion vassali*.

Measurements	Left
Otolith Length, OL	3.210 mm
Otolith Height, OH	2.317 mm
Otolith Area	5.430 mm^2
Otolith Weight	0.0179 g
OL/SL%	2.96%
Otolith relative size	0.464
Ellipticity	0.16157
Roundness	71.28862
Aspect Ratio	1.385412
Rectangularity	0.730212

P. vassali is an uncommon endemic fish in the Mediterranean Sea. It was initially described by the French scientist Antoine Risso in 1810. It was first recorded in Lebanese waters through a single specimen found in the stomach of *Scorpeana scorfa* (Mouneimné, 1977). In addition, it was recorded in several other locations in the Mediterranean Sea, including Tunisian waters (Capape *et al.,* 2016), Portugal (CARNEIRO *et al*., 2020), and the Adriatic Sea, which represents the northern arm of the

Mediterranean Sea (Lipej and Dulcic, 2010). Based on the latest updated list of bony fish species recorded from Libya (Elbaraasi *et al*., 2009), this is the first record *of Parophidion vassali* in Libyan waters.

Acknowledgments

The authors would like to express their warm thanks to Professor Antoni lombarte for assisting with the otolith classification of this species.

Conflict of Interest

The authors declare no conflict of interest

Author Contributions

Eman Alfergani contributed to sample collection and measurements; all authors contributed to manuscript writing and language review.

Ethics Approval

This research did not need ethical approval.

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